



# Draft Genome Sequence of *Tumebacillus* sp. Strain BK434, Isolated from the Roots of Eastern Cottonwood

Dana L. Carper,<sup>a</sup> Christopher W. Schadt,<sup>a</sup> Leah H. Burdick,<sup>a</sup> Udaya C. Kalluri,<sup>a</sup> Dale A. Pelletier<sup>a</sup>

<sup>a</sup>Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

**ABSTRACT** A Gram-positive bacterium was isolated from the root of an eastern cottonwood tree (*Populus deltoides*) in Georgia and identified as a *Tumebacillus* species with 99% 16S rRNA nucleotide identity to *Tumebacillus avium*. The genome is 4.6 Mbp and encodes 4,072 proteins and 251 RNAs.

*Tumebacillus* is a genus of Gram-positive rod-forming bacteria that was only recently described in the phylum *Firmicutes* (1). Since its discovery, eight species from this genus have been isolated from diverse environments, such as permafrost (1), soil (2, 3), river water (4), algal scum (5), and the gut of a vulture (6). Here, we report the first draft genome of a *Tumebacillus* species associated with a plant root.

The roots from a *Populus deltoides* (genotype WV94) (7) tree growing on a nursery site in Bellville, Georgia, were sampled in 2018. Fine roots (<2 mm) were excised from freshly harvested root samples and processed as described previously (8, 9). Nonsterilized roots were macerated in 10 ml of MgSO<sub>4</sub> (10 mM) and serially diluted onto Reasoner's 2A (R2A) agar (10). Cultures were isolated through three rounds of restreaking onto R2A medium at 28°C. The isolate was inoculated into R2A liquid medium and grown for 2 days at 30°C. Genomic DNA was isolated with the Qiagen DNeasy blood and tissue kit according to the manufacturer's instructions. An initial identification was carried out using Sanger sequencing of the 16S rRNA amplicon of strain BK434 with primers 27F and 1492R (11). Based on an NCBI BLAST (12) search of the nonredundant/nucleotide database, the 16S rRNA amplicon of strain BK434 was found to have 99.15% nucleotide identity, across 1,414 bp, to *Tumebacillus avium*. Because the genus *Tumebacillus* has very few genome-sequenced representatives, we proceeded with genome sequencing to allow greater exploration of the genetic potential of this group. The draft genome of *Tumebacillus* was generated at the U.S. Department of Energy (DOE) Joint Genome Institute (JGI) using Illumina technology (13). A standard shotgun library was constructed; briefly, DNA was sheared by ultrasonication (Covaris, Woburn, MA) to a 600-bp average fragment size, and an Illumina shotgun library was prepared with the Kapa Biosystems library preparation kit (Roche, Wilmington, MA) and sequenced using the NovaSeq Xp reagent kit v1.0 with 2 × 151-bp reads on the Illumina NovaSeq platform, which generated 9,668,990 reads totaling 1,460,017,490 bp. Default parameters were used for all software unless otherwise specified. Raw Illumina reads were quality filtered to remove known sequencing artifacts and contaminants and read depth was normalized using BBTools (14). The filtered reads were assembled using SPAdes (v3.12.0) (phred-offset, 33; cov-cutoff, auto; t, 16; m, 64; careful; k, 25,55,95) (15), and for quality purposes, contigs of <1 kbp were discarded (BBTools reformat.sh: minlength). The final draft assembly contained 34 contigs (*L*<sub>50</sub>, 3; *N*<sub>50</sub>, 708,514 bp) in 33 scaffolds, totaling 4,646,936 bp with a GC content of 57.94%. The final assembly was based on 1,431,753,368 bp of Illumina data with a mapped coverage of 305.7×. The genome was annotated using the IMG Microbial Genome Annotation Pipeline (MGAP) (v4.16.4) (16). A total of 4,072 protein-coding genes were predicted, with 251 RNA

**Citation** Carper DL, Schadt CW, Burdick LH, Kalluri UC, Pelletier DA. 2020. Draft genome sequence of *Tumebacillus* sp. strain BK434, isolated from the roots of eastern cottonwood. *Microbiol Resour Announc* 9:e00351-20. <https://doi.org/10.1128/MRA.00351-20>.

**Editor** Kenneth M. Stedman, Portland State University

**Copyright** © 2020 Carper 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 Udaya C. Kalluri, kalluriudayc@ornl.gov, or Dale A. Pelletier, pelletierda@ornl.gov.

**Received** 3 April 2020

**Accepted** 7 May 2020

**Published** 28 May 2020

genes and 7 CRISPR arrays. Protein-coding regions included those involved in spore germination, which is common in *Firmicutes*, and in flagellar motion. Flagella have been characterized for some species of *Tumebacillus* (5, 17), including *Tumebacillus avium* (6), the strain that is 99.15% similar based on 16S rRNA findings.

**Data availability.** The assembly has been deposited in GenBank under accession number [SLXS00000000](#) and is also available from the IMG/M database under accession number [2795386103](#). Raw sequences have been deposited in the NCBI Sequence Read Archive under BioProject number [PRJNA520069](#) and run number [SRR8861549](#).

## ACKNOWLEDGMENTS

We thank Kerrie Barry and Nicole Shapiro (JGI) for project management and Tse-Yuan S. Lu (Oak Ridge National Laboratory) for help with strain isolation.

This manuscript has been authored by UT-Battelle, LLC, under contract DE-AC05-00OR22725 with the U.S. DOE. Part of this research was funded by the U.S. DOE Office of Biological and Environmental Research, Genomic Science Program, as part of the Plant Microbe Interfaces Scientific Focus Area (<http://pmi.ornl.gov>). The Bellville field site maintenance was funded by the BioEnergy Science Center project. The BioEnergy Science Center is a U.S. DOE Bioenergy Research Center supported by the Office of Biological and Environmental Research in the U.S. DOE Office of Science. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. DOE under contract DE-AC05-00OR22725. The work conducted by the U.S. DOE JGI, a DOE Office of Science User Facility, is supported under contract DE-AC02-05CH11231. The data were generated for JGI proposal 503495 under U.C.K.

## REFERENCES

- Steven B, Chen MQ, Greer CW, Whyte LG, Niederberger TD. 2008. *Tumebacillus permanentifrigoris* gen. nov., sp. nov., an aerobic, spore-forming bacterium isolated from Canadian high Arctic permafrost. Int J Syst Evol Microbiol 58:1497–1501. <https://doi.org/10.1099/ijs.0.65101-0>.
- Kim JH, Kim W. 2016. *Tumebacillus soli* sp. nov., isolated from non-rhizosphere soil. Int J Syst Evol Microbiol 66:2192–2197. <https://doi.org/10.1099/ijs.0.001009>.
- Her J, Srinivasan S, Lee SS. 2015. *Tumebacillus luteolus* sp. nov., isolated from soil. Int J Syst Evol Microbiol 65:4107–4112. <https://doi.org/10.1099/ijs.0.000549>.
- Prasad RV, Bhumika V, Anil Kumar P, Srinivas N. 2015. *Tumebacillus lipolyticus* sp. nov., isolated from river water. Int J Syst Evol Microbiol 65:4363–4368. <https://doi.org/10.1099/ijs.0.000576>.
- Wu YF, Zhang B, Xing P, Wu QL, Liu SJ. 2015. *Tumebacillus algifaecis* sp. nov., isolated from decomposing algal scum. Int J Syst Evol Microbiol 65:2194–2198. <https://doi.org/10.1099/ijs.0.000240>.
- Sung H, Kim HS, Lee JY, Kang W, Kim PS, Hyun DW, Tak EJ, Jung MJ, Yun JH, Kim MS, Shin NR, Whon TW, Rho JR, Park SD, Shim HE, Bae JW. 2018. *Tumebacillus avium* sp. nov., isolated from the gut of a cinereous vulture, *Aegypius monachus*. Int J Syst Evol Microbiol 68:1659–1664. <https://doi.org/10.1099/ijs.0.002725>.
- Coyle DR, Coleman MD, Durant JA, Newman LA. 2006. Survival and growth of 31 *Populus* clones in South Carolina. Biomass Bioenergy 30:750–758. <https://doi.org/10.1016/j.biombioe.2005.08.005>.
- Gottel NR, Castro HF, Kerley M, Yang Z, Pelletier DA, Podar M, Karpinets T, Uberbacher E, Tuskan GA, Vilgalys R, Doktycz MJ, Schadt CW. 2011. Distinct microbial communities within the endosphere and rhizosphere of *Populus deltoides* roots across contrasting soil types. Appl Environ Microbiol 77:5934–5944. <https://doi.org/10.1128/AEM.05255-11>.
- Weston DJ, Pelletier DA, Morrell-Falvey JL, Tschaplinski TJ, Jawdy SS, Lu TY, Allen SM, Melton SJ, Martin MZ, Schadt CW, Karve AA, Chen JG, Yang X, Doktycz MJ, Tuskan GA. 2012. *Pseudomonas fluorescens* induces strain-dependent and strain-independent host plant responses in defense networks, primary metabolism, photosynthesis, and fitness. Mol Plant Microbe Interact 25:765–778. <https://doi.org/10.1094/MPMI-09-11-0253>.
- Reasoner DJ, Geldreich EE. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol 49:1–7. <https://doi.org/10.1128/AEM.49.1.1-7.1985>.
- Utturkar SM, Cude WN, Robeson MS, Yang ZK, Klingeman DM, Land ML, Allman SL, Lu T-Y, Brown SD, Schadt CW, Podar M, Doktycz MJ, Pelletier DA. 2016. Enrichment of root endophytic bacteria from *Populus deltoides* and single-cell-genomics analysis. Appl Environ Microbiol 82:5698–5708. <https://doi.org/10.1128/AEM.01285-16>.
- 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).
- Bennett S. 2004. Solexa Ltd. Pharmacogenomics 5:433–438. <https://doi.org/10.1517/14622416.5.4.433>.
- Bushnell B. BBTools software package. <http://bbtools.jgi.doe.gov>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin 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>.
- Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen IMA, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The standard operating procedure of the DOE-JGI Microbial Genome Annotation Pipeline (MGAP v.4). Stand Genomic Sci 10:86. <https://doi.org/10.1186/s40793-015-0077-y>.
- Wang QY, Xie NZ, Huang YY, Song LF, Du QS, Yu B, Chen D, Huang RB. 2014. Genome sequence of *Tumebacillus flagellatus* GST4, the first genome sequence of a species in the genus *Tumebacillus*. Genome Announc 2:e01189-14. <https://doi.org/10.1128/genomeA.01189-14>.