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GENOME SEQUENCES

Draft Genome Sequence of *Pseudomonas citronellolis* LA18T, a Bacterium That Uses Levulinic Acid

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ABSTRACT *Pseudomonas citronellolis* LA18T catabolizes levulinic acid (LA) from cellulosic biomass hydrolysate via acetyl-coenzyme A (acetyl-CoA) and propionyl-CoA. This study reports the 7.22-Mbp draft genome sequence of *P. citronellolis* LA18T. The draft genome sequence will aid the study of the LA catabolic pathway, which will allow for more applications of LA-utilizing bacteria.

Levulinic acid (LA) is a C_5 γ-keto acid that can be obtained from biomass resources and is a promising building block for biobased chemical products (1, 2). The recent discovery of a unique operon responsible for LA catabolism in *Pseudomonas putida* KT2440 has led to an interest in more efficient utilization of LA (3). Previously, we isolated several LA-utilizing bacteria, including *Pseudomonas* sp. strain LA18T, and identified several metabolites during LA catabolism (4, 5). Strain LA18T can utilize not only reagent-grade LA but also cedar-derived LA via acetic acid and propionic acid as intermediates (4, 6, 7); however, the molecular basis of LA catabolism in LA18T remains unclear.

Strain LA18T was isolated from a soil sample, as previously reported (4). The single colony of LA18T was picked up and cultured for preparation of a whole-genome sample. Total DNA was extracted from cultured suspension using DNeasy blood and tissue kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The draft genome sequence of LA18T was generated with the MiSeq next-generation sequencing platform (Illumina, San Diego, CA). Both paired-end and mate pair DNA libraries (insert size, ~500 bp) were prepared using a NEBNext Ultra DNA library prep kit for Illumina (New England BioLabs, Ipswich, MA) and a Nextera mate pair sample prep kit (Illumina), respectively, and sequenced on a MiSeq platform using the MiSeq reagent kit version 2 (Illumina). Sequence data totaling 3.13 million paired-end and 0.12 million mate pair reads, each approximately 250 and 2,000 bp in length, respectively, were generated. Genomic sequence assembly using ALLPATHS-LG version 46449 (8) with default settings generated 29 scaffolds composed of 179 contigs and a 7.22-Mb draft genome sequence at 100-fold coverage from both the paired-end and mate pair libraries. The length of the longest scaffold was 848,153 bp, and the N_{50} length was 3,751,832 bp, with six scaffolds. A total of 6,360 protein-coding genes were predicted using Glimmer 3.02 (9), with default settings, and annotated using NCBI blast 2.2.29 (blastp) with RefSeq version 65 (10, 11). The cutoff values used for gene annotation were an E value of ≤1e-10 and ≥25% amino acid sequence identity. A total of 60 tRNA- and 9 rRNA-encoding genes were also identified by tRNAscan-SE 1.3.1, with the general tRNA model option and RNAmmer 1.2, with default settings, respectively (12, 13). The

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16S rRNA gene sequence of LA18T shared >99.3% identity with that of *Pseudomonas citronellolis* P3B5. (14). To date, no possible LA catabolism gene operon similar to that in *P. putida* KT2440 has been found in the LA18T genome. Hence, the transcriptome analysis based on the draft genome sequence may provide some novel information on LA catabolism genes in *Pseudomonas* species.

Data availability. The *P. citronellolis* LA18T genome sequence has been deposited as 179 contigs and 29 scaffolds (accession numbers BGPP01000001 to BGPP01000029) in DDBJ/EMBL/GenBank. The version described in this paper is the first version.

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REFERENCES

1. Bozell JJ, Moens L, Elliott DC, Wang Y, Neuenscwander GG, Fitzpatrick SW, Bilski RJ, Jarnefeld JL. 2000. Production of levulinic acid and use as a platform chemical for derived products. Resour Conserv Recycl 28:227–239. https://doi.org/10.1016/S0921-3449(99)00047-6.
2. Pileidis FD, Titirici MM. 2016. Levulinic acid biorefineries: new challenges for efficient utilization of biomass. ChemSusChem 9:562–582. https://doi.org/10.1002/cssc.201501405.
3. Rand JM, Pisithkul T, Clark RL, Thiede JM, Mehrer CR, Agnew DE, Campbell CE, Markley AL, Price MN, Ray J, Wetmore KM, Suh Y, Arkin AP, Deutschbauer AM, Amador-Noguez D, Pfleger BF. 2017. A metabolic pathway for catabolizing levulinic acid in bacteria. Nat Microbiol 2:1624–1634. https://doi.org/10.1038/s41564-017-0028-z.
4. Habe H, Sato S, Morita T, Fukuoka T, Kirimura K, Kitamoto D. 2015. Bacterial production of short-chain organic acids and trehalose from levulinic acid: a potential cellulose-derived building block as a feedstock for microbial production. Bioresour Technol 177:381–386. https://doi.org/10.1016/j.biortech.2014.11.048.
5. Habe H, Sato S, Morita T, Fukuoka T, Kirimura K, Kitamoto D. 2015. Isolation and characterization of bacterial strains with the ability to utilize high concentration of levulinic acid, a platform chemical from inedible biomass. Biosci Biotechnol Biochem 79:1552–1555. https://doi.org/10.1080/09168451.2015.1031076.
6. Habe H, Kondo S, Sato Y, Hori T, Kanno M, Kimura N, Koike H, Kirimura K. 2017. Electrodialytic separation of levulinic acid catalytically synthesized from biomass for use in microbial conversion. Biotechnol Prog 33:448–453. https://doi.org/10.1002/btpr.2425.
7. Nemoto K, Tominaga K, Sato K. 2015. Facile and efficient transformation of lignocelluloses into levulinic acid using an $AlCl_3{\cdot}6H_2O/H_3PO_4$ hybrid acid catalyst. Bull Chem Soc Jpn 88:1752–1754. https://doi.org/10.1246/bcsj.20150266.
8. Butler J, MacCallum I, Kleber M, Shlyakhter IA, Belmonte MK, Lander ES, Nusbaum C, Jaffe DB. 2008. ALLPATHS: de novo assembly of whole-genome shotgun microreads. Genome Res 18:810–820. https://doi.org/10.1101/gr.7337908.
9. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23:673–679. https://doi.org/10.1093/bioinformatics/btm009.
10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
11. Tatusova T, Ciufo S, Fedorov B, O'Neill K, Tolstoy I. 2014. RefSeq microbial genomes database: new representation and annotation strategy. Nucleic Acids Res 42:D553–D559. https://doi.org/10.1093/nar/gkt1274.
12. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25:955–964.
13. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 35:3100–3108. https://doi.org/10.1093/nar/gkm160.
14. Remus-Emsermann MNP, Schmid M, Gekenidis M-T, Pelludat C, Frey JE, Ahrens CH, Drissner D. 2016. Complete genome sequence of *Pseudomonas citronellolis* P3B5, a candidate for microbial phyllo-remediation of hydrocarbon-contaminated sites. Stand Genomic Sci 11:75. https://doi.org/10.1186/s40793-016-0190-6.