

Genome Sequence of *Pseudomonas citronellolis* SJTE-3, an Estrogen- and Polycyclic Aromatic Hydrocarbon-Degrading Bacterium

Daning Zheng, Xiuli Wang, Pingping Wang, Wanli Peng, Nannan Ji, Rubing Liang

State Key Laboratory of Microbial Metabolism, and School of Life Sciences & Biotechnology, Shanghai Jiaotong University, Shanghai, China

D.Z. and X.W. contributed equally to this article.

***Pseudomonas citronellolis* SJTE-3, isolated from the active sludge of a wastewater treatment plant in China, can utilize a series of environmental estrogens and estrogen-like toxicants. Here, we report its whole-genome sequence, containing one circular chromosome and one circular plasmid. Genes involved in estrogen biodegradation in this bacterium were predicted.**

Received 12 October 2016 Accepted 14 October 2016 Published 8 December 2016

Citation Zheng D, Wang X, Wang P, Peng W, Ji N, Liang R. 2016. Genome sequence of *Pseudomonas citronellolis* SJTE-3, an estrogen- and polycyclic aromatic hydrocarbon-degrading bacterium. *Genome Announc* 4(6):e01373-16. doi:10.1128/genomeA.01373-16.

Copyright © 2016 Zheng et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Rubing Liang, icelike@sjtu.edu.cn.

Estrogens are one of the most dangerous environmental endocrine-disrupting compounds, and biodegradation is considered to be the efficient strategy (1–4). Although some estrogen-degrading bacteria have been isolated, the degradation mechanisms vary in different strains and remain poorly characterized (5, 6).

Pseudomonas citronellolis SJTE-3 isolated from active sludge of a wastewater treatment plant was capable of utilizing different estrogens (17 β -estradiol, estrone, estriol, and 17 α -ethynylestradiol), estrogenic chemicals, and some polycyclic aromatic hydrocarbons (PAHs) (naphthalene, bisphenol A, phenanthrene, fluorene, and anthracene) and transforming them into nonestrogenic chemicals (unpublished data). Therefore, strain SJTE-3 may play an important role in the bioremediation of estrogen-polluted environments; its genome sequencing will provide great insights into its genetic variability and assist in the study of its biodegradation mechanisms.

Genomic DNA from *P. citronellolis* SJTE-3 was extracted using the QIAamp DNA minikit (Qiagen, CA), and two libraries were constructed. One was a 10-kb-insert SMRTbell library sequenced on the Pacific Biosciences (PacBio, CA) RSII platform using two single-molecule real-time (SMRT) cells with a single 180-min movie (475,094 reads for 1,488,681,633 bp) using the PacBio RSII sequencer (7). Another one was a 400-bp-insert library, sequenced with paired-end sequencing mode (2 \times 251 bp) using Illumina Miseq platform (12,700,872 reads in 3,044,385,325 bp) (8). The gaps were closed by specific PCR and Sanger sequencing. The data of the PacBio RSII platform were assembled using the Celera Assembler and PBjelly software (9, 10), and the data of the Illumina MiSeq platform were corrected with Kmer and assembled with Newbler (11, 12). Finally, all the contigs were emended with Pilon (13). The coding sequences (CDSs) were predicted using Glimmer 3.0 (14); tRNA and rRNA were identified with tRNAscan-SE (15) and RNAmmer (16), respectively. The genome sequence was annotated using the RAST (17) and PGAAP (18) databases. Gene functions were annotated with the NCBI-NR (19), eggNOG (20), Swiss-Prot (21), and

KEGG (22) databases. The clustered regularly interspaced short palindromic repeats (CRISPRs) were identified by the CRISPR recognition tool (CRT) (23).

The genome of SJTE-3 contained one circular chromosome and one plasmid, pRBL16, with 67.04% and 56.57% G+C contents, respectively. The coding regions covering 86.21% of the genome (7,309,421 bp) contained 6,756 genes, encoding 6,667 proteins; 199 proteins were encoded by the 370,338-bp plasmid pRBL16. Also, the genome encodes five 5S-16S-23S rRNA operons, 69 tRNAs, 5 noncoding RNAs (ncRNAs), and 2 CRISPR clusters. The predicted proteins were classified into 36 COG categories. The six most abundant subsystems are related to amino acid metabolism ($n = 817$), carbohydrate metabolism ($n = 546$), cofactors, vitamins, and pigments ($n = 388$), protein metabolism ($n = 300$), fatty acids, lipids, and isoprenoids ($n = 272$), and aromatic compound metabolism ($n = 270$). Many CDSs are involved in membrane transport, stress response, motility, chemotaxis, regulation, and cell signaling. According to the proposed degradation pathways (24, 25), several enzymes involved in estrogen degradation were annotated, such as 3-ketosteroid-delta-dehydrogenase, hydroxysteroid dehydrogenase, and Rieske dioxygenase.

In conclusion, the whole-genome sequence of *P. citronellolis* SJTE-3 will enrich the genome database of estrogen degradation microorganism and shed a light on the biodegradation mechanism study of estrogenic chemicals.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers CP015878 (chromosome) and CP015879 (plasmid pRBL 16). The versions described in this paper are the first versions. The strain is available from the China General Microbiological Culture Collection Center (CGMCC) under the GenBank accession no. 12720.

ACKNOWLEDGMENTS

We acknowledge Shanghai Personal Biotech Co., Ltd. for genome sequencing.

This work was supported in part by the National Natural Science Foundation of China (grants 31370152 and d31570099), the Shanghai Pujiang Program (grant 14PJD020), and the Chen Xing Grant of Shanghai Jiaotong University.

FUNDING INFORMATION

This work, including the efforts of Rubing Liang, was funded by Shanghai Pujiang Program (14PJD020). This work, including the efforts of Rubing Liang, was funded by Chen Xing Grant of Shanghai Jiaotong University. This work, including the efforts of Rubing Liang, was funded by National Natural Science Foundation of China (NSFC) (31370152 and 31570099).

REFERENCES

- Combalbert S, Hernandez-Raquet G. 2010. Occurrence, fate, and biodegradation of estrogens in sewage and manure. *Appl Microbiol Biotechnol* 86:1671–1692. <http://dx.doi.org/10.1007/s00253-010-2547-x>.
- Sweeney MF, Hasan N, Soto AM, Sonnenschein C. 2015. Environmental endocrine disruptors: effects on the human male reproductive system. *Rev Endocr Metab Disord* 16:341–357. <http://dx.doi.org/10.1007/s11154-016-9337-4>.
- Lv X, Xiao S, Zhang G, Jiang P, Tang F. 2016. Occurrence and removal of phenolic endocrine disrupting chemicals in the water treatment processes. *Sci Rep* 6:22860. <http://dx.doi.org/10.1038/srep22860>.
- Lust M, Makinia J, Stensel HD. 2012. A mechanistic model for fate and removal of estrogens in biological nutrient removal activated sludge systems. *Water Sci Technol* 65:1130–1136. <http://dx.doi.org/10.2166/wst.2012.958>.
- Kurisu F, Ogura M, Saitoh S, Yamazoe A, Yagi O. 2010. Degradation of natural estrogen and identification of the metabolites produced by soil isolates of *Rhodococcus* sp. and *Sphingomonas* sp. *J Biosci Bioeng* 109:576–582.
- Sang Y, Xiong G, Maser E. 2012. Identification of a new steroid degrading bacterial strain H5 from the Baltic sea and isolation of two estradiol inducible genes. *J Steroid Biochem Mol Biol* 129:22–30. <http://dx.doi.org/10.1016/j.jsbmb.2011.01.018>.
- Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323:133–138. <http://dx.doi.org/10.1126/science.1162986>.
- Glenn TC. 2011. Field guide to next-generation DNA sequencers. *Mol Ecol Resour* 11:759–769. <http://dx.doi.org/10.1111/j.1755-0998.2011.03024.x>.
- Denisov G, Walenz B, Halpern AL, Miller J, Axelrod N, Levy S, Sutton G. 2008. Consensus generation and variant detection by Celera assembler. *Bioinformatics* 24:1035–1040. <http://dx.doi.org/10.1093/bioinformatics/btn074>.
- English AC, Richards S, Han Y, Wang M, Vee V, Qu J, Qin X, Muzny DM, Reid JG, Worley KC, Gibbs RA. 2012. Mind the gap: upgrading genomes with Pacific Biosciences RS long-read sequencing technology. *PLoS One* 7:e47768. <http://dx.doi.org/10.1371/journal.pone.0047768>.
- Fletez-Brant C, Lee D, McCallion AS, Beer MA. 2013. kmer-SVM: a Web server for identifying predictive regulatory sequence features in genomic data sets. *Nucleic Acids Res* 41:W544–W556. <http://dx.doi.org/10.1093/nar/gkt519>.
- Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437:376–380. <http://dx.doi.org/10.1038/nature03959>.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One* 9:e112963. <http://dx.doi.org/10.1371/journal.pone.0112963>.
- Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. *Nucleic Acids Res* 27:4636–4641. <http://dx.doi.org/10.1093/nar/27.23.4636>.
- 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. <http://dx.doi.org/10.1093/nar/25.5.0955>.
- Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of rRNA genes. *Nucleic Acids Res* 35:3100–3108. <http://dx.doi.org/10.1093/nar/gkm160>.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
- Pruitt KD, Tatusova T, Klimke W, Maglott DR. 2009. NCBI reference sequences: current status, policy and new initiatives. *Nucleic Acids Res* 37:D32–D36. <http://dx.doi.org/10.1093/nar/gkn721>.
- Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2008. GenBank. *Nucleic Acids Res* 36:D25–D30. <http://dx.doi.org/10.1093/nar/gkm929>.
- Powell S, Forslund K, Szklarczyk D, Trachana K, Roth A, Huerta-Cepas J, Gabaldón T, Rattei T, Creevey C, Kuhn M, Jensen LJ, von Mering C, Bork P. 2014. eggNOG v4.0: nested orthology inference across 3686 organisms. *Nucleic Acids Res* 42:D231–D239. <http://dx.doi.org/10.1093/nar/gkt1253>.
- Boutet E, Lieberherr D, Tognolli M, Schneider M, Bansal P, Bridge AJ, Poux S, Bougueleret L, Xenarios I. 2016. UniProtKB/Swiss-Prot, the manually annotated section of the UniProt KnowledgeBase: how to use the entry view. *Methods Mol Biol* 1374:23–54. http://dx.doi.org/10.1007/978-1-4939-3167-5_2.
- Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, Yamanishi Y. 2008. KEGG for linking genomes to life and the environment. *Nucleic Acids Res* 36:D480–D484. <http://dx.doi.org/10.1093/nar/gkm882>.
- Bland C, Ramsey TL, Sabree F, Lowe M, Brown K, Kyrpidis NC, Hugenholtz P. 2007. CRISPR recognition tool (CRT): a tool for automatic detection of clustered regularly interspaced palindromic repeats. *BMC Bioinformatics* 8:209. <http://dx.doi.org/10.1186/1471-2105-8-209>.
- Horinouchi M, Hayashi T, Kudo T. 2012. Steroid degradation in *Comamonas testosteroni*. *J Steroid Biochem Mol Biol* 129:4–14. <http://dx.doi.org/10.1016/j.jsbmb.2010.10.008>.
- Yu CP, Roh H, Chu KH. 2007. 17 β -Estradiol-degrading bacteria isolated from activated sludge. *Environ Sci Technol* 41:486–492. <http://dx.doi.org/10.1021/es060923f>.