



Draft Genome Sequence of the Redox-Active Enteric Bacterium *Citrobacter portucalensis* Strain MBL

 Lev M. Tsy-pin,^a Scott H. Saunders,^a Yinon Bar-On,^b Jared R. Leadbetter,^{c,d}  Dianne K. Newman^{a,d}

^aDivision of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, USA

^bDepartment of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel

^cDivision of Engineering and Applied Science, California Institute of Technology, Pasadena, California, USA

^dDivision of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, USA

ABSTRACT We grew a soil enrichment culture to identify organisms that anaerobically oxidize phenazine-1-carboxylic acid. A strain of *Citrobacter portucalensis* was isolated from this enrichment and sequenced by both Illumina and PacBio technologies. It has a genome with a length of 5.3 Mb, a G+C content of 51.8%, and at least one plasmid.

During the Microbial Diversity 2017 program at the Marine Biological Laboratory (MBL), we aimed to isolate organisms that anaerobically oxidize phenazine-1-carboxylic acid (PCA), a process that has never been described. Topsoil samples from Falmouth, Massachusetts (41°36'58.9"N, 70°34'31.2"W; 41°32'42.8"N, 70°37'52.4"W; and 41°31'34.3"N, 70°39'05.3"W), were incubated anoxically in Balch tubes with a minimal medium containing acetate as a nonfermentable carbon source, reduced PCA and acetate as electron donors, and nitrate as the terminal electron acceptor (<https://doi.org/10.17504/protocols.io.bh4tj8wn>). PCA-oxidizing enrichments were serially passaged. One strain was isolated based on its ability to anaerobically oxidize PCA and sequenced. After isolation and for sequencing, it was grown in LB medium, Miller (product number 244620; BD Difco), at 30°C with shaking at 250 rpm. It was stored as 35% glycerol stocks in a -80°C freezer.

For Illumina sequencing, DNA was extracted using the DNeasy blood and tissue kit (product number 69504; Qiagen). The library was prepared using a NEBNext kit (product number E7335; New England Biolabs) and sequenced to 100× coverage (5 million 100-bp single-end reads) on a HiSeq 2500 instrument. Base calls were performed with RTA v1.13.48.0, followed by conversion to fastq files with bcl2fastq v1.8.4. The reads were concatenated into a single file, trimmed with Trimmomatic v0.39 (with the following parameters: leading, 27; trailing, 27; slidingwindow, 4:20; minlen, 80) (1), and analyzed for quality using FastQC v0.11.8 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). For PacBio sequencing, DNA was extracted with phenol-chloroform. The library was prepared using the SMRTbell Express template preparation kit v2.0 with barcoded overhang adapters and was sequenced in a multiplexed PacBio Sequel II single-molecule real-time (SMRT) cell. This yielded 4,335 reads with a mean length of 9,840 bases for approximately 8× coverage, with an N_{50} value of 103,465 nucleotides (nt) given by Canu v2.0 (set genomesize, 5.3m; mininputcoverage, 7; stoponlowcoverage, 7) (2, 3). The PacBio fastq and trimmed Illumina reads were coassembled using SPAdes v1.13.1 (4). The resulting scaffolds were further improved by comparison to nine reference *Citrobacter* genomes using MeDuSa v1.6 (Table 1) (5). Contaminant sequences from eukaryotes were identified using NCBI BLAST and removed from the genome, along with scaffolds shorter than 200 nt, and the remaining scaffolds were

Citation Tsy-pin LM, Saunders SH, Bar-On Y, Leadbetter JR, Newman DK. 2020. Draft genome sequence of the redox-active enteric bacterium *Citrobacter portucalensis* strain MBL. Microbiol Resour Announc 9:e00695-20. <https://doi.org/10.1128/MRA.00695-20>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2020 Tsy-pin 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 Dianne K. Newman, dkn@caltech.edu.

Received 16 June 2020

Accepted 8 July 2020

Published 6 August 2020

TABLE 1 Whole-genome comparisons of *C. portucalensis* MBL to references

Reference genome (GenBank accession no.)	BLAST ANI (%)	Proportion BLAST aligned (%)	MUMmer ANI (%)	Proportion MUMmer aligned (%)	Pearson's correlation coefficient for tetranucleotide usage
<i>C. portucalensis</i> A60 ^T (MVFY0000000.1)	98.42 ^a	84.79	98.78 ^a	85.43	0.99923 ^a
<i>C. portucalensis</i> Effluent_1 (NZ_CP039327.1)	97.81 ^a	82.92	98.31 ^a	83.43	0.99908 ^a
<i>Citrobacter braakii</i> FDAARGOS_253 (NZ_CP020448.2)	92.5	79.75	93.15	80.66	0.99707 ^b
<i>Citrobacter werkmanii</i> BF-6 (NZ_CP019986.1)	90.32	78.4	91.15	78.6	0.9963 ^b
<i>Citrobacter freundii</i> CFNIH1 (NZ_CP007557.1)	90.32	77.25	91.1	77.87	0.99596 ^b
<i>Citrobacter youngae</i> NCTC13709 (NZ_LR134485.1)	89.27	75.07	90.33	74.52	0.99759 ^b
<i>Citrobacter koseri</i> ATCC BAA-895 (NC_009792.1)	83.05	65.21	85.43	54.95	0.97119
<i>Citrobacter amalonaticus</i> Y19 (NZ_CP011132.1)	81.74	66.53	85.23	49.16	0.98547
<i>Citrobacter farmeri</i> AUSMDU00008141 (NZ_CP022695.1)	81.47	66.96	85.01	49.17	0.98683
<i>Citrobacter rodentium</i> ICC168 (NC_013716.1)	81.28	62.37	84.98	43.83	0.94412
<i>E. coli</i> O157:H7 Sakai (NC_002695.2)	80.28	62.16	84.67	38.66	0.97933

^a Values above the threshold for species identity (7).
^b Values within the range for species identity (7).

analyzed with QUASt v5.0.2 (6). Default parameters were used for all software unless otherwise specified.

We identified the isolate as a *Citrobacter portucalensis* strain (which we designated strain MBL) by average nucleotide identity (ANI) and tetranucleotide usage correlations

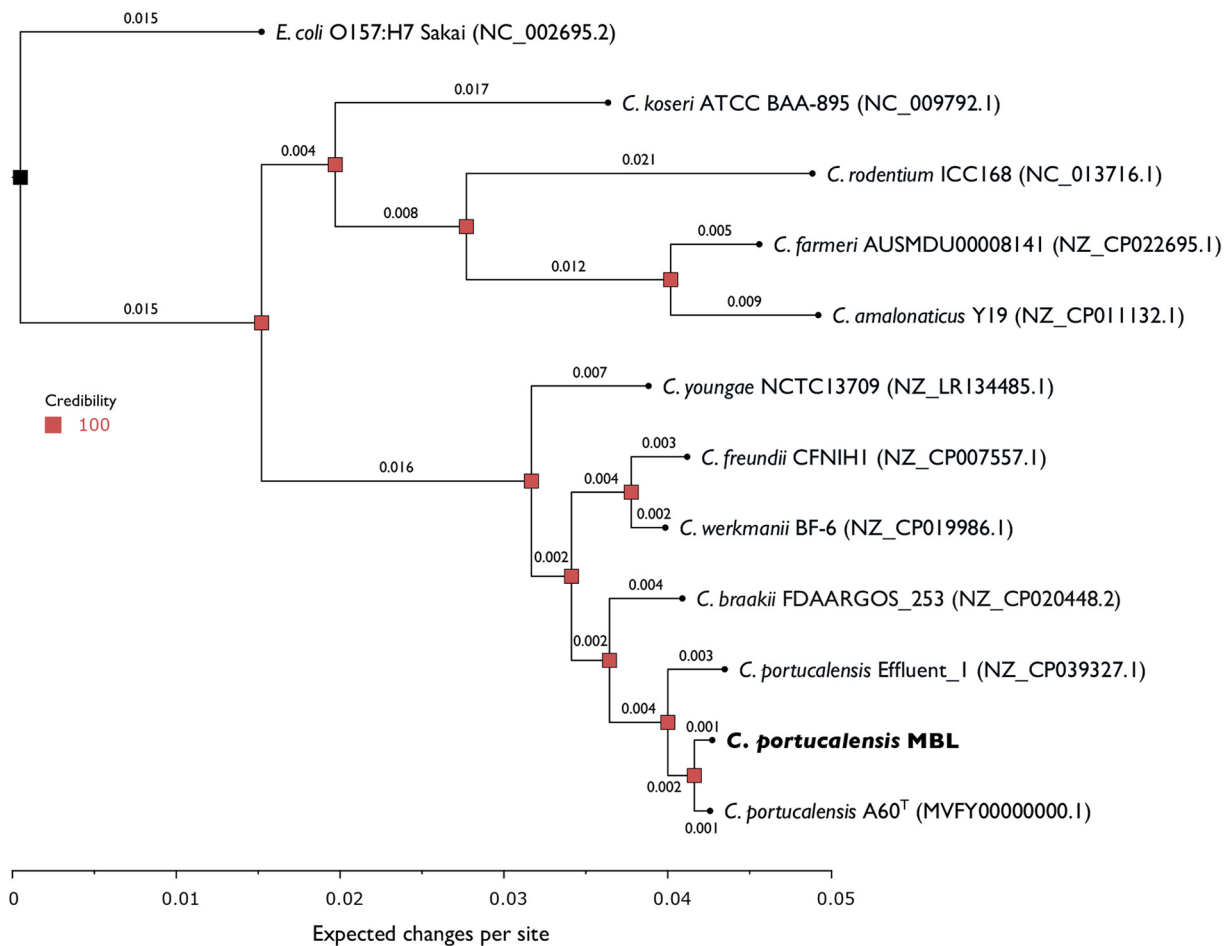


FIG 1 MLSA phylogeny of the genomes compared in Table 1. *C. portucalensis* MBL is shown in bold at the bottom of the tree. The *E. coli* strain is the outgroup and roots the tree. This phylogeny was calculated based on the alignment of concatenated HMM profiles of 32 single-copy nonribosomal housekeeping genes that are shared across all 12 genomes (PFAM accession numbers PF00709.21, PF00406.22, PF01808.18, PF00231.19, PF00119.20, PF01264.21, PF00889.19, PF01176.19, PF02601.15, PF01025.19, PF01725.16, PF01715.17, PF00213.18, PF01195.19, PF00162.19, PF02033.18, PF02565.15, PF00825.18, PF01193.24, PF01192.22, PF01765.19, PF02410.15, PF03652.15, PF00584.20, PF03840.14, PF00344.20, PF01668.18, PF00750.19, PF01746.21, PF02367.17, PF02130.17, and PF02699.15).

using JSpeciesWS (Table 1), and we validated this finding using multilocus sequence analysis (MLSA) (Fig. 1) (7). MLSA was performed using *anvi'o* v6.1 to generate hidden Markov model (HMM) profiles for 32 nonribosomal single-copy housekeeping genes common to all reference strains compared (Fig. 1) (8). These HMM profiles were aligned using MUSCLE, and a phylogeny was constructed with MrBayes v3.2.7a on the CIPRES Science Gateway, with the reference *Escherichia coli* strain as the outgroup (Fig. 1) (9–11). Both the MLSA and whole-genome comparisons returned the type strain *C. portucalensis* A60^T as the closest relative to *C. portucalensis* MBL. In the whole-genome comparisons, only *C. portucalensis* Effluent_1 and A60^T gave values above the thresholds for species identity (7, 12–16). The *C. portucalensis* MBL genome has a total length of 5,311,497 nt with seven total scaffolds, none of which is circularized. The N_{50} value is 5,245,291 nt and corresponds to the single chromosome scaffold. There are two putative plasmid scaffolds, which we named pCpMBL1 and pCpMBL2 (50,894 bp and 5,198 bp, respectively) and which we identified by homology; pCpMBL1 is likely an F plasmid and contains homologs to all components of the conjugation apparatus. We annotated the genome using the Prokaryotic Genome Annotation Pipeline (PGAP) (17, 18). Current research on *C. portucalensis* MBL is directed toward understanding its redox physiology.

Data availability. This genome has been deposited at DDBJ/ENA/GenBank under the accession number [JABVAY000000000](https://accession.ddbj.go.jp/acc/showacc.cgi?acc=JABVAY000000000). The version described in this paper is version [JABVAY010000000](https://accession.ddbj.go.jp/acc/showacc.cgi?acc=JABVAY010000000). The genome and raw reads are associated with BioProject [PRJNA638116](https://ncbi.nlm.nih.gov/bioproject/PRJNA638116). The SRA accession number for the Illumina reads is [SRR11952884](https://www.ncbi.nlm.nih.gov/sra/SRR11952884), and the SRA accession number for the PacBio reads is [SRR11952883](https://www.ncbi.nlm.nih.gov/sra/SRR11952883).

ACKNOWLEDGMENTS

We thank Melanie Oakes at the University of California, Irvine, Genomics High-Throughput Facility for PacBio library preparation and sequencing. Special thanks go to Igor Antoshechkin at the Caltech Millard and Muriel Jacobs Genetics and Genomics Laboratory for Illumina library preparation and sequencing and for giving us critical feedback on the manuscript. Megan Bergkessel and Kurt Dahlstrom provided helpful discussions throughout this work. Kurt Hanselmann and Gabriela Kovacikova were responsible for much of the success and joy of the Microbial Diversity 2017 program.

Funding for the Microbial Diversity 2017 program at the MBL was provided by the Hibbit Endowed Education Fund, the Moshe Shilo Memorial Scholarship Fund, the Holger and Friederun Jannasch Scholarship, the U.S. DOE, NASA, the NSF, and the Simons Foundation. L.M.T. was supported by the Rosen Endowment Fellowship at Caltech and an NSF Graduate Research Fellowship (grant DGE-1745301). Additional support came from NIH (1R01AI127850-01A1) and Army Research Office (W911NF-17-1-0024) grants to D.K.N.

REFERENCES

- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Wilson K. 2001. Preparation of genomic DNA from bacteria. *Curr Protoc Mol Biol* Chapter 2:Unit 2.4. <https://doi.org/10.1002/0471142727.mb0204s6>.
- Koren S, Walenz BP, Berlin K, Miller JR, Bergman NH, Phillippy AM. 2017. Canu: scalable and accurate long-read assembly via adaptive k-mer weighting and repeat separation. *Genome Res* 27:722–736. <https://doi.org/10.1101/gr.215087.116>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshtkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Bosi E, Donati B, Galardini M, Brunetti S, Sagot M-F, Lió P, Crescenzi P, Fani R, Fondi M. 2015. MeDuSa: a multi-draft based scaffold. *Bioinformatics* 31:2443–2451. <https://doi.org/10.1093/bioinformatics/btv171>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.
- Eren AM, Esen ÖC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO. 2015. *Anvi'o*: an advanced analysis and visualization platform for 'omics data. *PeerJ* 3:e1319. <https://doi.org/10.7717/peerj.1319>.
- Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113. <https://doi.org/10.1186/1471-2105-5-113>.
- Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic

- inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>.
11. Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, p 1–8. *In* 2010 Gateway Computing Environments Workshop (GCE). Institute of Electrical and Electronics Engineers, Piscataway, NJ. <https://doi.org/10.1109/GCE.2010.5676129>.
 12. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 106:19126–19131. <https://doi.org/10.1073/pnas.0906412106>.
 13. Teeling H, Meyerdierks A, Bauer M, Amann R, Glöckner FO. 2004. Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ Microbiol* 6:938–947. <https://doi.org/10.1111/j.1462-2920.2004.00624.x>.
 14. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
 15. Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. *Genome Biol* 5:R12. <https://doi.org/10.1186/gb-2004-5-2-r12>.
 16. Ribeiro TG, Gonçalves BR, da Silva MS, Novais Â, Machado E, Carriço JA, Peixe L. 2017. *Citrobacter portucalensis* sp. nov., isolated from an aquatic sample. *Int J Syst Evol Microbiol* 67:3513–3517. <https://doi.org/10.1099/ijsem.0.002154>.
 17. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
 18. Haft DH, DiCuccio M, Badretdin A, Brover V, Chetvernin V, O'Neill K, Li W, Chitsaz F, Derbyshire MK, Gonzales NR, Gwadz M, Lu F, Marchler GH, Song JS, Thanki N, Yamashita RA, Zheng C, Thibaud-Nissen F, Geer LY, Marchler-Bauer A, Pruitt KD. 2018. RefSeq: an update on prokaryotic genome annotation and curation. *Nucleic Acids Res* 46:D851–D860. <https://doi.org/10.1093/nar/gkx1068>.