Deciphering the root endosphere microbiome of the desert plant *Alhagi sparsifolia* for drought resistance-promoting bacteria

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Abstract

Drought is among the most destructive abiotic stresses limiting crop growth and yield worldwide. Although most research has focused on the contribution of plant-associated microbial communities to plant growth and disease suppression, far less is known about the microbes involved in drought resistance among desert plants. In the present study, we applied 16S rRNA gene amplicon sequencing to determine the structure of rhizosphere and root endosphere microbiomes of *Alhagi sparsifolia*. Compared to those of the rhizosphere, endosphere microbiomes had lower diversity but contained several taxa with higher relative abundance; many of these taxa were also present in the roots of other desert plants. We isolated a *Pseudomonas* strain (LTGT-11-2Z) that was prevalent in root endosphere microbiomes of *A. sparsifolia* and promoted drought resistance during incubation with wheat. Complete genome sequencing of LTGT-11-2Z revealed 1-aminocyclopropane-1-carboxylate deaminases, siderophore, spermidine, and colanic acid biosynthetic genes, and type VI secretion system genes, which are likely involved in biofilm formation and plant-microbe interactions. Together, these results indicate that drought-enduring plants harbor bacterial endophytes favorable to plant drought resistance, and suggest that novel endophytic bacterial taxa and gene resources may be discovered among these desert plants.

Importance

Understanding microbe-mediated plant resistance to drought is important for sustainable agriculture. We performed 16S rRNA gene amplicon sequencing and culture-dependent functional analyses of *Alhagi sparsifolia* rhizosphere and root endosphere microbiomes, and identified key endophytic bacterial taxa and their genes facilitating drought resistance in wheat. This study improves our understanding of plant drought resistance and provides new avenues for drought resistance improvement in crop plants under field conditions.

Introduction

Drought stress is among the most destructive abiotic stresses as its intensity and frequency increases with climate change, drought is expected to threaten more than 50% of the Earth's arable land by 2050 (1, 2). Because water scarcity is a rapidly growing sustainability problem worldwide, it is impossible to combat drought by simply increasing irrigation infrastructure (3). Climate change and an increasing global population will further worsen this condition; therefore, there is an urgent need to improve plant resistance to drought under limited water resource availability. To date, the creation of drought-tolerant cultivars has been the predominant approach for mitigating the negative effects of drought stress on crop growth and yield (4, 5). Although conventional breeding techniques and genetic engineering have promoted the development of drought-tolerant crop varieties, each method has disadvantages and neglects the complex ecological context of the plant growth environment (5−7).

In nature, plants harbor a diverse bacterial community in the rhizosphere that affects plant growth and health (2, 8). Some rhizobacteria can transcend the endodermis barrier and colonize internal tissues to thrive as endophytes in roots, stems, leaves, and other organs (9, 10). Endophytic bacteria can also originate from the phyllosphere or be transmitted through seeds (11). Because endophytic bacteria are relatively protected from the competitive and high-stress soil environment and achieve intimate contact with plant tissues, they are considered to have major interactions with host plants (9, 11, 12). Although the functional capacities of rhizospheric and endophytic bacteria in plant growth promotion and disease control have been widely reported (11, 13, 14), their roles in protecting plant resistance to abiotic stresses such as drought are only beginning to gain attention (3, 5, 7). Recent studies have shown that some plant growth-promoting (PGP) bacteria can increase drought resistance in crop plants such as wheat, maize, tomato, lettuce, chick pea, and beans (12, 15−17).

Northwestern China, which has typical arid and semi-arid regions, accounts for

73 approximately 38% of the Chinese territory. Water deficiency is a major factor limiting
74 the yield of *Triticum aestivum* L. (winter wheat), which is among the most important
75 crops grown in semi-arid areas of northwestern China (18). *Alhagi sparsifolia* Shap.
76 (Leguminosae), a major drought-tolerant plant in the desert ecosystem of northwestern
77 China (19), is an ideal host species for the discovery of novel microbial symbionts that
78 confer drought resistance in crop plants for local agriculture. Hence, the aim of this study
79 was (i) to decipher the root endosphere microbiome of the desert plant *A. sparsifolia* to
80 identify drought resistance-promoting microbes and (ii) to obtain a better understanding
81 of the mechanisms by which these bacteria colonize plants and contribute to drought
82 stress mitigation. To meet these objectives, we applied a combination of
83 culture-dependent and -independent approaches to identify key bacterial taxa in the root
84 endosphere microbiome of *A. sparsifolia* that showed the ability to increase drought
85 resistance in wheat. We then performed genome sequencing and comparative genomics
86 analysis of a drought resistance-promoting strain to investigate the potential mechanisms
87 of bacterial colonization and enhancement of drought resistance in wheat.
88
89 **Results**
90 *Alpha and beta diversity*
91 We applied Illumina HiSeq2500 high-throughput sequencing of the V3−V4 regions of
92 16S rRNA genes to analyze the diversity of rhizospheric and endophytic bacterial
93 communities associated with *A. sparsifolia*. After deletion of chloroplast and
94 mitochondrial-derived 16S rRNA gene amplicons, high-quality reads were assembled
95 (Table S1). The rarefaction curves (Fig. S1) were close to saturation, indicating that the
96 sequencing depth was sufficient to cover the diversity of microbial populations in the
97 rhizosphere soil and plant tissue samples. We observed clear variation between the
98 rhizosphere soil samples and root endosphere samples in terms of richness and diversity
99 (Table S2). The observed amplicon sequence variants (ASVs) and diversity, indicated by
100 the Chao1, abundance-based coverage estimator, and Shannon and Simpson indices,

were much lower in the root endosphere than in the rhizosphere ($P < 0.05$) (Table S2).

We evaluated beta diversity in terms of ASV composition and relative abundance. High dissimilarity between rhizospheric and endophytic microbial communities was revealed by principal coordinate analysis (PCoA), which showed clear separation of rhizosphere soil samples from endosphere samples (Fig. S2). A PCoA plot based on phylum abundance showed that PCo1 explained 73.02% of the Bray-Curtis dissimilarity. Compared to rhizosphere samples, endosphere samples were closer to each other, resulting in much greater dissimilarity among microbial communities in the rhizosphere than among endosphere microbial communities (Fig. S2).

Microbial community composition

Only a small number of sequences in each sample (< 0.1%) were assigned to the phylum Euryarchaeota (Archaea). Within the domain Bacteria, Proteobacteria were further classified into class level. Most of the sequences in the endosphere samples was dominated by *Gammaproteobacteia* (94.8−98.2%) (Fig. 1). While in rhizosphere samples, *Actinobacteria* (28.2−37.1%) was the most abundant phylum, followed by *Alphaproteobacteria* (14.3−19.5%), *Gammaproteobacteria* (13.2−19.0%), *Bacteroidetes* (8.2−11.7%), and *Firmicutes* (7.4−11.6%) (Fig. 1). We evaluated the phyla abundance difference between the rhizosphere and the endosphere by two-tailed Student's t-test followed by Benjamini-Hochberg correction (Table S3). All phyla with relative abundances greater than 0.02% in the rhizosphere were more abundant in the rhizosphere than that in the endosphere except the *Gammaproteobacteria* that comprised 96.39% of the endosphere community (Table S3).

We further examined microbial community composition at the genus level (Fig. S3). A large proportion of rhizosphere sequences could not be unambiguously classified at the genus level (18.56-21.42%); however, only a small proportion of sequences from the root

128 endosphere remained unclassified (< 0.78%; Fig. S3). We evaluated the top 30 abundant
129 genera by two-tailed Student's t-test followed by Benjamini-Hochberg correction to test
130 the effects of the plant compartment (rhizosphere vs endosphere) on their relative
131 abundances (Table S4). We detected significantly higher abundance ($P < 0.05$) of the
132 genera *Kocuria* (18.802%), *Halomonas* (2.545%), *Pseudomonas* (2.504%), *Truepera*
133 (1.079%), and *Planococcus* (1.050%) in the rhizosphere than in the endosphere (Table
134 S4). In contrast, the genera *Pseudomonas* (80.00%), *Stenotrophomonas* (5.816%),
135 *Achromobacter* (3.589%), *Undibacterium* (2.099%), and *Providencia* (1.912%) were
136 significantly more abundant in the root endosphere than in the rhizosphere ($P < 0.05$;
137 Table S4).
138
139 *A* Pseudomonas *strain improved drought resistance in wheat*
140 In parallel to the culture-independent study, we isolated bacteria from *A. sparsifolia* roots
141 for PGP and drought resistance promotion. Based on the endophytic bacterial community
142 composition determined by the culture-independent approach, seven strains (*Serratia*
143 *marcescens* LTGR-2, *Stenotrophomonas maltophilia* LTGR-2-1Z, *Pseudoxanthomonas*
144 *wuyuanensis* LTGR-13Z, *Rhizobium massiliae* LTGR-20, *Pantoea dispersa*
145 LTGPAF-12F, *Acinetobacter oleivorans* LTGT-10, and *Pseudomonas* sp. LTGT-11-2Z)
146 were selected and characterized for PGP activities *in vitro*. To this end, *Pseudomonas* sp.
147 LTGT-11-2Z showed growth promotion when incubated with wheat. In addition,
148 activities related to PGP were examined, including siderophore production,
149 exopolysaccharide production, 1-aminocyclopropane-1-carboxylate (ACC) deaminase
150 activity, growth in 5% NaCl, growth in 20% PEG, and growth at 42°C, which further
151 supported the PGP of LTGT-11-2Z (Table S5).
152 We performed additional experiments to confirm the ability of LTGT-11-2Z to
153 improve drought resistance in wheat. After seven days of water deprivation,
154 non-inoculated control plants were severely affected, whereas plants inoculated with

LTGT-11-2Z were healthier and better hydrated (Fig. 2A). Under the similar field capacity (Fig. 2B), inoculated plants showed significantly higher shoot length, root length, total plant fresh weight, and dry weight compared with the non-inoculated stressed control (Fig. 2C-F). In addition, the colonization of LTGT-11-2Z on wheat roots were observed by confocal microscopy and bacterial cells were clearly detected in wheat root cells. LTGT-11-2Z was observed to have adhered to or colonized on wheat root surfaces (Fig. S4).

Genomics analysis of Pseudomonas *sp. LTGT-11-2Z*

More than 0.8% of the 16S amplicon sequencing reads of *A. sparsifolia* root endosphere microbiota were assigned to the 16S sequence of LTGT-11-2Z, which showed 100% similarity with an abundant ASV, suggesting that this bacterium was abundant in endosphere microbial communities. After BLASTn searching against gene sequences in NCBI-Nr database, the 16S rRNA gene of LTGT-11-2Z showed 100% similarity with that of *Pseudomonas fluorescens* 2P24, which was isolated from wheat roots and has been demonstrated to show PGP ability (20); 2P24 also produces several antifungal compounds, including 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide and siderophores (20).

To understand the mechanisms by which LTGT-11-2Z colonizes plants and promotes mitigation of drought stress, we performed complete genome sequencing of the *Pseudomonas* strain. The genome of *Pseudomonas* sp. LTGT-11-2Z consisted of a circular chromosome of 6,073,276 bp, with an overall GC content of 61.68%; its circular chromosome contained 5,702 genes, including 77 tRNA-coding genes, 17 rRNA genes, and 5,608 protein-coding genes (Table S6). Despite the high similarity between 16S rRNA gene sequences of LTGT-11-2Z and 2P24, average nucleotide identity (ANI) between their genomes was only 78.38%. The genome map of LTGT-11-2Z is shown in Fig. 3A.

To gain a better understanding of the functional profiles of LTGT-11-2Z, we compared its genome to those of previously reported plant endophytes, including *P. fluorescens* 2P24 (20), *Pseudomonas putida* MTCC5279 (17), *Klebsiella* sp. LTGPAF-6F (21), *Serratia proteamaculans* 568 (22), *Burkholderia phytofirmans* PsJN (23, 24), *Azospirillum* sp. B510 (25), *Klebsiella pneumoniae* 342 (26), *Methylobacterium populi* BJ001 (24), *P. putida* W619 (22), Enterobacter sp. 638 (27), *Pseudomonas stutzeri* A1501 (28), *Azoarcus* sp. BH72 (29), and *Gluconacetobacter diazotrophicus* Pal5 (30) (Table S7). These reference strains have been reported to play roles in the promotion of plant growth and drought resistance. For example, *P. putida* MTCC5279 promotes growth and drought stress alleviation of *Cicer arietinum* L. (chick pea) (17), *Klebsiella* sp. LTGPAF-6F improves growth and drought tolerance of wheat (21), and a series of genes involved in bacterial adaptation to plant tissue conditions, such as the limitation of amino acid and carbon source concentrations, were discovered in *K. pneumoniae* 342 (26). Functions of the reference strains relevant to rhizosphere competence, plant colonization, plant growth promotion, and stress resistance were analyzed and compared with those of LTGT-11-2Z. The results revealed that a number of these functions were present in LTGT-11-2Z; it possessed complete pathways encoding flagellar assembly and chemotaxis-related proteins, as well as genes for curli fiber biosynthesis, which may contribute to plant adhesion (24). A total of 222 glycoside hydrolase genes were detected, several of which may allow bacterial penetration of the plant cell wall and colonization of plant tissues (24). We also annotated biosynthetic genes relevant to plant growth promotion or abiotic stress resistance, which were represented by ACC deaminases and genes for the synthesis of siderophores and spermidine (Table S8), consistent with its activities revealed by experiments (Table S5).

We then sought to understand the specific functions of LTGT-11-2Z that mediate interactions with *A. sparsifolia*. The genome of this strain was compared with those of four previously reported plant endophytes also belonging to the genus *Pseudomonas*: *P.*

putida W619, an endophytic bacterium of poplar trees (22); *P. stutzeri* A1501, a rice root-associated bacterium (28); *P. putida* MTCC5279, a positive PGP rhizobacteria in chick pea (17); and the phylogenetically close strain *P. fluorescens* 2P24 (20). The resulting Venn diagram revealed that 1,760 KEGG genes were shared by LTGT-11-2Z, 2P24, W619, A1501, and MTCC5279 (Fig. 3B). LTGT-11-2Z possessed 63 unique KEGG genes (Fig. 3B; Table S9). In particular, the colanic acid biosynthesis glycosyl transferase *wcaI* was only present in LTGT-11-2Z; this gene is involved in colanic acid synthesis, which contributes to biofilm architecture and allows voluminous biofilm formation (31). In addition, two type VI secretion system (T6SS) encoding genes (*vasI* and *impI*) were only present in LTGT-11-2Z. An overview of predicted metabolic properties and important transport pathways for interactions between the strain and the host plant is summarized in Fig. 4.

Discussion

Studies of the contribution of plant-associated microbiomes to plant drought resistance are rare. In the present study, we performed high-throughput 16S rRNA gene amplicon sequencing to describe the rhizosphere and endosphere prokaryotic microbiomes of *A. sparsifolia*, a typical desert plant that inhabits poor and extremely dry soil environments in northwestern China. We integrated our high-throughput-based assessment of bacterial diversity in the endosphere and culture-dependent functional analyses to identify drought resistance-promoting endophytic bacteria and further mined its genes involved in endophytic colonization and promotion of plant drought resistance.

Bacterial ASV diversity and richness were higher in the rhizosphere than in the root endosphere of *A. sparsifolia*, indicating that only a limited number of bacteria can adapt to an endophytic lifestyle. A similar result was observed in a previous study (10), which demonstrated a great loss of bacterial diversity and richness from rhizosphere soil to the endosphere compartments of poplar trees. Fitzpatrick et al. (32) found that the

rhizosphere exhibited higher diversity and greater evenness of abundance than the endosphere across 30 plant species of 14 families (*Amaranthaceae*, *Apocynaceae*, *Asparagaceae*, *Asteraceae*, *Brassicaceae*, *Convolvulaceae*, *Fabaceae*, *Onagraceae*, *Plantaginaceae*, *Poaceae*, *Polygonaceae*, *Rosaceae*, *Solanaceae*, and *Asteraceae*). The rhizosphere of *Glaux maritima* (Primulaceae), a typical halophytic plant, was discovered to have greater richness and diversity than the endosphere microbial community (33). These results can be explained by the general perspectives that the soil-root interface acts as a selective barrier to determine endosphere community composition, and that plant endophytic colonization is limited to specific bacterial species (34).

Members of the phylum *Proteobacteria* have been found to be enriched in the rhizosphere and root endosphere of a wide range of desert plants, including *G. maritima* (33) and *Phoenix dactylifera* (35). Microbes isolated from the root tissues of *P. dactylifera* significantly increased plant growth under controlled drought stress (35). Consistently, our results revealed that the root endosphere of *A. sparsifolia* was dominated by *Proteobacteria*, which also dominated rhizosphere soil. However, unlike *G. maritima* (33), the relative abundance of *Alphaproteobacteria* was lower in the endosphere than in the rhizosphere of *A. sparsifolia*, and the relative abundance of *Gammaproteobacteria* was lower in the rhizosphere than in the endosphere, suggesting that members of *Gammaproteobacteria*, but not *Alphaproteobacteria*, are more effective endophytic colonizers of *A. sparsifolia*. Moreover, the root endosphere of *G. maritima* and *P. dactylifera* were reported to have an abundant *Actinobacteria* in endosphere (33, 35), which is also different from the case of *A. sparsifolia*. Together, the differences and similarities among the microbes of different desert plant species suggest that both drought environment and plant species influence the recruitment of bacterial endophyte communities in the rhizosphere.

At the genus level, rhizosphere communities were dominated primarily by *Kocuria*, which has been isolated from rhizosphere soil of various plants inhabiting different

environments (2, 5). In contrast, endophytic assemblages were dominated by *Pseudomonas*, followed by *Stenotrophomonas* and *Achromobacter*, all of which are members of the phylum *Proteobacteria.* Endophytic bacteria *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter* have been isolated from a variety of plant species and may provide beneficial effects for plant growth and health (35, 36). For example, *Pseudomonas* isolates from *Arabidopsis* roots showed the ability to adhere and colonize on *Arabidopsis* and grapevine rhizoplanes (36), and *Pseudomonas* isolates from *Suaeda salsa* increased salt stress tolerance and plant growth in cucumber and rice plants (37). These results may indicate common functions among certain microbial taxa in plant roots.

The drought resistance-promoting strain LTGT-11-2Z possesses broad PGP potential, as revealed by comparative genome analysis. Siderophore production is a well-known PGP property (38); polyamine production may contribute to the improvement of plant growth under water stress conditions (39); and lowering plant ethylene levels through ACC deaminase activity is among the major mechanisms employed by PGP bacteria to protect plants against a wide range of environmental stresses (40, 41). LTGT-11-2Z also possesses genes responsible for flagellum biosynthesis, chemotaxis, curli fiber production, and plant cell wall-degrading enzymes, which may be involved in plant adhesion and colonization (24, 27). Certain genes, including T6SS and biofilm formation-related genes, are specific for LTGT-11-2Z to a greater extent than for closely related *Pseudomonas* strains, implying unique mechanisms that mediate interactions between LTGT-11-2Z and plants. Therefore, our results suggest that LTGT-11-2Z has the potential for use as a biotechnological agent to improve drought resistance in crop plants for arid land agriculture.

Conclusion

In the present study, we revealed the structure of rhizosphere and root endosphere

290 microbiomes of the desert plant *A. sparsifolia*. Endosphere microbiomes showed lower
291 diversity than those of the rhizosphere, but contained several microbial taxa that were
292 also present in roots of previously reported desert plants. The *Pseudomonas* strain
293 LTGT-11-2Z, isolated from *A. sparsifolia* roots, improved drought resistance in wheat,
294 likely due to synergistic effects of multiple activities related to plant growth promotion
295 and stress resistance. Comparative genomics analysis revealed a subset of genes involved
296 in rhizosphere competence, plant colonization, plant growth promotion, and plant
297 protection. Taken together, these results provide a basis for more detailed studies of the
298 molecular mechanisms responsible for bacteria-mediated drought resistance in plants.
299 Further research is needed to evaluate whether drought resistance improvement in crop
300 plants can be achieved by these bacteria under field conditions, which is a prerequisite
301 for their application in agricultural practice to combat drought.
302
303 **Materials and Methods**
304 *Sampling*
305 *A. sparsifolia* Shap. was collected in August 2014 from Taklamakan Desert, Xinjiang
306 Uyghur Autonomous Region, northwest China. 16 independent plant samples with soil
307 attached to the roots were collected at the sampling site and placed in sterile bags. After
308 being transported to the laboratory within 48 h, the rhizosphere soil was collected and
309 stored at −80ºC, while the plant material was carefully washed in running water to
310 remove external soil and debris. After drying at room temperature, the roots were
311 separated and subjected to a five-step surface sterilization procedure as described
312 previously (42). Sterility checks were performed by plating the final wash water and
313 placing pieces of the surface-sterilized tissues on Trypticase soy agar (TSA) plates.
314 Sterilized tissues were used immediately for the isolation of endophytic bacteria and
315 stored at −80ºC for molecular analysis.
316

DNA isolation and high-throughput 16S rRNA gene amplicon sequencing

Rhizosphere soil from 16 *A. sparsifolia* individuals was separately subjected to DNA extraction by using the E.Z.N.A. Soil DNA Kit (Omega Bio-Tek Inc., USA) according to the manufacturer's instructions. Extraction of endobacterial DNA from the root tissues of the 16 independent plants was carried out by using a modified bacterial cell enrichment method according to Nissinen et al. (43). For each rhizosphere soil and plant root sample, DNA was extracted in triplicate, and the resulting DNA extracts were mixed together and stored at -20ºC for downstream manipulation. Subsequently, 32 DNA samples, including 16 biological replicates, were subjected to PCR amplification. The V3-V4 hypervariable region of bacterial 16S rRNA gene was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GGACTACHVGGGTATCTAAT-3′) (44). The PCR reaction was performed in 50 µl containing 100 ng of template DNA, 1.5 µl of primers (5 µM), 1 µl of KOD DNA polymerase (2.5 U μl^{-1}) (Toyobo, Osaka, Japan), 5 µl of 10 × KOD Buffer, and 5 µl of dNTPs (2.5 mM). The PCR amplification was performed under the following cycling conditions: initial denaturation at 95ºC for 2 min, followed by 27 cycles at 98ºC for 10 s, 62ºC for 30 s and 68 ºC for 30 s, and a final extension at 68ºC of 10 min. Each DNA sample was amplified in triplicate, mixed into one PCR product, examined by 2% agarose gel, and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The purified PCR amplicons were quantified with a QuantiFluor™-ST fluorometer (Promega Corporation, Madison, WI, USA), pooled at equimolar concentrations, and finally sequenced on an Illumina HiSeq PE250 platform at Gene Denovo Ltd., Co (Guangzhou, China).

Sequence processing and statistical analysis

Quality control of the paired-end 16S rRNA amplicon reads was conducted using the NGS QC Toolkit (45). The cut-off value for high-quality filtering was 20. Reads containing more than 30% low-quality bases or unpaired reads were removed.

Quality-filtered reads were assembled into error-corrected ASVs using the DADA2 v1.4.0 software (46) to represent unique bacterial taxa. Merged reads were aligned to the SILVA database (47) implemented in the QIIME2 package (46). Taxonomic annotation at different taxonomic levels ranging from phylum to species was performed based on ASV composition and relative abundance. Chimeric sequences and mitochondrial and chloroplast ASVs were removed from all samples. Community richness and diversity indices and rarefaction curves were determined using the "qiime diversity core-metrics-phylogenetic" command for alpha and beta diversity analysis in the QIIME2 package. Relationships between communities were tested using PCoA implemented in the PAST software package based on Bray-Curtis distances (48). Differences in pairwise comparisons between the endosphere and rhizosphere were evaluated using the two-tailed Student's *t*-test, and all the phyla and the top 30 abundant genera showing significant differences were identified; *P* values of Student's *t*-tests were corrected using the Benjamini-Hochberg method.

A. sparsifolia *root strain isolation and identification*

After surface sterilization, *A. sparsifolia* root tissues were cut into small fragments and macerated using a sterile mortar and pestle in sterile distilled water. Macerated samples were serially diluted, spread-plated onto 10% tryptic soy agar (TSA) and reasoner's 2A Agar (Difco) plates supplemented with 50 μg mL^{-1} cycloheximide, and incubated at 28°C for 1 week. After incubation, colonies were picked from the plates and sub-cultured to obtain pure isolates. For 16S rRNA gene sequencing, genomic DNA was extracted, and the 16S rRNA gene sequence was amplified using the bacterial universal primers 27F and 1492R, as previously described (49). The obtained 16S rRNA gene sequences were compared with available 16S rRNA gene sequences from the EzBioCloud server using the BLASTn online tool (https://www.ezbiocloud.net/) (49). All isolates were assigned to the genus level based on the closest match in the NCBI online database.

371

372 *Screening of strains improving drought resistance in wheat and related experiments*

373 Seeds of the winter wheat *T. aestivum* were surface sterilized and germinated as
374 previously described (50). 3-day-old seedlings of uniform size were selected and planted
375 in a sterilized soil mixture (3:1 soil to sand) in a 14-cm plastic pot. Seedlings were
376 maintained in a growth chamber under a 14-h/10-h light/dark photoperiod. One wheat
377 individual was grown in the same pot. After seven days of growth under normal watering
378 conditions, seedlings were fertilized with single bacterial cultures in sterilized tap water
379 at 10^8 CFU g^{-1} soil, and subjected to normal watering for 10 days prior to drought stress
380 by withholding water irrigation. After observation for up to seven days, the PGP effects
381 of seven isolated bacterial strains were noted. ACC deaminase activity was determined
382 according to the methods by Penrose and Glick (51), which measures the amount of
383 a-ketobutyrate produced when ACC is cleaved by ACC deaminase; the abundance of
384 a-ketobutyrate was determined by comparing the absorbance at 540 nm of a sample to a
385 standard curve. Exopolysaccharide production and siderophore production, and resistance
386 to abiotic stresses (temperatures and osmotic stress), were performed as described in our
387 previous study (50).

388 After the selection of *Pseudomonas* sp. LTGT-11-2Z for further study, additional
389 experiments with more replicates were performed to confirm its PGP and drought
390 resistance promotion. Four wheat individuals were grown in the same pot and each
391 treatment included three pots as three replicates. After growth at under normal watering
392 conditions for seven days, inoculated plants and non-inoculated control plants were
393 regularly watered for 10 days and subjected to drought stress by withholding water
394 irrigation for up to seven days. Plants that were properly irrigated throughout the
395 experiment were also used as a positive control. When the non-inoculated plants had
396 become severely wilted, water irrigation was resumed for one day, and then plant health
397 was assessed and photographed. Plants exposed to each treatment were then harvested for

biomass and length measurement. Statistical analysis was performed using two-tailed Student's *t*-test to compare data from plants inoculated with LTGT-11-2Z and non-inoculated plants under drought treatment.

To visualize whether the bacteria could adhere and colonize on the wheat root, confocal microscopy was conducted. After seven days of water deprivation, only inoculated wheat root (17-day old) were harvested. The strain LTGT-11-2Z was labeled with green fluorescent protein (GFP) transformed with the pKEN-GFP-mut3 plasmid and inoculated on wheat roots. Wheat roots were gently washed to remove weakly bound bacteria and then stained with 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific, USA) by incubation in the dye (300 nM in phosphate-buffered saline) for 1 min. Bacterial cells adhering to the wheat root were observed using a laser-scanning confocal microscope (LSM710; Carl Zeiss, Germany). The excitation/emission wavelengths (nm) of the DAPI and GFP channels were 358/461 and 488/520, respectively.

Whole-genome sequencing and genome analysis of the selected Pseudomonas strain

Genomic DNA of *Pseudomonas* sp. LTGT-11-2Z was extracted as described previously (52). Whole-genome sequencing was performed by using PacBio RS II and Illumina HiSeq X Ten System. In the PacBio sequencing, a 10 kb insert size library was constructed, and in the Illumina sequencing, 350 bp short insert library was constructed. The genome coverage in the PacBio sequencing was > 100× and that in the Illumina sequencing was 500×. *De novo* assembly of the genome was conducted using SPAdes (version 3.12.0) (53), which assembles PacBio and Illumina sequences together using the options '--pacbio' and '--pe'. After assembly, each genome contained a single contig, and no plasmid sequences were identified.

Prediction of protein-coding genes was performed using the software prodigal

(version 2.6.3) (53). All the reference genomes used for comparison were downloaded from the NCBI gene bank. The endophyte genomes used as references was selected by referring to a previous work (24). The number of genes in the functional categories was calculated based on the annotations by searching against the COG (55), KEGG (55), CAZy (56), and NCBI-Nr databases on a local server. Protein-coding sequences were BLASTp searched against the KEGG database using an *E* value cut-off of 1e-7. The unique and common KEGG genes for the four *Pseudomonas* genomes were identified using Venn analysis (http://bioinformatics.psb.ugent.be/webtools/Venn/).

Nucleotide sequence accession numbers

The raw 16S rRNA gene amplicon sequences have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA515584. The genome sequence of LTGT-11-2Z has been deposited in GenBank under accession number CP033104.

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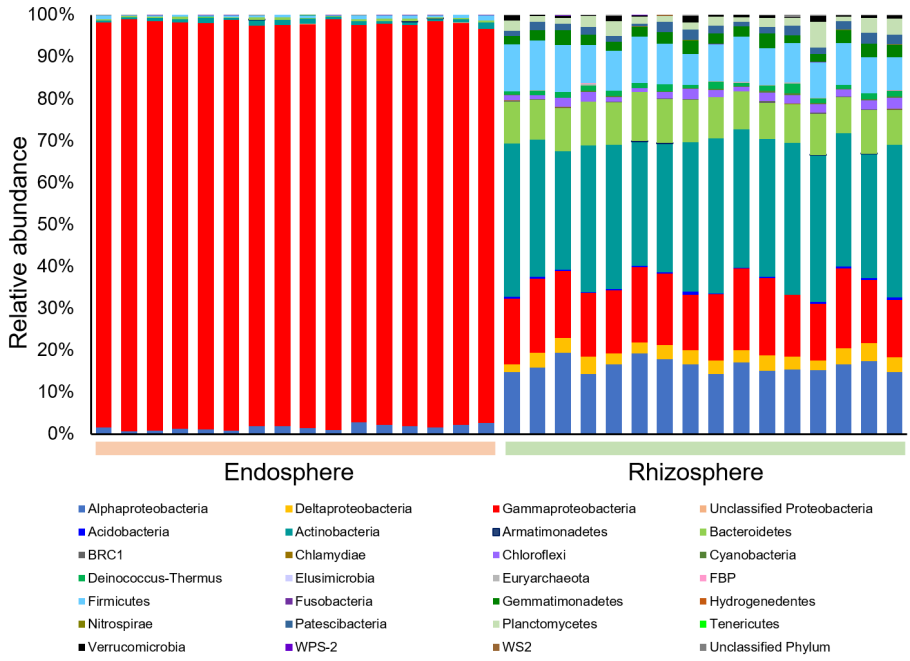
638 **Figures**



639

640 **Fig. 1** Community structures at the phylum level. The detailed relative abundances of the
641 phyla and differences between root rhizosphere and endosphere are listed in Table S3.
642 Data analysis was performed using DADA2 v1.4.0 implemented in QIIME2.

643

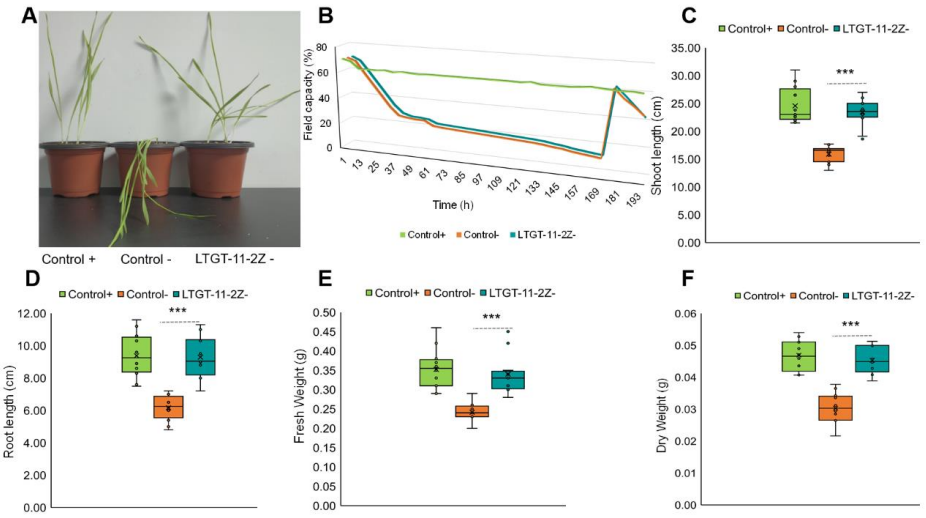
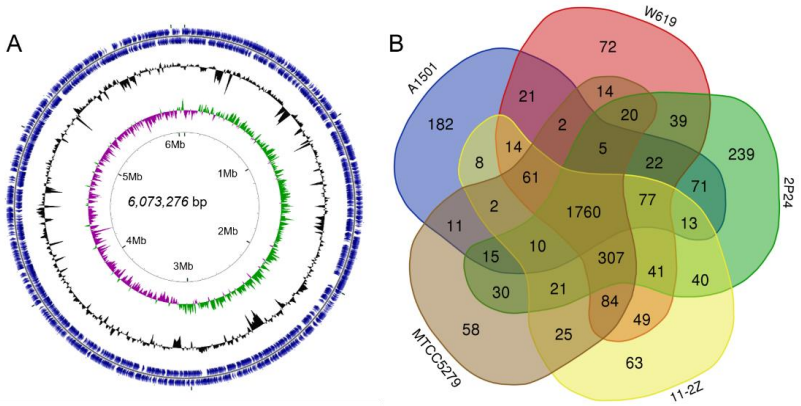


Fig. 2 *Pseudomonas* sp. LTGT-11-2Z improved wheat resistance to drought. '+', irrigated at soil water-holding capacity throughout the experiment; '−', grown without water for seven days and watered for one day. **A** Representative images of plants inoculated with LTGT-11-2Z compared with those of non-inoculated plants under water stress conditions. **B** Field capacity (%). **C** Shoot length (cm). **D** Root length (cm). **E** Plant fresh weight (g). **F** Plant dry weight (g). Statistical analysis between control (–) and LTGT-11-2Z (–) was performed using Student's t-test. ***$P \leq 0.001$.

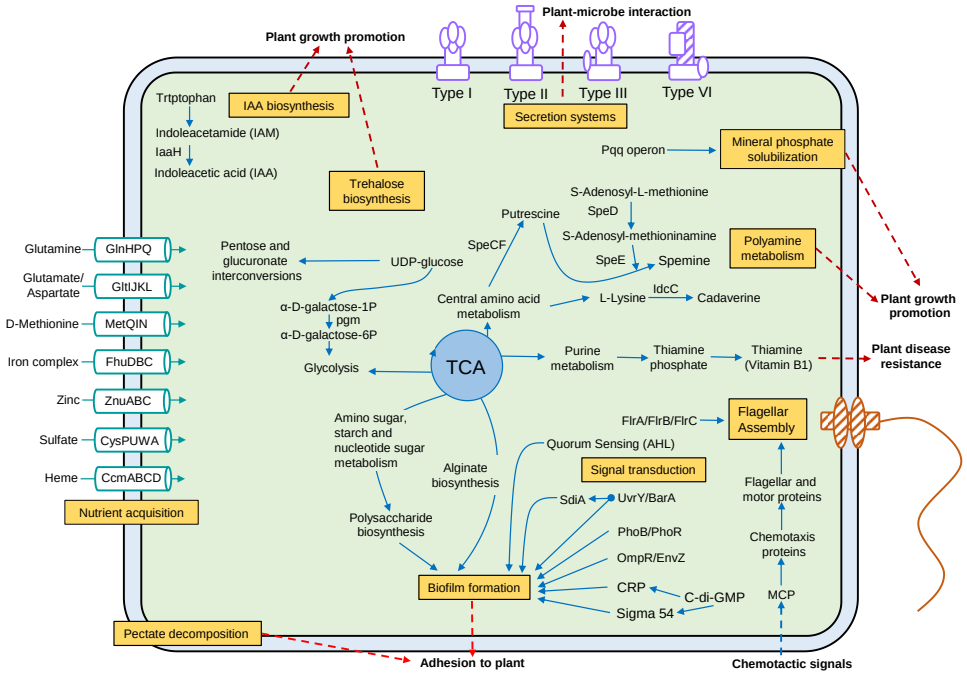


652

653 **Fig. 3** Complete genome sequencing and comparative analysis of *Pseudomonas* sp.
654 LTGT-11-2Z. **A** Circular representation of the genome; illustration is based on
655 visualization of sequence feature information by the CGView Server
656 (http://stothard.afns.ualberta.ca/cgview_server/). **B** Whole-genome comparison between
657 *Pseudomonas* sp. LTGT-11-2Z and four other endophytes: *Pseudomonas fluorescens*
658 2P24, *Pseudomonas putida* MTCC5279, *Pseudomonas putida* W619, and *Pseudomonas*
659 *stutzeri* A1501. Overlapping regions indicate the number of Kyoto Encyclopedia of
660 Genes and Genomes (KEGG) genes conserved within the specified genomes. Numbers in
661 non-overlapping portions of each ring indicate the number of KEGG genes unique to
662 each strain.

663

664



665

666 **Fig. 4** Overview of predicted metabolism and transport pathways in *Pseudomonas* sp.
667 LTGT-11-2Z. The metabolic pathways were constructed based on the genes of
668 LTGT-11-2Z annotated by the KEGG database (http://www.genome.ad.jp). The red
669 dashed arrows indicated putative functions or metabolism processes mediating
670 microbe-host interactions.

671