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Characterisation of Endophytic Bacteria from a Desert Plant *Lepidium perfoliatum* L.

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Abstract

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Sixty-two endophytic bacteria from the leaves, roots, and stems of healthy *Lepidium perfoliatum* L. were isolated and characterised. From the results, 89, 87, 90, and 97% isolates could tolerate 12% NaCl, 30% PEG 6000, 50°C and pH 10, respectively. 74% isolates could form a biofilm. Besides, 28 isolates could improve the germination rate of host seeds under different degree of drought stress. These data suggest that the endophyte isolates show considerable resistance to abiotic stress and assist their plant hosts to germinate under drought stress.

Keywords: *Lepidium perfoliatum* L.; endophytic bacterial diversity; biofilm; salt tolerance; drought tolerance

Abiotic stresses such as drought, salinity, heat, and soil pH are major limiting factors that affect crop productivity worldwide as a result of their debilitating effects on all plant functions. Endophytic bacteria colonise inner host tissues without damaging the host (Reinhold-Hurek & Hurek 2011). They have several beneficial effects on host plants such as plant growth promotion and increased resistance against plant pathogens and parasites (Hallmann *et al.* 2011). Furthermore, endophytic bacteria have recently been discovered to mitigate the adverse effects of abiotic stress on the host plant. Date palm root-associated bacteria promote plant growth under drought conditions (Cherif *et al.* 2015). Plant growth promoting endophytic bacteria, for example, *Burkholderia phytofirmans* PsJN improve the growth of wheat and maize under drought conditions (Naveed *et al.* 2014a, b). Endophytic bacterial strains *Achromobacter xylosoxidans* and *Bacillus pumilus* enhance the growth of sunflower seedlings under water stress (Forchetti *et al.* 2010). The use of bacterial endophytes (*Pseudomonas fluorescens* YsS6 and *P. migulae* 8R6) has the potential to facilitate tomato plant growth under high salt contents (Ali *et al.* 2014). Isolates from the halophyte *Limonium sinense* (Girard) Kuntze, which possessed ACC deaminase activity could enhance plant growth under saline stress conditions (Qin *et al.* 2014). The endophytic bacterium *A. xylosoxidans* that was isolated from the root tissue of *Catharanthus roseus* was able to mitigate salt stress in *C. roseus* (Karthikeyan *et al.* 2012). Inoculation of rice (*Oryza sativa* L.) with the endophytic bacterium *P. pseudoalcaligenes* that was isolated from the root tissue could increase shoot biomass at lower saline levels (Jha *et al.* 2011).

Lepidium perfoliatum L. (Brassicaceae) is an annual or biannual herb plant living in wilderness or desert (Yuan *et al.* 2013). It is the sand-fixing pioneer plant because of its fast growth and the resistance to drought and salinity (Huang *et al.* 2011). The previ-

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ous study of *L. perfoliatum* was focussed on its seed coat mucilage, which is important for germination in a harsh environment (Li-Li *et al.* 2008). To the best of our knowledge, there are currently no data on the culture-dependent bacterial endophyte diversity in *L. perfoliatum*. Thus, a better understanding of the presence of the endophytic bacteria in *L. perfoliatum* is important. These bacteria may have been adapted to the salt, drought, heat, and alkali stress conditions and could provide significant benefits to the host plants. Studying the bacterial endophytes could offer a few important clues on how the mutual interaction of these endopyte with their host helps them to withstand the desert environment successfully.

This study aims to isolate the endophytic bacteria associated with the desert plant *Lepidium perfoliatum* L., and to identify them using 16S rRNA gene sequence analysis. Also included in the objective is the characterisation of enzyme production, tests for salt, drought, heat and alkali tolerance, and biofilm formation in non-stressed and stressed conditions. Finally, whether these isolates could improve the germination rate of host seeds under different degree of drought stress was tested.

MATERIAL AND METHODS

Sampling of plant materials. *L. perfoliatum* was collected in its native habitat from a hill near Urumqi City in Xinjiang Province, China (43°50'N, 87°35'E) in May 2013. Annual precipitation in this area is less than 200 mm, however, the evaporation capacity is as high as 2600 mm, and the relative humidity is only 30% which is the typical ecoclimatic condition in thr area of drought and desert. After digging into a depth of 5–30 cm around the main root of plants, all whole plants were then immediately put in sterile polystyrene bags and brought to the laboratory on ice.

Isolation of endophytic bacteria. Fresh healthy *L. perfoliatum* plants were washed thoroughly with tap water to remove adhering soil and debris. One gram of leaves, stems and roots was weighed individually and immersed in 75% ethanol for 3 min three times, and then treated with 0.1% mercuric chloride for 3 min and finally rinsed 6 times in sterile distilled water (5 min each rinse). In order to examine the effect of surface sterilisation, aliquots of the final rinsing water were spread on beef extract peptone medium plates containing (in g/l) beef extract 3, peptone 10, NaCl 5, pH 7.2, and cultured at 37°C. The absence of bacteria after 6 days of incubation was taken to confirm that the surface disinfection process was successful. Ten grams of each surface-sterilised tissue were ground using sterilised mortar and pestle with 10 ml of sterilised potassium phosphate buffer (pH 7.0). The suspension was used for serial dilution plating on beef extract peptone medium plates that were then incubated at 37°C. Bacterial colonies from each tissue were passed through four rounds of single colony isolation by streaking them on beef extract peptone medium to ensure purity of the organism. Pure cultures were stored in 20% glycerol at –80°C.

16S rRNA gene amplification, bacterial identification and phylogenetic analysis. Fresh cultures (3 ml) of the isolates in beef extract peptone medium were used for total DNA extraction (Sambrook 1989). Genomic DNA was used as a template for 16S rRNA gene amplification with universal primer set 27F and 1492R (Chelius & Triplett 2001) in a polymerase chain reaction (PCR). Purified PCR products were sequenced with an ABI 3730 automated sequencer (Invitrogen, Shanghai, China). All the 16S rRNA gene sequences of the isolates were identified using the EzTaxon algorithm (http://www.ezbiocloud.net/eztaxon/identify). Multiple sequence alignment was performed with Clustal X software (Thompson *et al.* 1997). A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei 1987) using MEGA 3 (Kumar *et al.* 2004).

Physiological and biochemical characterisation. All isolates were initially tested for their phenotypic features. Gram's reaction was performed, and the cell shape was observed under a light microscope by taking a drop of bacterial culture suspension in sterile physiological solution (9 g/l NaCl). The growth of the isolates at different temperatures was determined in beef extract peptone liquid medium at the following temperatures: 4, 20, 37, 45, 50, 55, and 60°C. The pH range was tested on beef extract peptone liquid medium set at different pH values, using the buffers homopiperazine-N,N'-bis-2-(ethanesulfonic acid) (Homopipes; 25 mmol/l) for pHs ranging between 4 and 5, 2-(N-morpholino)ethanesulfonic acid (MES; 20 mmol/l) for pHs ranging between 5 and 7, 2-(N-morpholino)propanesulfonic acid (MOPS; 20 mmol/l) for pHs ranging between 7 and 8, and adjusted with NaOH for pHs up to 11 (Fterich *et al.* 2012).

Catalase activity was determined by observation of O_2 evolution from cell suspensions following the addition of 0.5 mM H_2O_2 (Rorth & Jensen 1967). To determine amylase activity, the isolates were in-

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oculated on starch agar. And after bacterial growth, 5 ml of a 1% iodine–potassium iodide solution was added to each plate, allowing the visualisation of clear halos around the colonies (Krishnan *et al.* 2012). To determine esterase activity, previously sterilised Tween 80 was added to the sterilised culture medium containing (in g/l) peptone 10, NaCl 5, CaCl·H_2O 0.1, pH 7.4 to a final concentration of 1% (v/v) to evaluate the bacterial esterase activity. The presence of halos was considered to indicate enzymatic activity (Castro *et al.* 2014). To determine protease activity, we used a culture medium containing skim milk; and the formation of a halo around the colony was considered indicative of proteolytic activity (Castro *et al.* 2014).

Abiotic stress resistance. Resistance to salt was assessed by adding 2, 5, 7, 10, 12, and 15% (w/v) of sodium chloride (NaCl) to beef extract peptone liquid medium and incubating at 37°C overnight. Tolerance to drought stress was evaluated by adding 10, 20, and 30% (m/v) of polyethylene glycol (PEG 6000) to beef extract peptone medium and the growth was checked after 16 h incubation at 37°C. Alkali resistance was assessed by adjusting medium pH with saturated NaOH from 7 to 10 and the isolates

Table 1. Endophytic bacteria isolated from surface-sterilised *Lepidium perfoliatum* L.

Isolates	GenBank Accession No.[a]	Closest phylogenetic relative (GenBank Accession No.)	Identity (%)	No. of isolates
Isolates from leaves				
NB3-1, NB3-2, NB3-4, NB3-5, NB3-7, NB4-2, NB4-4, NB4-5, NB4-6, NB4-7, NB4-8, NZ2-4-1, NZ2-4-2, NZ2-6, NL1-3-1, NL1-3-2	KR999901, KR999902, KR999904, KR999905, KR999907, KR999910, KR999912, KR999913, KR999914, KR999915, KR999916, KR999921, KR999922, KR999924, KR999932, KR999933	*Bacillus subtilis* subsp. *inaquosorum* KCTC 13429^T (EU138467)	99.79, 99.79, 99.65, 99.65, 99.93, 99.58, 99.93, 99.72, 99.93, 99.93, 99.58, 99.31, 99.58, 99.57, 99.72, 99.93	16
NB3-3, NB3-6, NB3-8, NB4-1, NB4-3, NB4-9, NZ2-3, NZ4-2, NZ4-3	KR999903, KR999906, KR999908, KR999909, KR999911, KR999917, KR999920, KR999930, KR999931	*Bacillus flexus* IFO 15715^T (AB021185)	98.29, 99.72, 99.58, 99.93, 99.65, 99.86, 100, 99.58, 99.93	9
NZ2-1	KR999918	*Bacillus cereus* ATCC 14579^T (NR_074540)	99.93	1
NZ2-2, NZ3-2, NL3-1	KR999919, KR999927, KR999934	*Bacillus licheniformis* ATCC 14580^T (X68416)	99.58, 99.37, 99.58	3
NZ2-5	KR999923	*Bacillus safensis* FO-36b^T (AF234854)	99.51	1
NZ2-7, NZ4-1-1, NZ4-1-2	KR999925, KR999928, KR999929	*Bacillus mojavensis* RO-H-1^T (JH600280)	99.79, 99.86, 99.65	3
NZ3-1	KR999926	*Bacillus sonorensis* NBRC 101234^T (AF302118)	99.16	1
Isolates from roots				
NG3-1, NG3-2, NG3-3, NG3-4, NG3-5, NG3-6, NG3-7, NG3-8, NG3-9, NG3-10, NG4-1, NG4-3, NG4-4, NG4-5, NG4-6, NG4-7, NG4-8, NG4-9, NG4-10, NG4-11, NG4-12, NG4-14, NG4-15, NG4-16, NG4-17	KR999935, KR999936, KR999937, KR999938, KR999939, KR999940, KR999941, KR999942, KR999943, KR999944, KR999945, KR999947, KR999948, KR999949, KR999950, KR999951, KR999952, KR999953, KR999954, KR999955, KR999956, KR999958, KR999959, KR999960, KR999961	*Bacillus subtilis* subsp. *inaquosorum* KCTC 13429^T (EU138467)	99.44, 99.65, 99.72, 99.79, 99.57, 99.51, 99.86, 99.65, 99.86, 99.79, 99.44, 99.79, 99.93, 99.86, 99.72, 99.37, 99.93, 99.93, 99.65, 99.72, 99.58, 99.86, 99.36, 99.57, 99.86	25
NG4-2	KR999946	*Bacillus sonorensis* NBRC 101234^T (AF302118)	98.82	1
NG4-13	KR999957	*Bacillus licheniformis* ATCC 14580^T (X68416)	97.91	1
Isolates from stems				
NJ4-1	KR999962	*Bacillus sonorensis* NBRC 101234^T (AF302118)	99.03	1

[a]accession numbers correspond with the isolate order

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were incubated at 37°C overnight. Heat resistance was assessed by incubating overnight at 37, 45, 50, and 55°C, respectively.

Biofilm formation. Biofilm formation was quantified according to Tariq *et al.* (2014) with some modifications. Briefly, the strains were incubated at 37°C for 24 h

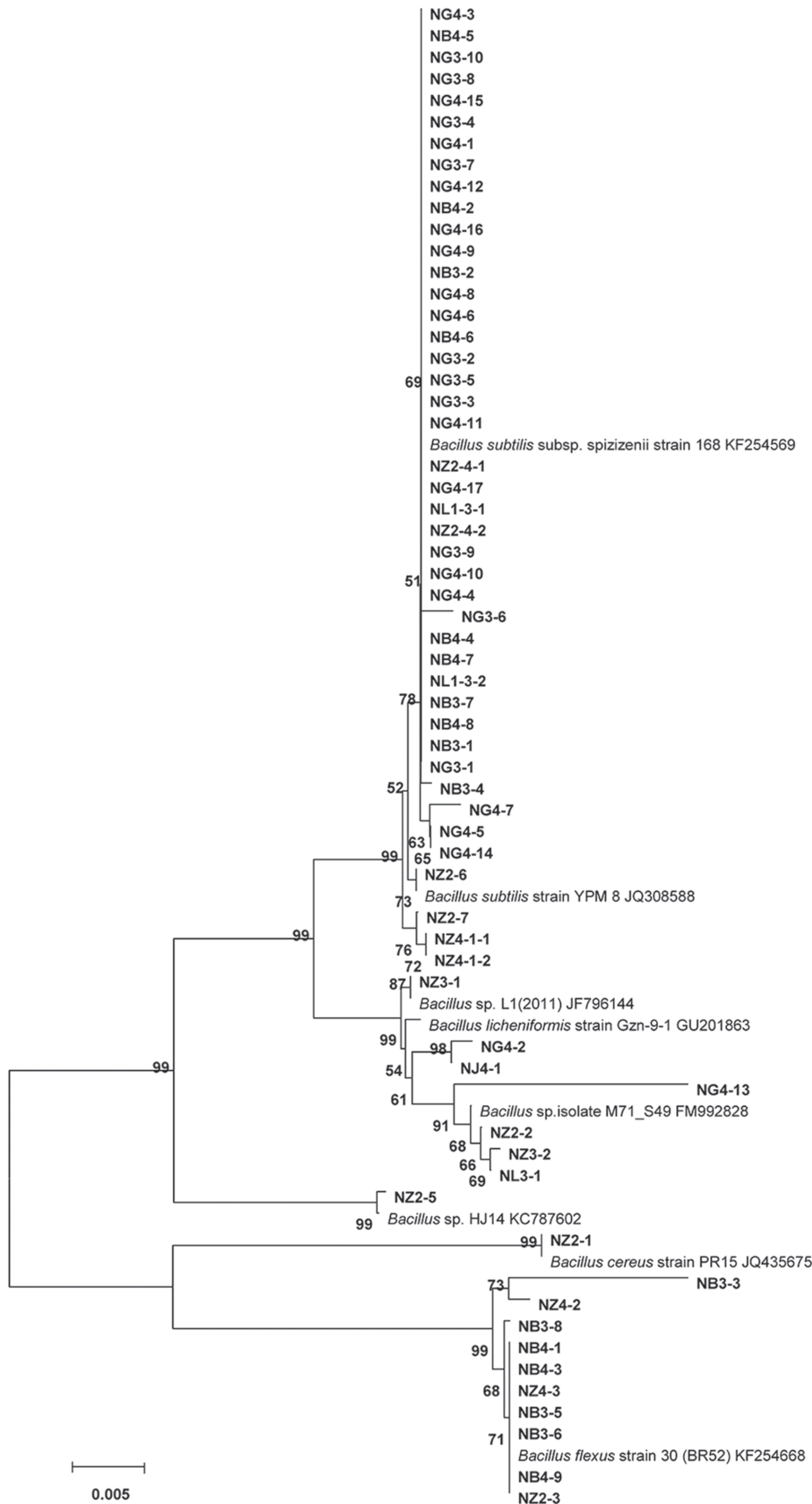

Figure 1. Neighbour-joining tree of partial 16S rDNA sequences of 62 endophytic isolates from *Lepidium perfoliatum* and their close relatives. Sequences obtained in this work are in bold. Numbers at nodes are bootstrap scores (above 50%) obtained from 1000 replications

Bar = 1 nt substitution per 200 nt

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in LB liquid medium. The cells were harvested and resuspended to an OD_{600} of 0.2. An aliquot (1 ml) of the bacterial cell suspension was added to test tubes and incubated at 37°C for 20 hours. Each isolate was repeated 3 times. Finally, the liquid of the cultures was discarded carefully and the biofilm remained attached to the wall of the tube. Then the biofilms were stained with 1 ml of 0.01% crystal violet for 40 minutes. The excess dye was washed away with three changes of sterile distilled water. The dye that stained the biofilm was then solubilised with 3 ml of 95% ethanol, and the amount of dye was quantified by measuring the absorbance at 570 nm. Means and standard errors of the mean were calculated.

All isolates were individually checked for biofilm formation by visually assessing its formation on the top of the medium in different salt, drought, heat and alkali stress conditions. For salt stress, the medium was supplemented with various concentrations of NaCl (0, 5, 10, and 15%). For drought stress, the medium was supplemented with PEG 6000 (0, 10, 20, and 30%). For alkali stress, the medium was supplemented with NaOH to adjust pH from 7 to 10. For heat stress, the test tubes were incubated at 37, 45, 50, and 55°C, respectively, for 24 hours. The experiment was carried out in four replicates.

Seed germination experiment. Seeds of *L. perfoliatum* were harvested in its native habitat. The seeds' mucilage was removed by soaking in 18 M H_2SO_4 for 5 min and then the seeds were rinsed in sterile distilled water for three times. The treated seeds were placed on two layers of filter paper which were moistened with 1.4 ml serial concentration of PEG 6000 (0, 5, 10, 15, 20, and 25%, w/v) and 0.7 ml beef extract peptone medium or 0.7 ml of the bacteria cultures (OD_{600} = 1.0) in 6 cm Petri dishes (Li-Li *et al.* 2008). Each treatment was performed in quadruplicate with 25 seeds per replicate. Germination rate was scored 8 days later after placing the Petri dishes in an illumination cultivation chamber at 20°C, 60~70% humidity, 2500–3000 lux illumination.

Statistical analysis. Analysis of variance (ANOVA) using SPSS 13.0 software was employed for the statistical analysis of biofilm formation and germination rate. Means among the treatments were compared by the least significant difference (*LSD*) test. Differences were considered to be significant at $P < 0.05$.

RESULTS

Endophytic bacteria isolation and phylogenetics. A total of 62 endophytic bacterial isolates were obtained from leaves, roots and stems of *L. perfoliatum*. The analysis of 16S rRNA gene sequences showed that all of them belonged to the genus *Bacillus* and their GenBank accession numbers weres KR999901 to KR999962 (Table 1). A phylogenetic tree constructed using the partial 16S rDNA sequences of the bacterial endophytes and taxa is shown in Figure 1.

Distribution and diversity of the bacterial endophytes are shown in Figure 2. Of the 62 isolates, 34 were from leaves, 27 were from roots, and 1 was from stems (Figure 2A). The relative amounts of the species isolated are as follows: species similar to *B. subtilis* (66%), *B. flexus* (14%), *B. licheniformis* (6%), *B. mojavensis* (5%), *B. sonorensis* (5%), *B. cereus* (2%), and *B. safensis* (2%) (Figure 2B).

As shown in Figure 3, the isolates were shared at least by 7 "species": similar to *B. sonorensis* were distributed in leaves, roots and stems; but similar to *B. subtilis* and *B. licheniformis* were obtained only from leaves and roots; and similar to *B. flexus*, *B. mojavensis*, *B. cereus*, and *B. safensis* were located in leaves only, suggesting higher diversity of bacteria living in leaves than in roots.

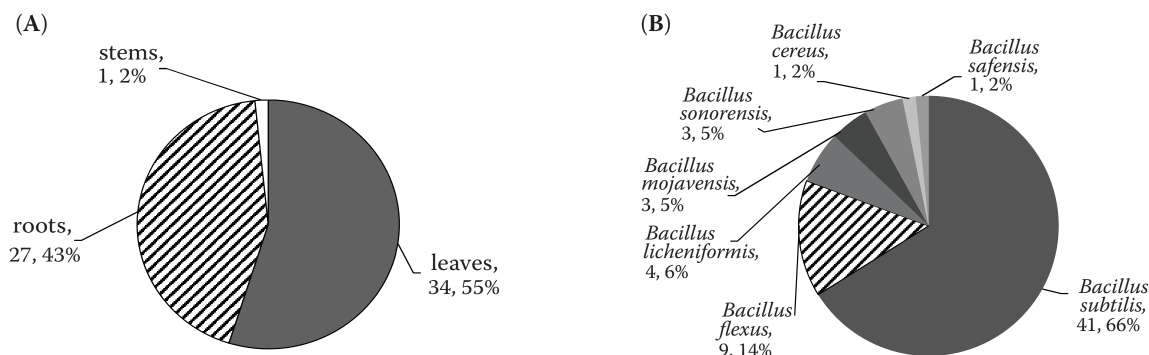

Figure 2. Number of obtained strains from different tissues (**A**) and endophytic bacterial diversity (**B**) of *Lepidium perfoliatum*

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Table 2. Morphology and biochemical characteristics of 62 endophytic isolates from *Lepidium perfoliatum*

Isolates	Gram reaction	Cell morohology	pH		Temperature		Tolerance (%)		Activity				Biofilm formation
			extent	optimum	extent	optimum	NaCl	PEG 6000	catalase	amylase	esterase	protease	
NB3-1	+	Rod	4–11	6–9	20–50	37	12	30	+	+	+	+	+
NB3-2	+	Rod	5–10	6–9	20–50	37	12	30	+	–	+	+	+
NB3-3	+	Rod	5–10	6–9	20–45	37	15	30	+	+	–	+	–
NB3-4	+	Rod	4–9	6–9	4–50	37	15	30	+	+	+	+	+
NB3-5	+	Rod	5–10	6–9	4–45	37	12	20	+	–	–	+	–
NB3-6	+	Rod	5–10	6–9	20–37	30	12	20	+	+	–	+	–
NB3-7	+	Rod	5–9	6–9	20–50	37	12	30	+	+	+	+	+
NB3-8	+	Rod	5–11	6–9	4–50	37	15	20	+	-	-	+	-
NB4-1	+	Rod	5–11	6–9	20–50	37	7	20	+	+	-	+	-
NB4-2	+	Rod	5–10	6–9	4–50	37	12	30	+	-	+	+	+
NB4-3	+	Rod	5–11	6–9	20–50	37	15	30	+	-	+	+	-
NB4-4	+	Rod	4–10	6–9	4–50	37	12	30	+	+	+	+	+
NB4-5	+	Rod	4–10	6–9	20–50	37	12	30	+	+	+	+	+
NB4-6	+	Rod	4–10	6–9	4–50	37	12	30	+	-	+	+	+
NB4-7	+	Rod	4–10	6–9	4–50	37	12	30	+	+	+	+	+
NB4-8	+	Rod	4–10	6–9	4–50	37	12	30	+	+	+	+	+
NB4-9	+	Rod	5–11	6–9	20–50	37	12	20	+	-	+	+	-
NZ2-1	+	Rod	4–11	6–9	20–50	37	5	30	+	-	-	+	-
NZ2-2	+	Rod	5–11	6–9	4–50	37	15	30	+	+	+	+	-
NZ2-3	+	Rod	5–11	6–9	20–50	37	12	20	+	+	+	+	-
NZ2-4-1	+	Rod	4–11	6–9	20–50	37	15	30	+	+	+	+	+
NZ2-4-2	+	Rod	4–10	6–9	4–50	37	15	30	+	+	+	+	+
NZ2-5	+	Rod	5–11	6–9	20–50	37	10	20	+	–	+	+	–
NZ2-6	+	Rod	5–11	6–9	20–50	37	5	30	+	+	+	+	–
NZ2-7	+	Rod	5–11	6–9	20–50	37	12	30	+	+	+	+	+
NZ3-1	+	Rod	6–11	6–9	20–50	37	15	30	+	–	+	+	+
NZ3-2	+	Rod	6–11	6–9	20–50	37	15	30	+	+	-	+	–
NZ4-1-1	+	Rod	5–10	6–9	20–50	37	10	30	+	–	+	+	+
NZ4-1-2	+	Rod	6–10	6–9	20–50	37	12	30	+	+	+	+	+
NZ4-2	+	Rod	6–11	6–9	20–37	30	7	20	+	–	–	+	–
NZ4-3	+	Rod	6–11	6–9	20–37	30	10	30	+	–	–	+	–
NL1-3-1	+	Rod	4–11	6–9	4–50	37	15	30	+	+	+	+	+
NL1-3-2	+	Rod	4–11	6–9	4–45	37	12	30	+	–	+	+	+
NL3-1	+	Rod	4–11	6–9	20–45	37	15	30	+	–	+	+	–
NG3-1	+	Rod	5–10	6–9	4–50	37	15	30	+	+	+	+	+
NG3-2	+	Rod	5–10	6–9	4–55	37	12	30	+	+	+	+	+
NG3-3	+	Rod	5–10	6–9	4–50	37	15	30	+	+	+	+	+
NG3-4	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG3-5	+	Rod	5–10	6–9	20–50	37	12	30	+	+	+	+	+
NG3-6	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG3-7	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG3-8	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG3-9	+	Rod	5–10	6–9	4–55	37	12	30	+	+	+	+	+
NG3-10	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG4-1	+	Rod	6–11	6–9	4–50	37	12	30	+	+	+	+	+
NG4-2	+	Rod	4–10	6–9	20–55	37	15	30	+	–	+	+	+
NG4-3	+	Rod	5–10	6–9	20–50	37	12	30	+	+	+	+	+
NG4-4	+	Rod	5–10	6–9	4–50	37	12	30	+	+	+	+	+
NG4-5	+	Rod	5–10	6–9	20–50	37	12	30	+	+	+	+	+
NG4-6	+	Rod	6–10	6–9	20–50	37	15	30	+	+	+	+	+
NG4-7	+	Rod	6–10	6–9	20–55	37	12	30	+	+	+	+	+

+ positive reaction; – negative reaction

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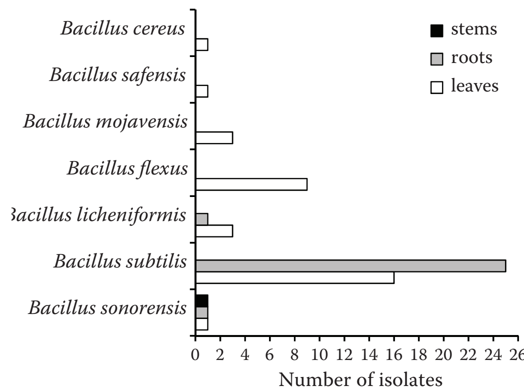

Figure 3. Frequency of bacterial endophytes in different parts of *Lepidium perfoliatum*

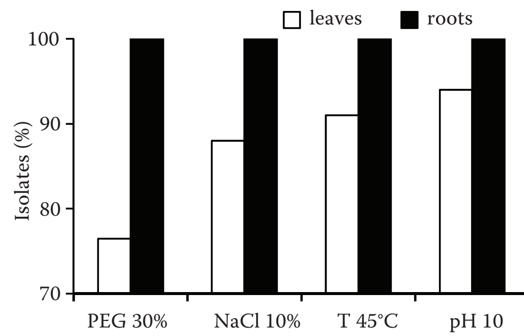

Figure 4. Percentage of isolates displaying the assayed abiotic stress tolerance of endophytic bacteria from leaves and roots of *Lepidium perfoliatum*

Phenotypic and functional characterisation of isolates. All endophytic isolates were individually tested for their gram-reaction, cell morphology, the range and optimal pH and temperature, salt and drought tolerance, abilities to hydrolyse starch, esters, protein, and degrade hydrogen peroxide (Table 2).

All of the isolates were Gram-positive. Microscopy showed that all isolates were rods. 100% isolates grew at 20 and 37°C, 95% at 45°C, 90% at 50°C, and 6% at 55°C. None could grow at 60°C. However, it was discovered that all the isolates could survive after being incubated at 60°C for 3 h and then transferred to a temperature of 37°C, which was the optimal temperature for all isolates. For the pH test, 100% isolates grew at pH 6, 7, 8, and 9, 97% at pH 10, 84% at pH 5, 40% at pH 11, and 26% at pH 4; pH 6 to pH 9 was the optimal pH range for all isolates. For the NaCl test, 100, 97, 94, 89, and 25% isolates grew at 5, 7, 10, 12, and 15% NaCl, respectively. 100 and 87% isolates grew at 20 and 30% PEG 6000, respectively. Out of the 62 isolates, 44 showed amylolytic activity (89% isolates were from roots and 56% isolates from leaves). Fifty-three of the 62 isolates showed esterasic activity (100% isolates were from roots and 74% isolates from leaves) and all of them were protease and catalase positive. Only 74% isolates could form a biofilm (Table 2). Analysing the adaptability of isolates to abiotic stresses further (Figure 4) revealed that drought stress resistance was shown by 100% isolates from roots, and 76% isolates from leaves tolerated 30% PEG 6000. Within the bacterial collection, 100% isolates from roots and 88% isolates from leaves actively grew in the presence of 10% NaCl. 100% isolates from roots and 91% isolates from

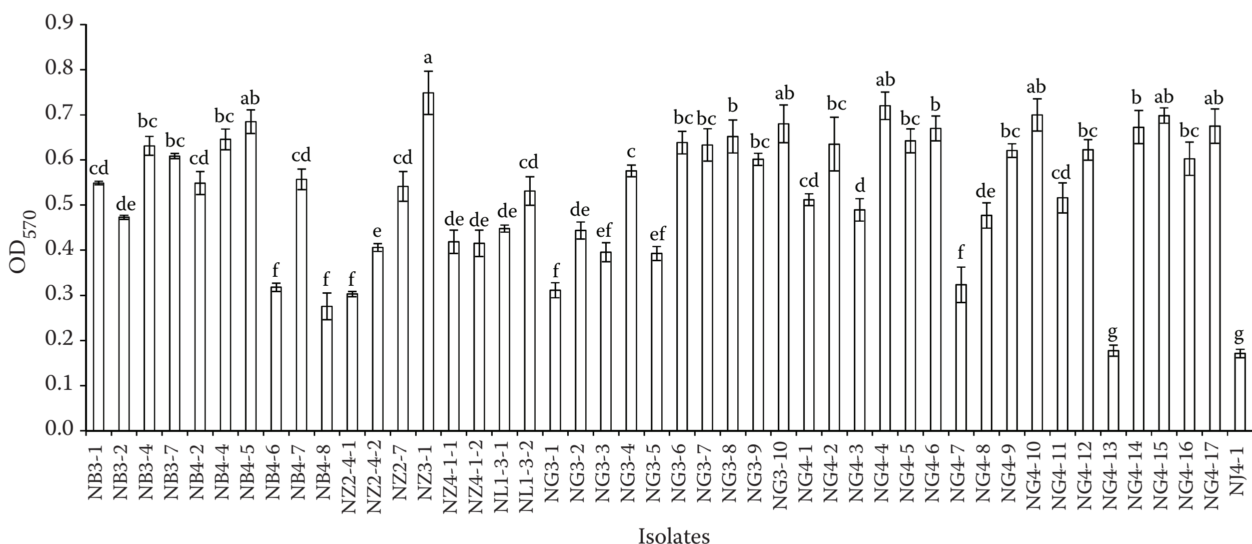

Figure 5. Quantity of the biofilm (absorbance 570 nm) of endophytic bacteria determined by crystal violet staining
Each value is plotted as the mean ± SE (n = 3)

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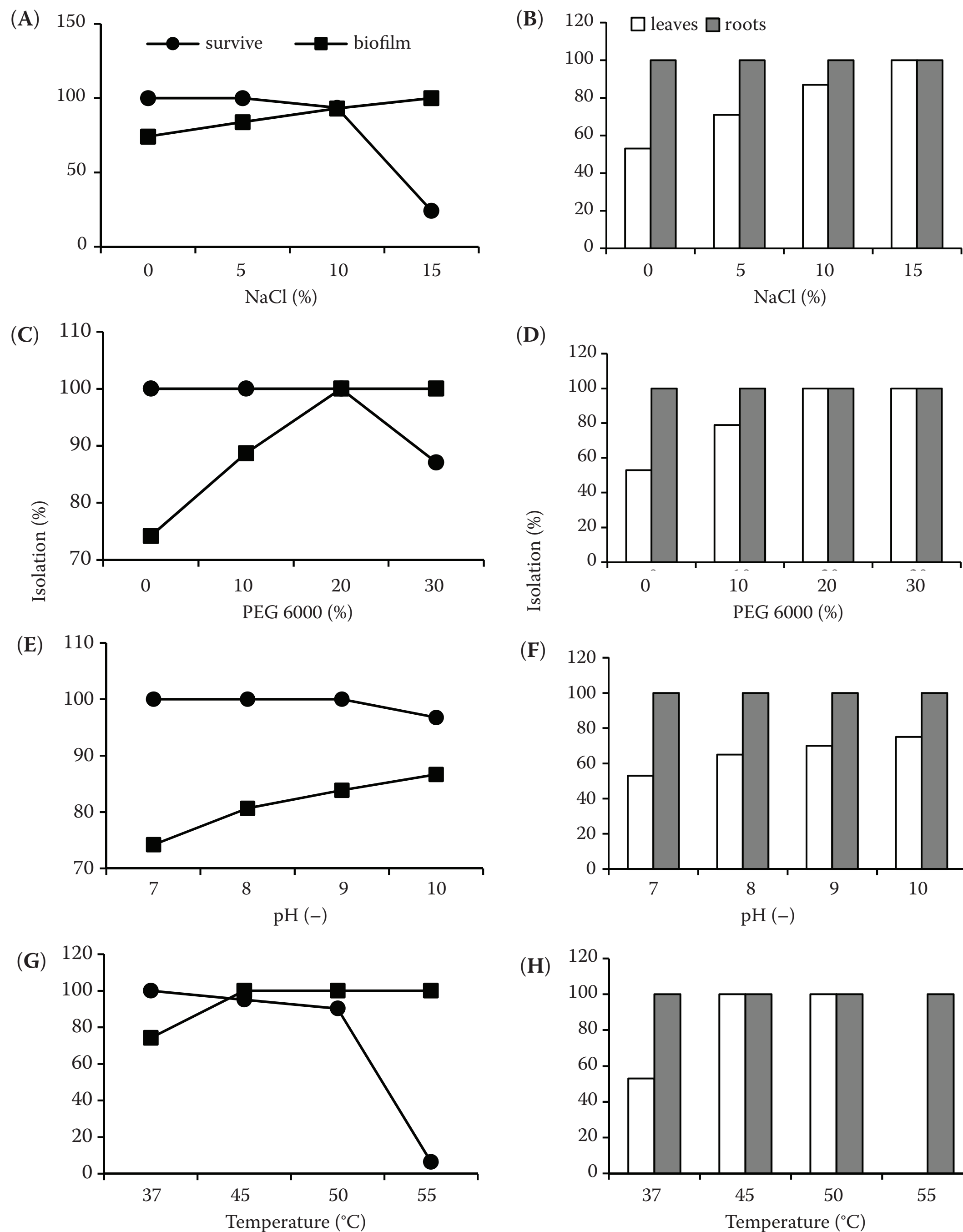

Figure 6. Percentage of survived isolates and biofilm formed in the survived isolates and percentage of biofilm formed by isolates from leaves and roots of *Lepidium perfoliatum* under different concentrations of NaCl (**A**, **B**), under different concentrations of PEG 6000 (**C**, **D**), at various pH values (**E**, **F**), and at various temperatures (**G**, **H**)

leaves were able to grow at 45°C. 100 and 94% of the strains from roots and leaves, respectively, were able to grow in basic media (up to pH 10). Isolates from roots were more tolerant than isolates from leaves.

Biofilm formation. In the non-stressed conditions, 46 of the 62 isolates exhibited significantly different biofilm formation at a varying OD_{570} level from 0.171 ± 0.009 to 0.749 ± 0.048. Bacterial isolates NZ3-1 and NG4-4 showed high efficiency in biofilm formation in glass test tubes (Figure 5).

The percentage of survived isolates decreased as the concentration of NaCl increased. However, of the survived isolates, the percentage of biofilm formation increased as the concentration of NaCl

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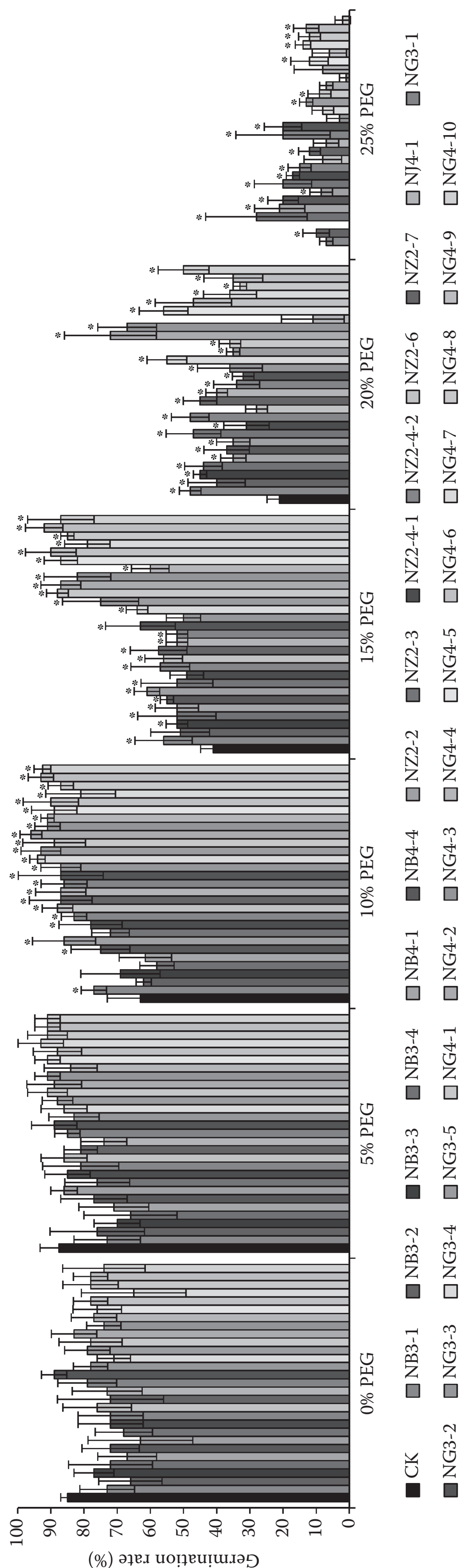

Figure 7. *Lepidium perfoliatum* seed' germinations rate at different concentration of PEG 6000 with or without endophytic bacteria
Each value is plotted as the mean ± SE (n = 4); *means P < 0.05

increased (Figure 6A). The same trend was observed in drought, alkali, and heat stress conditions (Figures 6C, 6E, 6G).

The formation of biofilm on survived isolates from roots was always 100% in non-stressed and various salt, drought, alkali and heat stress conditions (Figures 6B, 6D, 6F, 6H). The survived isolates from leaves showed biofilm formation that increased from 53% in non-stressed conditions to 100% at 15% NaCl (Figure 6B), from 53% in non-stressed condition to 100% at 20% PEG and at 30% PEG (Figure 6D), from 53% in non-stressed condition to 75% at pH 10 (Figure 6F), from 53% in non-stressed conditions to 100% at 45 and 50°C, but decreased to 0 at 55°C since no isolates from the leaves could survive at this temperature (Figure 6H).

Role of endophytic bacteria in seed germination rate under drought stress. Under drought stress conditions with 10, 15, 20, and 25% PEG 6000, there were 23, 25, 26 and 17 strains that could significantly improve seed germination, respectively (Figure 7). The result showed that under non-stressed conditions or lower drought stress conditions with 5% PEG 6000, no isolate could benefit to germination rate, however, as the concentration of PEG 6000 increased to 20%, more and more isolates that could enhance the germination rate. Even under the hightest concentration of PEG 6000, there were still 17 isolates that could improve seed germination. Among these useful isolates, twelve were helpful at all 10, 15, 20, and 25% PEG 6000, in which 1, 1, 10 isolates were identified as *B. licheniformis*, *B. mojavensis*, and *B. subtilis*, respectively.

DISCUSSION

Currently, endophytic bacteria are attracting increasing attention not only for their promotion of plant growth and control of plant diseases (Hallmann *et al.* 2011), but also for their stress tolerance and improvement of plant growth in an extreme environmen (Forchetti *et al.* 2010; Jha *et al.* 2011; Karthikeyan *et al.* 2012; Ali *et al.* 2014; Naveed *et al.* 2014a, b; Qin *et al.* 2014; Cherif *et al.* 2015). *Phoenix dactylifera* L. root endophytic bacteria that were isolated from the environment of desert oasis promoted plant growth under drought conditions (Cherif *et al.* 2015). However, studies focusing on the isolation and characterisation of endophytic bacteria from "pioneering plant" in the desert are scarce. Therefore, we examined the *L. perfoliatum* grown in

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the desert of Xinjiang China for the presence of endophytic bacteria and tried to determine their abiotic stress resistance.

In this study, we demonstrated that the culturable bacterial endophytes belong to the genus *Bacillus*, which are frequently isolated from plant tissues and as endophytic microorganisms (Misaghi & Donndelinger 1990; Sturz 1995; Araújo *et al.* 2001; Forchetti *et al.* 2007). Because of their spore-forming ability, they could resist adverse ecological conditions, and this feature is evident in the isolates obtained from *L. perfoliatum* growing in extreme drought, saline, heat and alkali conditions. This kind of abundance has also been observed in endophytic bacteria from roots of the halophyte *P. strombulifera* (Sgroy *et al.* 2009).

We found that the diversity of endophytic bacteria varied greatly within different tissues of *L. perfoliatum*. Endophytes were mainly located in the leaves followed by the roots and then the stems. Our results concur with a previous finding that the highest diversity of endophytic bacteria was from leaf tissues (Shi *et al.* 2013).

Eighty-nine percent isolates were able to grow at 12% NaCl, and this is similar to the finding that 84% isolates from the halophyte *Limonium sinense* could grow at 13% NaCl (Qin *et al.* 2014). Also, 100% isolates could grow in 20% PEG, agreeing with the result that 100% strains from the root of *Phoenix dactylifera* L. located in a desert oasis grew at low water activities in the presence of 20% PEG (Cherif *et al.* 2015). We noticed that the vast majority of the isolates (95%) were able to grow at 45°C, higher than the observations reported for the isolates from nodules of *Acacia tortilis* subsp. *raddiana* in arid soils of Tunisia (Fterich *et al.* 2012). All of the isolates from the root of *L. perfoliatum* tolerated 50°C whereas none of the strains from the root of *Phoenix dactylifera* L. located in a desert oasis grew at 50°C (Cherif *et al.* 2015). Ninety-seven percent isolates showed tolerance to pH values from 6 to 10, which is in agreement with the report of Fterich *et al*. (2012). The results highlighted the remarkable adaptability of *L. perfoliatum* isolates that occurs in extreme arid zones of China. High salt, drought and alkali tolerance and T_{max} values are very important characteristics for survival under the hot and dry conditions. These findings suggest that salt-, drought-, alkali- and heat-tolerant endophytes might protect their host plant against abiotic stress.

We have demonstrated that 71, 85, and 100% endophytic bacteria possess the ability to degrade the plant polymer starch, esterase and protein, respectively. These results indicate the endophyte' potential for nutrient acquisition and hydrolysis of a macromolecule into a micromolecule to protect the plant from abiotic stress.

Biofilms are surface-attached communities of bacteria, which wrap bacteria inside with a self-produced extracellular matrix that protects them from environmental stress (Costerton *et al.* 1995, 1999; Stanley & Lazazzera 2004). Biofilm formation enables rhizobia to withstand various abiotic stress conditions, thereby contributing to an efficient symbiosis (Hirsch 2010). Biofilm formation by plant growth-promoting rhizobacteria (PGPR) improved PGPR effects (Seneviratne *et al.* 2010). Our data show that 74% of endophytic bacterial isolates from *L. perfoliatum* could form a biofilm and the percentage kept growing in various stress conditions (Figures 6A, 6C, 6E, and 6G), indicating that biofilm formation helps endophytic bacteria grow in the assayed abiotic stress. Exopolysaccharides (EPS), important components of the biofilm matrix, have multiple functions such as surface attachment, protection against abiotic or biotic stress factors and nutrient gathering (Becker 2015). Loss-of-function EPS mutants of *Sinorhizobium meliloti* and *Mesorhizobium tianshanense* show impaired biofilm formation and delay nodule formation or reduce nodulation capacity (Fujishige *et al.* 2006). Similarly, the nitrogen-fixing endophyte *Gluconacetobacter diazotrophicus* secretes EPS, which play a role in biofilm formation and the endophytic colonization of graminaceous plants (Meneses *et al.* 2011). Disruption of *ptsI* in *B. cereus*, which was isolated from the root systems of healthy wheat varieties, caused a 70% reduction in biofilm formation and a 1000-fold reduction in colonisation (Xu *et al.* 2014). Biofilm formation of *Halomonas meridiana* PAa6 helped the bacterial strains to colonise better the *Cicer arietinum* var. CM 98 roots in saline soil (Qurashi & Sabri 2012). Symbiotic genes are important for biofilm-forming rhizobia (Fujishige *et al.* 2008). It seems that biofilm formation is positively connected with the microbe-plant symbiosis. Our data show that 100% of isolates from roots form a biofilm no matter the stress conditions (Figures 6B, 6D, 6F, and 6H), which may assist themselves to colonise in the roots of *Lepidium perfoliatum* L.

In summary, this study describes the morphological, molecular, enzymatic, stress tolerance and biofilm formation characteristics of culturable bacterial endophytes isolated from leaves, roots and stems of *L. perfoliatum*. The biofilm formation was confirmed in 74% isolates in non-stressed conditions, and the percentage increased in salt, drought, heat and alkali

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stress conditions, suggesting their fine adaption to the harsh environment. Besides, 28 isolates could improve the seed germination rate under different drought stress. These data are valuable and essentially show that the endophytic bacteria from *L. perfoliatum* could assist their host to resist a harsh environment.

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