

UC Merced

UC Merced Previously Published Works

Title

Complete Genome Sequence of *Serratia quinivorans* Strain 124R, a Facultative Anaerobe Isolated on Organosolv Lignin as a Sole Carbon Source

Permalink

<https://escholarship.org/uc/item/82v1r3sn>

Journal

Microbiology Resource Announcements, 8(18)

ISSN

2576-098X

Authors

Chaput, Gina

Ford, Jacob

DeDiego, Lani

et al.

Publication Date

2019-05-02

DOI

10.1128/mra.00409-19

Peer reviewed



Complete Genome Sequence of *Serratia quinivorans* Strain 124R, a Facultative Anaerobe Isolated on Organosolv Lignin as a Sole Carbon Source

Gina Chaput,^a Jacob Ford,^b Lani DeDiego,^b Achala Narayanan,^b Wing Yin Tam,^b Meghan Whalen,^b Marcel Huntemann,^c Alicia Clum,^c Alex Spunde,^c Manoj Pillay,^c Krishnaveni Palaniappan,^c Neha Varghese,^c Natalia Mikhailova,^c I-Min Chen,^c Dimitrios Stamatis,^c T. B. K. Reddy,^c Ronan O'Malley,^c Chris Daum,^c Nicole Shapiro,^c Natalia Ivanova,^c Nikos C. Kyrpides,^c Tanja Woyke,^c Tijana Glavina del Rio,^c Kristen DeAngelis^a

^aDepartment of Microbiology, University of Massachusetts, Amherst, Amherst, Massachusetts, USA

^bDepartment of Biology, University of Massachusetts, Amherst, Amherst, Massachusetts, USA

^cU.S. Department of Energy Joint Genome Institute, Walnut Creek, California, USA

ABSTRACT The complete genome sequence of the gammaproteobacterial isolate *Serratia quinivorans* 124R consists of 5 Mb over 2 scaffolds and a G+C content of 52.85%. Genes relating to aromatic metabolism reflect its isolation on organosolv lignin as a sole carbon source under anoxic conditions as well as the potential for lignin biorefinery applications.

Lignin is an abundant, natural resource for aromatic chemical production; yet, only 1% to 2% of lignin produced annually in the paper and pulp industry is processed (1). Bacterial anoxic depolymerization techniques need further development to valorize lignin, but exact mechanisms remain largely undefined (2–4). Known enzymes include glutathione S-transferases (GSTs) in the β -etherase system (5–8); however, isolation and characterization of anaerobic lignin-degrading bacteria will help advance understanding of and discover new mechanisms.

In this study, temperate forest soil was used to inoculate minimal medium (9) containing organosolv lignin as the sole carbon source under anoxic conditions and was transferred onto fresh medium every 4 to 9 weeks for 465 days. Consortia were diluted to 1 to 5 cells/ml onto a 0.001% five-carbon mixture (10) incubated anaerobically in the dark at 25°C for 6 weeks and then were streaked onto R2A agar for colony isolation. To screen for lignin depolymerization capabilities, isolates were grown anoxically on R2A plates containing lignin-mimicking dyes, malachite green and Congo red (11). Isolate 124R was selected for genome sequencing due to the formation of clearing zones for both dyes.

Genomic DNA was extracted using the Qiagen Genomic-tip protocol for bacteria. A >10-kbp PacBio SMRTbell library was constructed and sequenced on the PacBio RS II platform (12). This generated 296,135 filtered subreads, totaling 591,980,396 bp. Raw reads were assembled using Hierarchical Genome Assembly Process 3 (HGAP3; SMRT Analysis v2.3.0.p5) (13). The final draft assembly contained 2 contigs in 2 scaffolds, covering a total of 5,025,603 bp, with an N/L_{50} value of 1/4,986,851, a G+C content of 52.85%, and an average sequence coverage of 86.8 \times . Gene prediction and functional annotation were performed using the Department of Energy Joint Genome Institute (DOE JGI) annotation pipeline (14), available through the Integrated Microbial Genomes data management system (15, 16). 124R contains 4,636 predicted protein-coding sequences, of which 86.06% were assigned a function, as well as 85 tRNAs and 7 rRNA operons. Putative aromatic metabolic pathways were analyzed using the MetaCyc and KEGG databases via the Integrated Microbial Genomes and Microbiomes (IMG/M) database

Citation Chaput G, Ford J, DeDiego L, Narayanan A, Tam WY, Whalen M, Huntemann M, Clum A, Spunde A, Pillay M, Palaniappan K, Varghese N, Mikhailova N, Chen I-M, Stamatis D, Reddy TBK, O'Malley R, Daum C, Shapiro N, Ivanova N, Kyrpides NC, Woyke T, Glavina del Rio T, DeAngelis K. 2019. Complete genome sequence of *Serratia quinivorans* strain 124R, a facultative anaerobe isolated on organosolv lignin as a sole carbon source. *Microbiol Resour Announc* 8:e00409-19. <https://doi.org/10.1128/MRA.00409-19>.

Editor Christina Cuomo, Broad Institute of MIT and Harvard University

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply.

Address correspondence to Kristen DeAngelis, deangelis@microbio.umass.edu.

Received 9 April 2019

Accepted 12 April 2019

Published 2 May 2019

(16). Seventy-six enzymes were identified (encompassing 41 functions under KEGG map 01220), including complete metabolism of benzoate and 4-hydroxyphenylacetate. Genes for anaerobic degradation of gallate, phenylacetate, and 4-coumarate were found within MetaCyc. Additionally, two homologues to the *Nu*-class GSTs were identified via IMG/M NCBI BLAST (16).

The 16S rRNA gene of 124R was queried using NCBI BLASTn (17) and shared 99% sequence identity with *Serratia quinivorans* strain 4364 and *Serratia proteamaculans* DSM 4543, which are in the class *Gammaproteobacteria* and family *Yersiniaceae*. However, 124R shared <89% average nucleotide identity (18) and <95% two-way average amino acid identity (19) with *Serratia quinivorans* (strains NCTC13194, NCTC13189, and NCTC11544) and *Serratia proteamaculans* (strains 568, MFPA44A14, and NCTC10861). DNA-DNA hybridization (DDH) calculations (20) resulted with a <34% DDH estimate across comparisons, further supporting this species demarcation (21). These findings suggest that 124R is a distinct *Serratia* species with both lignin depolymerization and catabolic potential for lignin biorefinery applications.

Data availability. This whole-genome shotgun project has been deposited in GenBank under accession no. [NZ_SHMO00000000](https://www.ncbi.nlm.nih.gov/nuccore/NZ_SHMO00000000) (SRA accession no. [SRX5216996](https://www.ncbi.nlm.nih.gov/sra/SRX5216996)). The version described in this paper is the first version, NZ_SHMO01000000.

ACKNOWLEDGMENTS

Funding for this project came from the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, and the Joint BioEnergy Institute (JBEI), both supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231. This publication was also developed under Assistance Agreement no. FP-91782301-0, awarded by the U.S. Environmental Protection Agency to G.C.

The views expressed in this document are solely those of the authors and do not necessarily reflect those of the EPA. EPA does not endorse any products or commercial services mentioned in this publication. The manuscript has not been formally reviewed by EPA.

We thank JBEI for providing the organosolv lignin.

REFERENCES

- Melián Rodríguez M. 2016. Lignin biomass conversion into chemicals and fuels. PhD dissertation. Technical University of Denmark, Lyngby, Denmark.
- Abdelaziz OY, Brink DP, Prothmann J, Ravi K, Sun M, García-Hidalgo J, Sandahl M, Hultheberg CP, Turner C, Lidén G, Gorwa-Grauslund MF. 2016. Biological valorization of low molecular weight lignin. *Biotechnol Adv* 34:1318–1346. <https://doi.org/10.1016/j.biotechadv.2016.10.001>.
- Xu R, Zhang K, Liu P, Han H, Zhao S, Kakade A, Khan A, Du D, Li X. 2018. Lignin depolymerization and utilization by bacteria. *Bioresour Technol* 269:557–566. <https://doi.org/10.1016/j.biortech.2018.08.118>.
- Beckham GT, Johnson CW, Karp EM, Salvachúa D, Vardon DR. 2016. Opportunities and challenges in biological lignin valorization. *Curr Opin Biotechnol* 42:40–53. <https://doi.org/10.1016/j.copbio.2016.02.030>.
- Kamimura N, Sakamoto S, Mitsuda N, Masai E, Kajita S. 2019. Advances in microbial lignin degradation and its applications. *Curr Opin Biotechnol* 56:179–186. <https://doi.org/10.1016/j.copbio.2018.11.011>.
- Ohta Y, Nishi S, Hasegawa R, Hatada Y. 2015. Combination of six enzymes of a marine *Novosphingobium* converts the stereoisomers of β -O-4 lignin model dimers into the respective monomers. *Sci Rep* 5:15105. <https://doi.org/10.1038/srep15105>.
- DeAngelis KM, Sharma D, Varney R, Simmons BA, Isern NG, Markillie LM, Nicora CD, Norbeck AD, Taylor RC, Aldrich JT, Robinson EW. 2013. Evidence supporting dissimilatory and assimilatory lignin degradation in *Enterobacter lignolyticus* SCF1. *Front Microbiol* 4:280. <https://doi.org/10.3389/fmicb.2013.00280>.
- Orellana R, Chaput G, Markillie LM, Mitchell H, Gaffrey M, Orr G, DeAngelis KM. 2017. Multi-time series RNA-seq analysis of *Enterobacter lignolyticus* SCF1 during growth in lignin-amended medium. *PLoS One* 12:e0186440. <https://doi.org/10.1371/journal.pone.0186440>.
- Buyer JS. 1995. A soil and rhizosphere microorganism isolation and enumeration medium that inhibits *Bacillus mycoides*. *Appl Environ Microbiol* 61:1839–1842.
- Cho JC, Giovannoni SJ. 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine Gammaproteobacteria. *Appl Environ Microbiol* 70:432–440. <https://doi.org/10.1128/AEM.70.1.432-440.2004>.
- Bandounas L, Wierckx NJP, de Winde JH, Ruijsenaars HJ. 2011. Isolation and characterization of novel bacterial strains exhibiting ligninolytic potential. *BMC Biotechnol* 11:94. <https://doi.org/10.1186/1472-6750-11-94>.
- 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, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D, Zhao P, Zhong F, Korlach J, Turner S. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323:133–138. <https://doi.org/10.1126/science.1162986>.
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long read SMRT sequencing data. *Nat Methods* 10:563. <https://doi.org/10.1038/nmeth.2474>.
- Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen IMA, Pati A, Nielsen T. 2015. The standard operating procedure of the DOE-JGI microbial genome anno-

- tation pipeline (MGAP v. 4). *Stand Genomic Sci* 10:86. <https://doi.org/10.1186/s40793-015-0077-y>.
15. Chen IM, Markowitz VM, Palaniappan K, Szeto E, Chu K, Huang J, Ratner A, Pillay M, Hadjithomas M, Huntemann M, Mikhailova N, Ovchinnikova G, Ivanova NN, Kyrpides NC. 2016. Supporting community annotation and user collaboration in the integrated microbial genomes (IMG) system. *BMC Genomics* 17:307. <https://doi.org/10.1186/s12864-016-2629-y>.
 16. Chen I-MA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang J, Andersen E, Huntemann M, Varghese N, Hadjithomas M, Tennessen K, Nielsen T, Ivanova NN, Kyrpides NC. 2017. IMG/M: integrated genome and metagenome comparative data analysis system. *Nucleic Acids Res* 45:D507–D516. <https://doi.org/10.1093/nar/gkw929>.
 17. 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](https://doi.org/10.1016/S0022-2836(05)80360-2).
 18. Lee I, Kim YO, Park SC, Chun J. 2016. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 66:1100–1103. <https://doi.org/10.1099/ijsem.0.000760>.
 19. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Preprints* 4:e1900v1. <https://doi.org/10.7287/peerj.preprints.1900v1>.
 20. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>.
 21. Thompson CC, Chimetto L, Edwards RA, Swings J, Stackebrandt E, Thompson FL. 2013. Microbial genomic taxonomy. *BMC Genomics* 14:913. <https://doi.org/10.1186/1471-2164-14-913>.