



Draft Genome Sequence for *Klebsiella michiganensis* B199A, Originally Identified as *Enterobacter aerogenes*

Matthew J. Igo,^{a*}  Donald W. Schaffner^a

^aDepartment of Food Science, Rutgers University, New Brunswick, New Jersey, USA

ABSTRACT Here, we report the draft genome sequence of a strain of *Klebsiella michiganensis* originally identified as *Enterobacter aerogenes* B199A. This strain has been used as a *Salmonella* surrogate to study the effectiveness of handwashing and measure cross-contamination to and from a wide variety of surfaces and foods.

We report the draft genome sequence of *Klebsiella michiganensis* strain B199A originally identified as *Enterobacter aerogenes*. B199A was first used to study microbial cross-contamination in the kitchen (1) and originally obtained from Vivolac Cultures, Greenfield, Indiana. Commercial use was for fermenting egg white prior to drying to reduce Maillard browning (2), and the original source was likely naturally fermented egg whites (3). Ribotyping of B199A by Vivolac in 2017 identified it as *Klebsiella pneumoniae*, and they discontinued its sale (personal communication was from WD Sing). We have used this culture to study handwashing (4), cross-contamination to and from hands (5) and surfaces (6), and survival on different surfaces (7).

B199A was revived from our culture collection on tryptic soy agar with nalidixic acid (TSA-na) (Fisher Scientific, Waltham, MA) from -80°C and incubated at 37°C for 24 h. A single colony was transferred into 10 mL of TSB-na (Fisher Scientific) for 24 h at 37°C . Genomic DNA was extracted using the Promega (Madison, WI) Wizard DNA extraction kit (<https://www.promega.com/resources/protocols/technical-manuals/0/wizard-genomic-dna-purification-kit-protocol/>). Extracted DNA was refrigerated and sent for sequencing (Genewiz, South Plainfield, NJ). Results were returned as raw FASTQ files. Illumina MiSeq 2 × 150-bp sequencing was used for the sequencing with coverage depth set to 50× using pre-existing *Enterobacter* data. An analysis was conducted on GalaxyTrkr with default parameters unless otherwise stated. Trimmomatic v. 0.38 (8) using the SLIDINGWINDOW default parameters removed adapter sequences. Sequence reads were *de novo* assembled using SPAdes v. 3.12.0 (9) with k-mers autodetected as 31, 45, 59, 73, and 87. The average read length of the sequence was 120 bp. QUAST v. 5.0.2 (10) determined statistics on the assembled genome. AMRFinder v. 3.8.28 (11) determined antimicrobial resistant genes. FastANI v. 1.33 (12) analysis was performed against *Enterobacteriaceae* until it was determined that the strain was *K. michiganensis* based on an average nucleotide identity (ANI) value of 98.7. This result was confirmed upon deposit to GenBank. *K. michiganensis* B199A had a sequence length of 6,117,020 bp, with trimmed reads containing an average of 120 bp and a total read count of 7,034,327 for a coverage of 138× with a GC content of 55.65%. The genome contained 72 contigs with an N_{50} of 212,765 bp. Antibiotic-resistant genes include a class A extended-spectrum beta-lactamase, OXY-1-1, and an aminoglycoside O-phosphotransferase, *aph(3')-Ia*. Assembled sequences were annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) v. 6.0.

Data availability. The sequences were deposited in GenBank under SRA accession number [SRR18059839](https://www.ncbi.nlm.nih.gov/sra/SRR18059839) and BioProject [PRJNA807804](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA807804). This whole-genome shotgun project was deposited at DDBJ/ENA/GenBank under the accession [JAKSGB000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAKSGB000000000).

Editor David Rasko, University of Maryland School of Medicine

Copyright © 2022 Igo and Schaffner. 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 Donald W. Schaffner, don.schaffner@rutgers.edu.

*Present address: Matthew J. Igo, US Food and Drug Administration, Center for Drug Evaluation and Research, Silver Spring, Maryland, USA.

The authors declare no conflict of interest.

Received 29 March 2022

Accepted 3 May 2022

Published 18 May 2022

ACKNOWLEDGMENTS

We thank Michael Doyle, Regents Professor of Food Microbiology (Retired), University of Georgia, for providing the original culture. We also thank Wesley D. Sing, Chief Executive Officer, Vivolac Cultures Corporation, Greenfield Indiana, for background on the history of the original culture.

REFERENCES

1. Zhao P, Zhao T, Doyle M, Rubino J, Meng J. 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. *J Food Prot* 61:960–963. <https://doi.org/10.4315/0362-028X-61.8.960>.
2. Stuart LS, Goresline HE. 1942. Bacteriological studies on the “natural” fermentation process of preparing egg white for drying. *J Bacteriol* 44:541–549. <https://doi.org/10.1128/jb.44.5.541-549.1942>.
3. Stuart L, Goresline HE. 1942. Studies of bacteria from fermenting egg white and the production of pure culture fermentations. *J Bacteriol* 44:625–632. <https://doi.org/10.1128/jb.44.6.625-632.1942>.
4. Jensen DA, Danyluk MD, Harris LJ, Schaffner DW. 2015. Quantifying the effect of hand wash duration, soap use, ground beef debris, and drying methods on the removal of *Enterobacter aerogenes* on hands. *J Food Prot* 78:685–690. <https://doi.org/10.4315/0362-028X.JFP-14-245>.
5. Jensen DA, Danyluk MD, Harris LJ, Schaffner DW. 2017. Quantifying bacterial cross-contamination rates between fresh-cut produce and hands. *J Food Prot* 80:213–219. <https://doi.org/10.4315/0362-028X.JFP-16-240>.
6. Miranda RC, Schaffner DW. 2016. Longer contact times increase cross-contamination of *Enterobacter aerogenes* from surfaces to food. *Appl Environ Microbiol* 82:6490–6496. <https://doi.org/10.1128/AEM.01838-16>.
7. Igo MJ, Schaffner DW. 2019. Quantifying the influence of relative humidity, temperature, and diluent on the survival and growth of *Enterobacter aerogenes*. *J Food Prot* 82:2135–2147. <https://doi.org/10.4315/0362-028X.JFP-19-261>.
8. 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>.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyskhin 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>.
10. 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>.
11. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu C-H, McDermott PF. 2019. Using the NCBI AMRFinder tool to determine antimicrobial resistance genotype-phenotype correlations within a collection of NARMS isolates. *bioRxiv* <https://doi.org/10.1101/550707>.
12. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 9:5114. <https://doi.org/10.1038/s41467-018-07641-9>.