



Short communication

Complete genome sequence of the drought resistance-promoting endophyte *Klebsiella* sp. LTGPAF-6F



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ARTICLE INFO

Article history:

Received 10 November 2016

Received in revised form 10 February 2017

Accepted 10 February 2017

Available online 14 February 2017

Chemical compounds studied in this article:

Indole-3-acetic acid (PubChem CID: 802)

Acetoin (PubChem CID: 179)

2,3-Butanediol (PubChem CID: 262)

Spermidine (PubChem CID: 1102)

Trehalose (PubChem CID: 7427)

Keywords:

Drought resistance-promoting

Bacterial endophyte

Complete genome

Nitrogen fixation

Spermidine

Trehalose

ABSTRACT

Bacterial endophytes with capacity to promote plant growth and improve plant tolerance against biotic and abiotic stresses have importance in agricultural practice and phytoremediation. A plant growth-promoting endophyte named *Klebsiella* sp. LTGPAF-6F, which was isolated from the roots of the desert plant *Alhagi sparsifolia* in north-west China, exhibits the ability to enhance the growth of wheat under drought stress. The complete genome sequence of this strain consists of one circular chromosome and two circular plasmids. From the genome, we identified genes related to the plant growth promotion and stress tolerance, such as nitrogen fixation, production of indole-3-acetic acid, acetoin, 2,3-butanediol, spermidine and trehalose. This genome sequence provides a basis for understanding the beneficial interactions between LTGPAF-6F and host plants, and will facilitate its applications as biotechnological agents in agriculture.

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Some bacterial endophytes have been shown to promote plant growth and enhance plant resistance to biotic and abiotic stresses (Coleman-Derr and Tringe, 2014; Mesa et al., 2015). Application of such bacteria will be of great importance in biocontrol of phytopathogens, promoting plant growth and also in bioremediation of soil. Drought has emerged as one of the major environmental stresses that limit crop growth and productivity worldwide, and is expected to cause damage to crop production on more than half of the earth's arable lands by 2050 (Vinocur and Altman, 2005). The roles of endophytic bacteria in the management of abiotic stresses

such as drought are only beginning to gain attention (Ngumbi and Kloepper, 2016). Several recent studies have shown that endophytic bacteria can increase drought resistance in crop plants such as wheat, maize, tomato, lettuce and beans (Gagné-Bourque et al., 2016).

During the course of looking for plant-beneficial microorganisms associated with drought-tolerant plants, strain LTGPAF-6F was isolated from the roots of the desert plant *Alhagi sparsifolia* Shap. (*Leguminosae*) collected from the Taklamakan Desert, Xinjiang Uyghur Autonomous Region in north-west China. Strain LTGPAF-6F was able to grow on nitrogen-free medium and the *nifH* gene was detected by PCR amplification, suggesting the nitrogen-fixing capability of this strain. Strain LTGPAF-6F also exhibited other plant growth promoting traits and stress resistance capabilities, including production of indole-3-acetic acid (IAA), acetoin, exopolysaccharides, protease and ammonia, and tolerance to osmotic stress (10% PEG), salt (7% NaCl) and high temperature (45 °C). Furthermore, strain LTGPAF-6F was found to improve the

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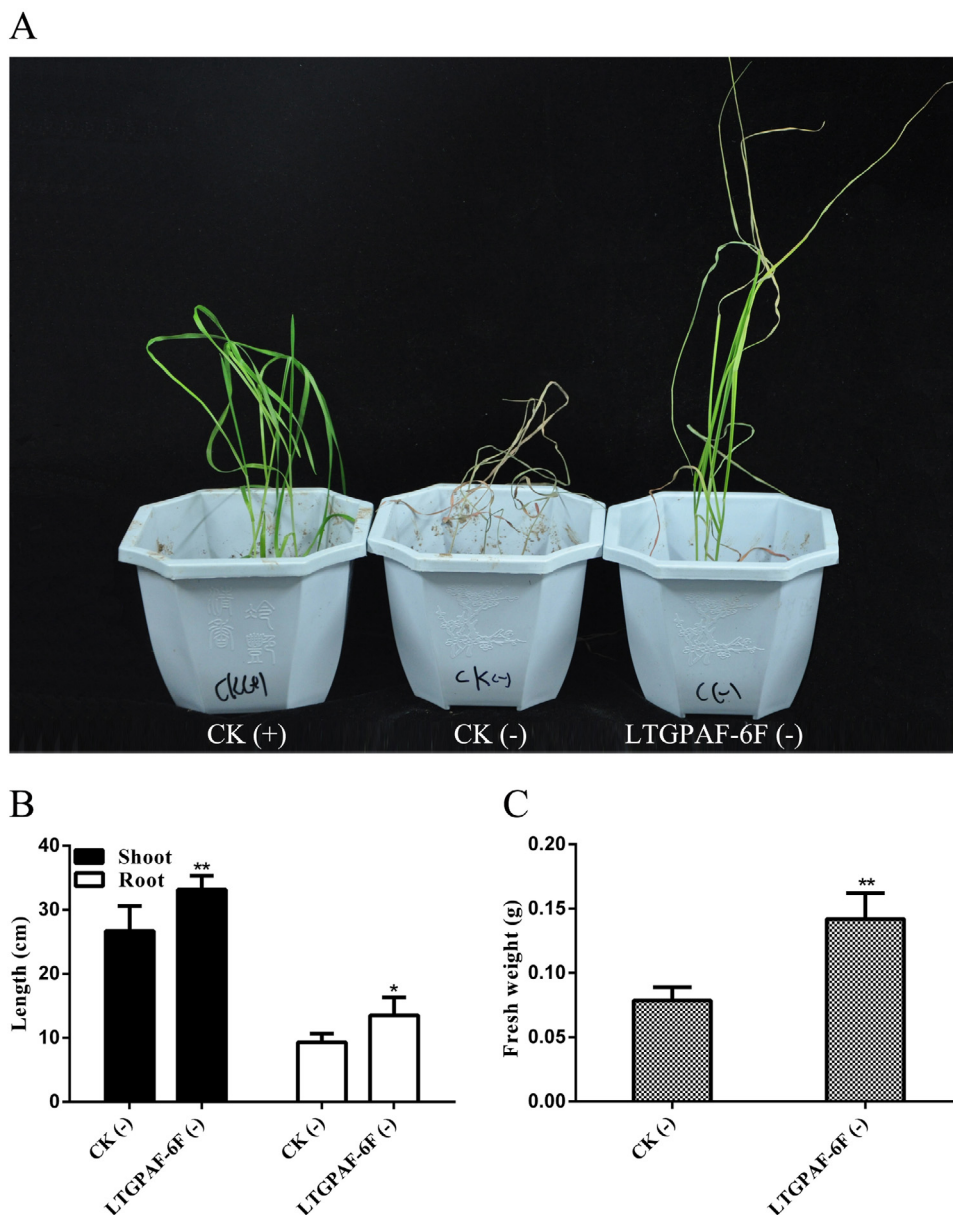


Fig. 1. Improvement of drought tolerance in wheat by strain LTGPAF-6F. The uniform-sized wheat seedlings were planted in sterilized field soil, three plants per plastic pot. After one week, the seedlings were inoculated with the bacterial suspension of strain LTGPAF-6F in sterilized tap water at the concentration of 10^8 cells g^{-1} of soil, while non-inoculated seedlings were watered with sterilized tap water. One week after bacteria inoculation, watering was terminated for 12 days. CK, non-inoculated controls; (+), kept well-watered throughout the experiment; (-), subjected to drought stress by withholding watering for 12 days. (A) Representative images of non-inoculated and inoculated plants grown without water for 12 days and the non-stressed controls. (B) Shoot and root length. (C) Plant fresh weight. Data represent the mean \pm SD from three biological replicates. Statistical analysis was performed using Student's *t*-test. *, $P \leq 0.05$; **, $P \leq 0.01$.

growth of wheat under drought stress (Fig. 1). After 12 d of drought treatments, the control plants became severely wilted and desiccated, whereas most of the inoculated plants remained full and green (Fig. 1A). In addition, the inoculation with LTGPAF-6F resulted in 45.3%, 24.2% and 80.7% increase of root length, shoot length and total fresh weight, respectively (Fig. 1B, C). A phylogenetic tree based on 16S rRNA gene sequences, reconstructed with the neighbor-joining method in the software package MEGA version 5.1, showed that strain LTGPAF-6F was a member of the genus *Klebsiella*, with *K. michiganensis* and *K. oxytoca* strains as the closest phylogenetic relatives (Fig. 2).

In order to gain insights into the genetic elements involved in endophytic colonization and plant growth promoting mechanism, complete genome sequencing of *Klebsiella* sp. LTGPAF-6F was performed. Genomic DNA of *Klebsiella* sp. LTGPAF-6F was extracted as

described previously (Zhu et al., 2014). The genome of *Klebsiella* sp. LTGPAF-6F was sequenced by Pacific Bioscience (PacBio) RS II sequencing technology. One SMRT cell yielded 395,306,920 bases in 45,151 reads with a mean read length of 12,592 bases and average genome coverage of $\sim 53\times$. *De novo* assembly of the genome was conducted using the Hierarchical Genome Assembly Process 3 (HGAP3) (Chin et al., 2013) within the SMRT Analysis v2.2.0 software. To avoid the homo-polymer errors produced during sequencing by PacBio, we re-sequenced this strain using Illumina HiSeq sequencer. Gene prediction and annotation was carried out using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; http://www.ncbi.nlm.nih.gov/genome/annotation_prok/) with *ab initio* gene prediction algorithm (GeneMarkS+) and similarity-based gene detection approach (ProSplign).

Table 1
The general features of *Klebsiella* sp. LTGPAF-6F genome.

Features	Chromosome	Plasmid unnamed1	Plasmid unnamed2
Genome size (bp)	6,174,401	526,446	89,552
G + C content (%)	56	49	51
rRNAs	25	0	0
tRNAs	85	0	0
Total predicted CDSs	5570	480	86
Genes with predicted functions	4594	300	70

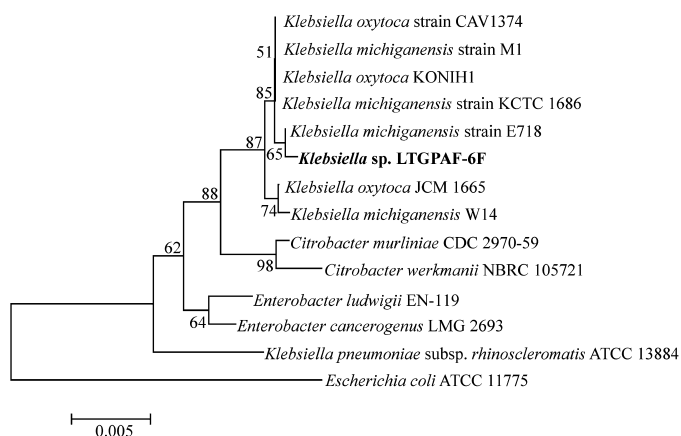


Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships of *Klebsiella* sp. LTGPAF-6F and closely related taxa. Dendrogram was generated by neighbor-joining and distance matrix was calculated by the Kimura two-parameter algorithm. The bar at the bottom indicates sequence divergence. Numbers at nodes indicate bootstrap percentages (based on 1000 resampled datasets).

The complete genome of *Klebsiella* sp. LTGPAF-6F consisted of a circular chromosome of 6,174,401 bp and two circular plasmids unnamed1 (526,446 bp) and unnamed2 (89,552 bp). The G + C content of the chromosome and the two plasmids was 56%, 49% and 51%, respectively. The chromosome contained 5570 predicted coding sequences (CDSs), 25 rRNA genes and 85 tRNA genes, while the two plasmids contained 480 and 86 predicted CDSs, respectively (Table 1). Among these CDSs, 4959 (80.8%) genes could be classified into 21 functional categories based on clusters of orthologous groups (COG) designations (Table S1).

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Genome analysis revealed that *Klebsiella* sp. LTGPAF-6F contains genes related to the plant growth promotion and stress tolerance, such as nitrogen fixation, production of IAA, acetoin, 2,3-butanediol, spermidine and trehalose. Nitrogenase genes and nitrogen fixation-associated genes were arranged in the 20-kb region of *Klebsiella* sp. LTGPAF-6F genome (BJF97_19155–BJF97_19245). In addition, two dinitrogenase iron-molybdenum cofactors (BJF97_02425, BJF97_31185) were found in the chromosome and the plasmid unnamed1, respectively. A potential IAA biosynthesis pathway, the indole-3-pyruvate (IPyA) pathway, was also detected in the *Klebsiella* sp. LTGPAF-6F genome. In this pathway, tryptophan is transaminated to IPyA via the aromatic amino acid aminotransferases (BJF97_01700 and BJF97_10435),

subsequently decarboxylated to indole acetaldehyde via IPyA decarboxylase (BJF97_20915), and then oxidized to IAA by aldehyde dehydrogenase (BJF97_22535). Moreover, the genome of *Klebsiella* sp. LTGPAF-6F has genes involved in the biosynthesis of the volatiles acetoin and 2,3-butanediol, which may function as plant growth promoter or signaling molecules mediating plant–microbe interactions (Ryu et al., 2003). The three genes encoding for acetolactate synthase (pyruvate to acetolactate; BJF97_16530), acetolactate decarboxylase (acetolactate to acetoin; BJF97_16525) and acetoin reductase (acetoin to 2,3-butanediol; BJF97_16535) are organized in an operon. Trehalose functions as an osmoprotectant and enables a variety of organisms to endure environmental stresses such as drought, high salinity, heat and low temperature (Duan et al., 2013). Plant growth-promoting bacteria overexpressing trehalose were reported to improve drought tolerance and biomass in crop plants (Suárez et al., 2008; Rodríguez-Salazar et al., 2009). Three trehalose biosynthetic pathways, including OtsA-OtsB (BJF97_18185 and BJF97_18190), TreS (BJF97_16360) and TreY-TreZ (BJF97_14650 and BJF97_14655) pathways, were identified in the genome of *Klebsiella* sp. LTGPAF-6F. Spermidine is a well-known plant growth regulator and was also newly identified as a protector against various abiotic stresses such as salt, drought, cold and oxidative stress (Alcázar et al., 2011). A spermidine-producing rhizobacterial strain *Bacillus megaterium* BOFC15 has been shown to improve plant growth and drought resistance (Zhou et al., 2016). In the genome of *Klebsiella* sp. LTGPAF-6F, the genes responsible for spermidine biosynthesis were found: *speA* (Arginine decarboxylase; BJF97_24825), *speB* (agmatinase; BJF97_22405 and BJF97_24820), *speD* (S-adenosylmethionine decarboxylase; BJF97_05380) and *speE* (spermidine synthase; BJF97_05385). In summary, *Klebsiella* sp. LTGPAF-6F contained nitrogen fixation genes and complete biosynthesis pathways of IAA, acetoin, 2,3-butanediol, spermidine and trehalose, which may explain the improvement of growth and drought tolerance in wheat by *Klebsiella* sp. LTGPAF-6F (Fig. 1).

Together, the whole genome data of *Klebsiella* sp. LTGPAF-6F will allow us to better understand the molecular basis for its beneficial effects in host plants and facilitate its potential applications as microbial inoculants in agriculture.

1. Nucleotide sequence accession number

The complete genome sequence of *Klebsiella* sp. LTGPAF-6F has been deposited in Genbank under the accession numbers of CP017450, CP017451 and CP017452 for chromosome, plasmid unnamed1 and plasmid unnamed2, respectively. The strain has been deposited at the China Center for Type Culture Collection under the accession number CCTCC M 2016053.

Acknowledgements

This work was supported by the National High Technology Research and Development Program of China (863 program, grant 2013AA102802), the National Natural Science Foundation of China (grant no. 31100001), National Natural Science Foundation of China (grant no. 31601047), National Natural Science Foundation of China (grant no. 31570133), the Natural Science Foundation of Shaanxi

Province, China (grant no. 2016JM3014), the Fundamental Research Funds for the Central Universities (grant no. 2452016010) and the Opening Project of the State Key Laboratory of Crop Stress Biology for Arid Areas (grant no. CSBAA2015005).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jbiotec.2017.02.008>.

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