

Isolation of a *Pseudomonas putida* strain that degrades *p*-hydroxybenzoic acid from the soil of a *Panax ginseng* field

Liang Shen

China Academy of Chinese Medical Sciences

Guangwei Zhu

China Academy of Chinese Medical Science

Shuai Guo

Henan University of Science and Technology

Xiwen Li

Chinese Academy of Chinese Medical Sciences, Institute of Chinese Materia Medica

Shuiming Xiao

Chinese Academy of Chinese Medical Sciences, Institute of Chinese Materia Medica

Jiang Xu (✉ jxu@icmm.ac.cn)

Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

Shilin Chen

Chinese Academy of Chinese Medical Sciences, Institute of Chinese Materia Medica

Research

Keywords: *Panax ginseng*, *Pseudomonas putida*, 16S rRNA phylogeny, *p*-hydroxybenzoic acid, allelochemical degradation

Posted Date: March 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-19021/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: *Panax ginseng* is a valuable herbal medicine which has been applied in eastern Asia to maintain their physical vitality for millennia. However, continuous cropping leads to the accumulation of allelochemicals, which subsequently reduces the quality and yield of *P. ginseng*, and arable soils for *P. ginseng* cultivation are getting scarce.

Methods: In this study, a strain highly efficient degrading *p*-hydroxybenzoic acid was isolated from the rhizosphere soil of a ginseng cultivation field. Subsequently, its draft genome was sequenced, and the key genes involving the *p*-hydroxybenzoic acid-degrading pathway were identified by bioinformatics software.

Results: Based on its biophysical/biochemical characteristics, analysis of the 16S rRNA sequence and *gyrB* gene authentication, strain LD6 was identified as *Pseudomonas putida* LD6. The initial *p*-hydroxybenzoic acid concentration in minimal salt medium was $100 \text{ mg}\cdot\text{L}^{-1}$, and after 8 d of bacterial culture in the laboratory, the degraded reached 97.35%. The *p*-hydroxybenzoic acid content was decreased by 35.21%, and the ginseng death rate was decreased by 38.46% in the rhizosphere soil of *P. ginseng* inoculated with strain LD6. The draft genome sequence consisted of 5,765,634 bp, and the genome comprised 5186 protein-coding genes, and the *pobA*, *catB*, *pcaG*, *UbiA*, and *UbiX* genes were annotated, and may play important roles in *p*-hydroxybenzoic acid degradation.

Conclusion: Isolation and manipulation of functional bacteria is an effective strategy to alleviate ginseng continuous cropping problems and enhance the quality and yield of *P. ginseng*.

Background

Panax ginseng C. A. Meyer is a valuable herbal medicine that has been used around the world to maintain human physical vitality for millennia [1]. Established beneficial activities include anti-tumor, anti-aging, anti-amnestic, and cardioprotective [2]. However, ginseng replanting in an attempt to perform continuous cropping usually fails, which reduces the quality and yield of *P. ginseng* raw materials, and soil conditions must be improved by more than 30 years of crop rotation before ginseng can be replanted [3]. Accumulation of allelochemicals is considered one of the main causes of *P. ginseng* mortality [4-8]. Allelochemicals, such as *p*-hydroxybenzoic acid can be extracted from ginseng rhizosphere soil and inhibit the growth of ginseng seedlings, and it was found in abundance in our test field which was secreted by the roots of ginseng. Eliminating the allelochemicals is an effective method to alleviate the continuous cropping obstacles and increase crop productivity of *P. ginseng*.

Microbial degradation is an important and effective strategy for the bioremediation of environmentally toxic substances [9]. As one example, Wu et al. [10] isolated a *Bacillus* sp. strain that can efficiently biodegrade di-n-butyl phthalate in a shallow aquifer environment. Microbial degradation of allelochemicals also occurs in the exudates produced during the growth of medicinal plants. Zhao et al. [11] isolated five bacterial strains possessing autotoxin-degrading activity from non-rhizospheric soil in which 6-year-old ginseng plants were grown, and the strains were used to degrade autotoxins in the

planting base of *P. ginseng*. Therefore, there is an urgent need to develop remediation with toxic substance-degrading bacteria to eliminate continuous cropping problems in *P. ginseng* plantation. However, little is known regarding how ginseng-associated bacteria degrade toxic substances, and thus, their degradation mechanisms require elucidation.

Microbial degradation would be helpful and remediation strategies could be developed to alleviate poor growth of *P. ginseng* caused by autotoxins. During the course of an investigation of a bacterial community in the rhizosphere soil obtained from a *P. ginseng* field, a *p*-hydroxybenzoic acid-degrading strain was isolated and designated strain LD6. Phenotypic, physiological, and phylogenetic analyses based on 16S rRNA gene sequences were performed, and LD6 was identified as a *P. putida*. In the current study, the strain degradation rate of the *p*-hydroxybenzoic acid from the bacterial culture and rhizosphere soils of *P. ginseng* was analyzed, and the draft genome sequence and degradation mechanism of *P. putida* LD6 were analyzed.

Materials And Methods

Materials and reagents

Rhizosphere soil adhering to the roots of *P. ginseng* was collected from a ginseng-cultivated field in Jilin, China (E: 126.85°, N: 42.39°) (Fig. S1 in Additional file 3). The ingredients used to create the mineral salt medium (MSM) have been previously described by Wang et al. [12]. The genomic DNA of bacterial strain was extracted and purification by Genomic DNA Purification Kit (Tiangen, Beijing, China). The *p*-hydroxybenzoic acid (99% purity) was procured from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultra-high performance liquid chromatography (UPLC) grade formic acid and methanol were obtained from CNW Technologies GmbH (Dusseldorf, Germany). Other chemical reagents were analytical grade and were purchased from local suppliers.

Strain isolation and culture conditions

The rhizosphere soil was dispelled by gently shaking the roots of *P. ginseng*, and then the soil was collected and homogenized through a 2-mm sieve and stored at 4°C for use. Next, one gram of fresh, intensively cultivated soil was homogenized in 100 ml MSM inorganic culture solution that contained allelochemicals (100 mg·L⁻¹) as the sole carbon source. In order to dilute and reduce possible carbon sources from soils, incubation and transfer were performed three times at 30°C for 5 days, each time on a rotary shaker operating at 150 r·min⁻¹. The final enriched bacteria were isolated following serial dilution with distilled water. Petri plates with separated single colonies were stored at 4°C for the following experiments [11]. One of the purified strains, designated LD6, displayed superior efficiency in the degradation of *p*-hydroxybenzoic acid, and was selected for further characterization. The isolate was deposited at -80°C in the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences.

Strain LD6 growth and *p*-hydroxybenzoic acid degradation

For the determination of the degradation efficiency of *p*-hydroxybenzoic acid, a single colony of strain LD6 was inoculated in 3 ml MSM (in a 15-ml plastic tube with cover), and incubated at 30°C with shaking (120 r·min⁻¹) until the OD600 of the culture solution reached 0.5. The culture solution was transferred to 100 ml MSM, with an initial concentration of *p*-hydroxybenzoic acid at 100 mg·L⁻¹, and bacterial strain at 1.4×10⁷ CFU·ml⁻¹ in 250-ml flasks, and incubated at 30°C with shaking at 120 r·min⁻¹. The OD600 of the culture solution, the autotoxin content, and the degradation in soil were analyzed after 0, 0.5, 1, 1.5, 2, 4, 6, and 8 d of incubation [11]. Except for isolation and identification experiments, other tests were repeated at least three times and with three parallel treatments.

Phenotypic and biochemical characteristics of LD6

The Gram reaction of strain LD6 was determined as previously described [13]. The bacterial morphology was determined by incubation on LB medium for 1 d, and then observation and recording using a Hitachi S-3400N scanning electron microscope. Growth was assessed in LB agar (LB, Difco) at 25°C. Catalase and oxidase utilization was determined as previously described [14]. The effect of pH on growth was analyzed in R2A broth using the three buffer tests (acetate buffer 5.0-5.5, phosphate buffer 6.0-8.0, and Tris buffer 8.5-10.0). To determine salt tolerance, the bacteria were grown on R2A agar with 0.5%-10% (w·v⁻¹) NaCl for 7 days. Physiological characteristics were determined according the method of Park et al. [15].

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of strain LD6 was amplified from bacterial genomic using the 27F and 1492R universal bacterial primers. Purified PCR products were analyzed using a 3730 XL sequencer (Applied Biosystems). The 16S rRNA gene sequences of other species were downloaded from GenBank and compared with the sequence of strain LD6 using the Clustal X program [16]. Phylogenetic trees based on the neighbor-joining algorithm were constructed using MEGA v4.0 software [17]. The evolutionary distances for strain LD6 were calculated using the maximum composite likelihood method [18].

Influences of strain LD6 on the replanting soil of *P. ginseng* after inoculation

To assess the degradation efficiency of LD6, we implemented a field experiment from June to August in the replant soil plantation base of *P. ginseng*. Three biological replicate blocks were used, and each block contained one field plot (1.2 m×5 m) with an initial *p*-hydroxybenzoic acid concentration of 1 mg·kg⁻¹. Two hundred 2-year old uniform seedlings were planted in each plot on May 1. Successively, 10 mL (10⁷ CFU mL⁻¹) of *P. putida* culture was added to the rhizosphere of uniform *P. ginseng* plants on June 1. The plot without the addition of cultures containing *P. putida* acted as a control (CK). After 60 d of inoculation, soil samples from each plot were collected and subsequently used for the analysis of the *p*-hydroxybenzoic acid degradation rate and the death rate of *P. ginseng* [4].

UPLC analytical methods

A reaction mixture containing *p*-hydroxybenzoic acid and LD6 was incubated in MSM at 30°C for 8 d. After incubating the reaction mixture until the designated date, 20 ml of reaction products was transferred to 100-ml flasks for detection. Liquid chromatographic analysis was performed with an Agilent 1290 Infinity II UPLC system (Agilent, USA) using a Thermo-C18 (150 mm × 2.1 mm, 2.6 μm) column. For UPLC analysis, the column temperature was maintained at 30°C. The auto sampler was conditioned at 25°C, the injection volume was 2 μl, and the flow rate was 0.3 ml·min⁻¹. The raw data were detected by a UPLC-diode array detector (DAD) at 254 nm [19]. The binary gradient elution system consisted of solvent A (0.25% formic acid water) and solvent B (methanol). Optimum separation was achieved using the following gradient program: 0-2 min, 4% (B); 2-5 min, 4-50% (B); 5-7 min, 50% (B); 7-8 min, 50-95% (B); and 8-12 min, 95% (B).

DNA preparation and genomic sequencing

Total genomic DNA was extracted using a T-Guide Bacteria Genomic DNA Kit (OSR-M502, TIANGEN, China), and purified by RNase-free DNase I (Takara, Japan). The DNA was sheared using a S220 focused ultrasonicator (Covaris, USA). Purified genomic DNA was analyzed using a hybrid sequencing strategy that combined the PacBio RS II and Illumina HiSeq sequencing platforms as previously described [20].

Bioinformatics analysis

After sequencing, the filtered low-quality reads were assembled with SOAPdenovo software to generate scaffolds. The cleaned reads were assembled using Velvet (version 1.2.07) and PrInSeS-G (version 1.0.0. beta) to correct sequencing errors [21]. Open reading frames (ORFs) were predicted and translated using MetaGene Annotator [22]. The nucleotide sequence was compared to the NCBI database (<http://www.ncbi.nlm.nih.gov/>). The tRNA and rRNA analyses were performed using tRNAscan-SE v.1.4 and RNAmmer, respectively [23]. Protein-coding gene prediction and gene annotation were conducted using RPSBLAST and Prokka, respectively [24]. Gene prediction and assembly were performed using Glimmer, and gene functions were annotated by BLASTP against the Clusters of Orthologous Groups (COG) database. A genomic overview of annotation information was created using Circos [25]. The genome alignment was performed by Progressive Mauve [26].

Nucleotide sequence accession number

The 16S rRNA gene sequence for strain LD6 determined in the present study was deposited in GenBank (GenBank accession no. MF588953). The *gyrB* sequence was submitted to GenBank (GenBank accession no. MF588954). Full genome sequences of *P. putida* KT2440 (NC_002947.4) and *P. putida* F1 (CP000712.1) were downloaded to GenBank.

Results

Phenotypic characteristics

In this study, morphological analysis of LD6 using scanning electron microscopy (SEM) indicated that the bacteria were consistent with the genus of *Pseudomonas*. Phenotypically, strain LD6 was shown to be short and rod-like in shape, an obligate aerobe that is oxidase- and catalase-positive, and Gram-stain-negative, and has a single polar-inserted flagellum (Fig. 1). Other phenotypic features of strain LD6 are summarized (Table S1 in Additional file 1).

Physiological characteristics

On nutrient agar, strain LD6 isolates grew at temperatures ranging from 4-41°C, with an optimum of 25-30°C. LD6 tolerated between 1% and 8% (w·v⁻¹) NaCl, and could grow within a pH range of 5-6. Cells utilized α -D-glucose, D-serine, D-glucuronic acid, and D-mannose as energy and carbon sources, but failed to utilize D-trehalose, D-maltose, D-fucose, D-galactose, turanose, or sucrose (Table S1 in Additional file 1).

Phylogenetic analysis

A complete 16S rRNA nucleotide sequence of 1198 bp was obtained. The phylogenetic position using the neighbor-joining method revealed that strain LD6 was allocated to the genus *Pseudomonas* and clustered with *P. putida* E16 (KC820813.1) with high bootstrap values of 100% (Fig. 2). The *gyrB* gene sequence (GenBank accession no. MF588954, 1149 bp) further supported the identity of LD6 as *P. putida*.

Strain LD6 growth and *p*-Hydroxybenzoic acid degradation

A degradation experiment was conducted in mineral salt medium (MSM) using allelochemicals, which it indicated that LD6 could use *p*-hydroxybenzoic acid as the sole carbon source in 50 mL of MSM (Fig. 3). The concentration of *p*-hydroxybenzoic acid in the MSM markedly decreased after inoculum in 1 d (71.33%), and 97.35% of the *p*-hydroxybenzoic acid was effectively degraded by strain LD6 after incubation for 8 d (Fig. 3b). The amount of *p*-hydroxybenzoic acid sharply decreased, accompanied by an increase in the density of the bacteria, indicating that the LD6 strain was highly effective in degrading *p*-hydroxybenzoic acid. The pH and salt data of experiments has not been decreased obviously. When a dual culture assay was used to screen microbial antagonists against *p*-hydroxybenzoic from the fresh rhizosphere soils of 2-year-old ginseng seedlings, the strain LD6 decreased the *p*-hydroxybenzoic content by 35.21%, and the ginseng death rate was decreased by 38.46% after 60 d (Fig. 3c and 3d). These data indicate that the continuous cropping obstacle of *P. ginseng* could be solved through the use of microbiologic control.

Genomic sequence of LD6

A draft genome of *P. putida* was sequenced based on its specific *p*-hydroxybenzoic acid-degrading characteristics. The genomic sequence of *P. putida* was deposited in the National Center for Biotechnology Information (NCBI) database (GenBank accession no. NPKG00000000). The linear, double-stranded genome of LD6 consists of a 5,765,634 bp draft genomic sequence with 62.54% G+C content

(Fig. S2). The coding sequences (CDSs) are 5,066,091 bp in length. The total number of predicted genes is 5271, which covers 5186 (98.39%) protein-coding genes and 85 RNA encoding genes, as well as 69 tRNA encoding genes and 16 rRNA (5S, 23S, and 16S) encoding genes (Table 1). The 4310 (83.11%) predicted genes were annotated and distributed in the 21 clusters of orthologous group (COGs) categories. The function of these predicted proteins included information storage and processing, cellular processes and signaling, and metabolism. The numbers of genes in the important categories were approximately 498 for amino acid transport and metabolism (11.55%), 387 for transcription (8.98%), 288 for inorganic ion transport and metabolism (6.68%), and 282 for energy production and conversion (6.54%) (Table S2 in Additional file 1, Fig. S3 in Additional file 3).

We conducted comparative genomics analysis among three strains: *P. putida* LD6, *P. putida* KT2440, and *P. putida* F1 (Fig. S4 in Additional file 3). Block outlines surround a region of the genome that is presumably homologous and internally free from genomic rearrangement. Regions outside blocks lack detectable homology among the input genomes. These three genomes were aligned with Progressive Mauve using default parameters. The genomic synteny between strain *P. putida* LD6 and *P. putida* KT2440 was higher. However, the genomic synteny between *P. putida* LD6 and *P. putida* F1 was much lower. The alignment analysis demonstrated that *P. putida* LD6 exhibited high affinity with *P. putida* KT2440 by Mauve software.

Table 1. Nucleotide content and gene count levels of the *P. putida* genome

Attribute	Value	% of total
Genome size (without gap) (bp)	5,765,634	99.88
DNA coding (bp)	5,066,091	87.87
DNA G+C (bp)	3,605,827.50	62.54
DNA scaffolds	1	0
Chromosome	1	0
Total genes	5271	100
Protein coding genes	5186	98.39
rRNA (operons)	16	0.30
tRNA	69	1.31
Genes assigned to COGs	4310	83.11
CRISPR repeats	3	0

Discussion

Ginseng is an herbaceous, perennial medicinal plant that requires continuous cultivation in fixed plots for 5-6 years. During this long growing process, allelochemicals accumulation, soil-borne diseases, and deterioration of soil physicochemical properties pose threats to the health of *P. ginseng*. Because successful replanting of *P. ginseng* requires 30 years of soil restoration, arable soil for *P. ginseng* cultivation is becoming scarce [5]. The inability to successfully replant *P. ginseng* and enable the crop to thrive is termed the continuous cropping obstacle. Allelochemicals are considered the primary cause of this problem, and the use of microorganisms to control these allelochemicals is an effective method [27]. In this study, the bacterial strain LD6 was isolated from the rhizosphere soil of ginseng, and it degraded *p*-hydroxybenzoic acid at a high rate, which indicates that microbes was a method to solve the continuous cropping obstacle of *P. ginseng*. Based on the morphological, biochemical, 16S rRNA and *gyrB* gene, as well as previously published studies, strain LD6 was confirming as *P. putida* [28-29]. However, the fact that *p*-hydroxybenzoic acid content in soil samples from the field experiment decreased about 35.21% in 60 days. It's indicate that the positive effect of added *P. putida* on ginseng wasn't higher, the influence factors should research in the future.

P. putida is a widely distributed *Pseudomonas* bacterium, and possesses the ability to degrade the nicotine from tobacco as well as the *p*-coumaric acid from rice [30-31]. Bertani et al. [32] found a *p*-hydroxybenzoate hydroxylase gene (*pobA*) of *P. putida* WCS358 involved in the catabolism of *p*-hydroxybenzoic acid. Our results indicated that *P. putida* has the ability to degrade *p*-hydroxybenzoic acid from *P. ginseng* planting soil. If additional genes or enzymes involved in *p*-hydroxybenzoic acid biodegradation were characterized, this strain could be exploitable as inoculum used degradation of *p*-hydroxybenzoic acid, and this strain could be developed and applied to autotoxin degradation agents. However, a single microbial species is unlikely to completely degrade all of the *p*-hydroxybenzoic acid in *P. ginseng* fields. For any bacteria with promising degradation capabilities, the stability in farmland soil and ability to quickly grow should also be studied.

Bacterial genome sequencing analysis could offer the most important information for the identification of species' unique genes and pathways to provide powerful genetic evidence to support the exploitation of functional bacteria for desirable abilities [33]. In this study, the *pobA* gene that catalyzed the conversion of *p*-hydroxybenzoic acid into 3,4-dihydroxybenzoic acid, and the *catB* and *pcaG* genes that catalyzed 3,4-dihydroxybenzoic acid into 3-carboxy-cis,cis-muconate, were annotated in the KEGG metabolic pathway of the strain LD6 genome (Fig. S5 in Additional file 3). Additionally, the *p*-hydroxybenzoic acid degradation by *pobA* [34], *catB* [35] and *pcaG* [36] was similarity with other *P. putida* research results. In the regulation pathway, the *UbiA* gene regulated the conversion of *p*-hydroxybenzoic acid into 4-hydroxy-3-polyprenylbenzoate, and *UbiX* regulation of 4-hydroxy-3-polyprenylbenzoate into 2-polyprenylphenol was forecast (Fig. S5 in Additional file 3), and the results were the same as those of Suzuki et al. [37]. Mauve software indicated that the gene sequence of *P. putida* LD6 had high affinity with *P. putida* KT2440, and the *pobA*, *catB*, *pcaG*, *UbiA*, and *UbiX* genes might played significant roles in *p*-hydroxybenzoic acid degradation.

Conclusions

In this study, a highly effective *p*-hydroxybenzoic acid-degrading bacterium was isolated from rhizosphere soil of *P. ginseng*. Based on morphological, physiological, 16S rDNA and *gyrB* gene authentication, the bacterial strain LD6 was identified as *P. putida*. The *P. putida* metabolized *p*-hydroxybenzoic acid completely at higher concentrations, suggesting that *P. putida* represents a novel tool for removing allelochemicals from rhizosphere soil of *P. ginseng*. And the draft genome sequence of *P. putida* LD6 was determined and analysis, the results will help to reveal useful basic information regarding the *p*-hydroxybenzoic acid degradation mechanism of *P. putida*. Isolation and manipulation of functional bacteria is an effective strategy to alleviate ginseng continuous cropping problems and enhance the quality and yield of *P. ginseng*.

Declarations

Acknowledgements

The authors wish to thank Li Yong for help with sampling for the manuscript.

Authors' contributions

XJ and CSL conceived and designed the study. SL, ZGW and GS collected and performed the data analysis. SL, LXW and XSM wrote the manuscript. All authors are responsible for reviewing data. All authors read and approved the final manuscript.

Funding

Project supported by the China Postdoctoral Science Foundation (Grant No. 2017M611128),

Project supported by the National Natural Science Foundation of China (No. 81403053),

Project supported by the National Key Research and Development Program of China (No. 2017YFC1702100).

Availability of data and materials

The readers can use data and materials in this manuscript by quotation of author names and Journal of Chinese Medicine.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All of authors consent to publication of this study in Journal of Chinese Medicine.

Competing interests

No conflicts of interest have been declared.

Author details

¹ Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China. ² Department of Science Research, Beijing Museum of Natural History, Beijing 100050, China. ³ College of Agricultural, Henan University of Science and Technology, Luoyang 471023, China.

References

1. Ernst E. *Panax ginseng*: an overview of the clinical evidence. *J Ginseng Res.* 2010;34(4):259-63.
2. Leung KW, Wong AST. Pharmacology of ginsenosides: a literature review. *Chinese medicine.* 2010;5(1):20.
3. Wu LJ, Zhao YH, Guan YM, Pang SF. A review on studies of the reason and control methods of succession cropping obstacle of *Panax ginseng* CA Mey. *Special Wild Economic Animal Plant Research.* 2008;2:68-72.
4. Li Y, Huang XF, Ding WL. Autotoxicity of *Panax ginseng* rhizosphere and non-rhizosphere soil extracts on early seedlings growth and identification of chemicals. *Allelopathy J.* 2011;28(2):145-54.
5. Li Y, Ying Y, Zhao DY, Ding WL. Influence of allelochemicals on microbial community in ginseng cultivating soil. *Chin Herb Med.* 2014;6(4):313-8.
6. Chen CB, Wang YY, Liu JY, Yan S, Xu SQ, Zhang LX. The identify for the allelochemical of the soil of ginseng rhizosphere. *Special Wild Economic Animal and Plant Research.* 2006;2:12-4.
7. Lei FJ, Zhang AH, Xu YH, Zhang LX. Allelopathic effects of ginsenosides on in vitro growth and antioxidant enzymes activity of ginseng callus. *Allelopathy J.* 2010;26(1):13-22.
8. Wu B, Long QL, Gao Y, Wang Z, Shao TW, Liu YN, Li Y, Ding WL. Comprehensive characterization of a time-course transcriptional response induced by autotoxins in *Panax ginseng* using RNA-Seq. *BMC genomics.* 2015;16(1):1010.
9. Wu Y, Li T, Yang L. Mechanisms of removing pollutants from aqueous solutions by microorganisms and their aggregates: a review. *Bioresource Technol.* 2012;107:10-8.
10. Wu Q, Liu H, Ye LS, Li P, Wang YH. Biodegradation of Di-n-butyl phthalate esters by *Bacillus* SASHJ under simulated shallow aquifer condition. *Int Biodeter Biodegr.* 2013;76:102-7.
11. Zhao DY, Li Y, Ding WL. Isolation and characteristics of *Panax ginseng* autotoxin-degrading bacterial strains. *China Journal of Chinese Materia Medica.* 2013;38(11):1703-6.
12. Wang J, Zhang MY, Chen T, Zhu Y, Teng Y, Luo YM, Peter C. Isolation and identification of a Di-(2-Ethylhexyl) phthalate-degrading bacterium and its role in the bioremediation of a contaminated soil. *Pedosphere.* 2015;25(2):202-11.

13. Gregersen, T. Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biot.* 1978;5(2):123-7.
14. Cappuccino JG, Sherman N. *Microbiology: a Laboratory Manual* 9th edn Benjamin Cummings, San Francisco, 2010.
15. Park SJ, Lee JJ, Lee SY, Lee DS, Kim MK, Ten LN, Jung HY. *Larkinella harenaesp* nov, Isolated from Korean beach soil. *Curr Microbiol.* 2017;74(7):798-802.
16. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 1997;25(24):4876-82.
17. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol.* 2007;24(8):1596-9.
18. Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol.* 1981;17(6):368-76.
19. Chen YG, Luo HY, Guo XD, Xian YP, Luo DH, Wu, YL. Determination of total maleic acid, fumaric acid, *p*-hydroxybenzoic acid and benzoic acid in food by ultra-performance liquid chromatography-tandem mass spectrometry. *Anal Methods.* 2014;6(13):4803-9.
20. Koren S, Schatz MC, Walenz BP, Martin J, Howard JT, Ganapathy G, Wang Z, Rasko DA, McCombie WR, Jarvis ED, Phillippy AM. Hybrid error correction and de novo assembly of single-molecule sequencing reads. *Nat Biotechnol.* 2012;30(7):693-700.
21. Massouras A, Hens K, Gubelmann C, Uplekar S, Decouttere F, Rougemont J, Cole ST, Deplancke B. Primer-initiated sequence synthesis to detect and assemble structural variants. *Nat Methods.* 2010;7(7):485-6.
22. Noguchi H, Taniguchi T, Itoh T. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. *DNA Res.* 2008;15(6):387-96.
23. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 1997;25(5):955-64.
24. Seemann T. Prokka: rapid prokaryotic genome Bioinformatics. 2014;30(14):2068-9.
25. Krzywinski M, Schein J, Birol I, Connors J, Gascoyne R, Horsman D, Jones SJ, Marra MA. Circos: an information aesthetic for comparative genomics. *Genome Res.* 2009;19(9):1639-45.
26. Darling AE, Mau B, Perna NT. Progressive Mauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One.* 2010;5(6):
27. Dong LL, Xu J, Li Y, Fang HL, Niu WH, Li XW, Zhang YJ, Ding WL, Chen SL. Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*. *Soil Biol. Biochem.* 2018;125:64-74.
28. Yamamoto S, Harayama S. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* Appl

Environ Microb. 1995;61(3):1104-9.

29. Ramos-González MI, Campos MJ, Ramos JL. Analysis of *Pseudomonas putida*KT2440 gene expression in the maize rhizosphere: in vitro expression technology capture and identification of root-activated promoters. J Bacteriol. 2015;187(12):4033-41.
30. Yu H, Tang HZ, Wang LJ, Yao YX, Wu G, Xu P. Complete genome sequence of the nicotine-degrading *Pseudomonas putida* strain S16. J Bacteriol. 2011;193(19):5541-2.
31. Zhang ZY, Pan LP, Li HH. Isolation, identification and characterization of soil microbes which degrade phenolic allelochemicals. Appl. Microbiol. 2010;108(5):1839-49.
32. Bertani I, Milan K, Vittorio V. Regulation of the *p*-hydroxybenzoic acid hydroxylase gene (*pobA*) in plant-growth-promoting *Pseudomonas putida* Microbiology. 2001;147(6):1611-20.
33. Pieper DH, dos Santos VAPM, Golyshin PN. Genomic and mechanistic insights into the biodegradation of organic pollutants. Curr Opin Biotech. 2004;15(3):215-24.
34. Morales G, Linares JF, Beloso A, Albar JP, Martínez JL, Rojo F. The *Pseudomonas putida* Crc global regulator controls the expression of genes from several chromosomal catabolic pathways for aromatic compounds. J Bacteriol. 2004;186(5):1337-44.
35. Jiménez JI, Miñambres B, García JL, Diaz E. Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* Environ microbiol. 2002;4(12):824-41.
36. Cronin CN, Kim J, Fuller JH, Zhang X, Meintire WS. Organization and sequences of *p*-hydroxybenzaldehyde dehydrogenase and other plasmid-encoded genes for early enzymes of the *p*-cresol degradative pathway in *Pseudomonas putida* NCIMB 9866 and 9869. DNA Sequence. 1999;10(1):7-17.
37. Suzuki K, Ueda M, Yuasa M, Nakagawa T, Kawamukai M, Matsuda H. Evidence that *Escherichia coli* *ubiA* product is a functional homolog of yeast COQ2, and the regulation of *ubiA* gene expression. Biosci biotech bioch. 1994;58(10):1814-9.

Figures

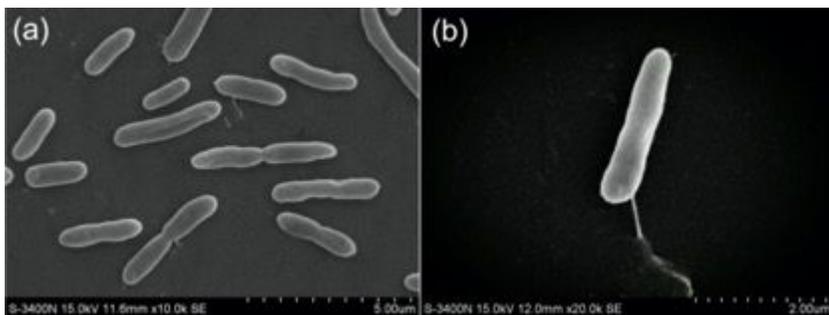


Figure 1

Morphological features of strain LD6. (a) group; (b) individual.

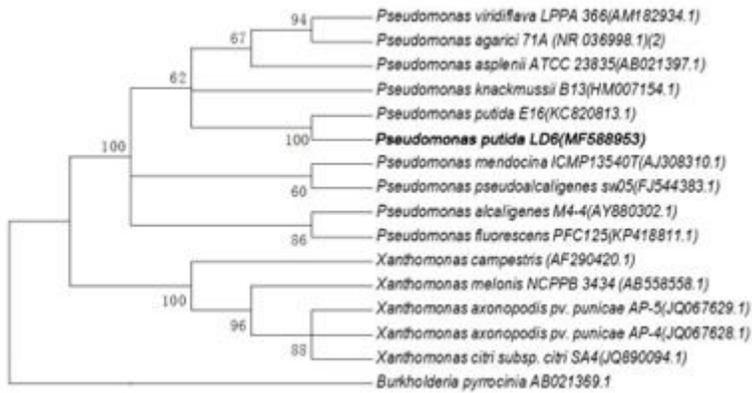


Figure 2

Neighbor-joining phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationship between strain LD6 and related strains. *Burkholderia pyrrocinia* (AB021369.1) was used as an outgroup. Asterisks indicate the clades that were conserved when the maximum likelihood method was used to construct the phylogenetic trees. Bootstrap values are expressed as percentages of 1000 replications; only bootstrap values greater than 50% are shown. Bar, 0.02 substitutions per nucleotide position.

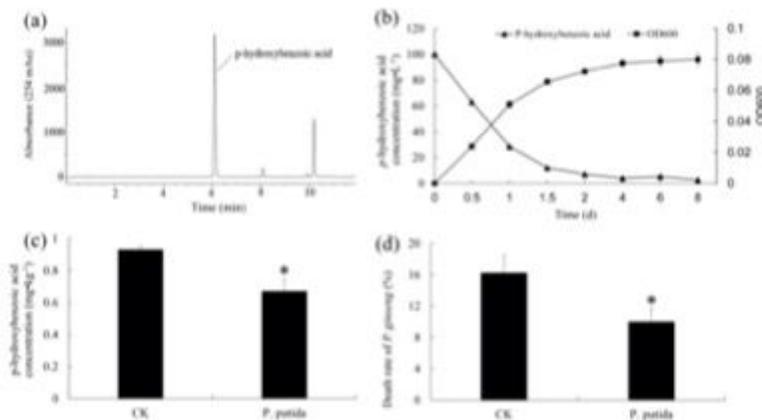


Figure 3

Degradation assays of p-hydroxybenzoic acid-degrading bacteria. (a) p-Hydroxybenzoic acid degradation kinetics and *P. putida* LD6 population in minimal salt medium recorded as optical density at 600 nm (OD 600), (b) reverse phase UPLC profile, (c) p-hydroxybenzoic acid concentration in the soil of *P. ginseng* plantation, and (d) death rate of *P. ginseng*. Vertical bars indicate standard deviations of the mean values (n=3).