AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

# Complete Genome Sequence of *Enterobacter asburiae* Strain AEB30, Determined Using Illumina and PacBio Sequencing

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**ABSTRACT** The complete genome sequence of *Enterobacter asburiae* strain AEB30 is presented. The strain was isolated from store-bought ginger in Albany, CA, in 2016.

*Enterobacter asburiae* is one of six species in the *Enterobacter cloacae* complex (1) discovered in clinical specimens in 1986 (2) and has been shown to inhibit the growth of *Escherichia coli* O157:H7 and *Salmonella enterica* on sprouting seeds (3, 4) and tomatoes (5). *E. asburiae* strain AEB30 was isolated from store-bought ginger in Albany, CA, in 2016, as described (6). A single colony was cultured in tryptic soy broth (Oxoid, Basingstoke, England) at 37°C for 24 h. *E. asburiae* AEB30 genomic DNA was extracted from 100 ml of an overnight culture by sucrose-Tris with phenol-chloroform cleanup extractions (7).

A PacBio SMRTbell library was prepared from 10 $\mu$g of bacterial genomic DNA fragmented using a g-TUBE device (Covaris, Woburn, MA), following the standard PacBio 20-kb library preparation procedure (8) but with 1× AMPure beads (PacBio) cleanup and an extra DNA repair step after BluePippin size selection with 0.75% DF marker S1 high-pass 6 to 10-kb v3 cassette (Sage Science, Beverly, MA). Each sample was run in one single-molecule real-time (SMRT) cell with the 0.1 nM on-plate concentration, P6/C4 sequencing chemistry, MagBead "One Cell Per Well" v1 collection protocol, and 360-min data collection mode. The Illumina library was prepared from 1.5 $\mu$g of bacterial genomic DNA fragmented using a microtube (Covaris) to 700- to 770-bp fragments at 30 lb/in$^2$ for 40 s following the manufacturer's protocol for the LTP library preparation kit (KAPA Biosystems, Wilmington, MA) (9). Sequencing was performed using a 2 × 250-cycle, paired-end format and the v2 reagent kit on a MiSeq instrument (Illumina, San Diego, CA). The PacBio RS II platform produced 86,804 total reads, of which 81,187 were used for assembly according to the RS Hierarchical Genome Assembly Process (HGAP) v3.0 in SMRT Analysis v2.2.0 (Pacific Biosciences, Menlo Park, CA). PacBio DNA internal control complex P6 was used as an internal sequencing control, and the read quality control was conducted using FastQC (Pacific Biosciences). The PacBio assembly resulted in one chromosomal contig. The Illumina MiSeq platform yielded 2,226,178 total reads, 2,051,277 of which were trimmed using a quality score threshold of 30 or higher (Q30) and assembled with the PacBio chromosomal contig within Geneious Prime v2020.0.4 (Biomatters, Ltd., Auckland, New Zealand). Single nucleotide polymorphisms (SNPs) between the PacBio assembly and the MiSeq reads were addressed using the Annotate and Predict/Find SNPs module (minimum coverage = 50; minimum variant frequency = 0.8). The genes encoding proteins, rRNAs, and tRNAs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4.10 (10), with additional manual annotation based on the genome sequence of *Enterobacter cloacae* complex strain 35734 (GenBank accession number CP012162).

The whole genome of *E. asburiae* strain AEB30 consists of a single circular chromosome of 4,748,641 bp (mean coverage, 261.5×, combined from PacBio and Illumina platforms; GC content, 55.8%). It is predicted to have 4,363 coding sequences (CDS), 8 rRNA operons, and 83 tRNAs. There are 1 intact, 1 questionable, and 1 incomplete prophage regions in the

**Citation** Tran TD, Hnasko R, Huynh S, Parker CT, Gorski L, McGarvey JA. 2021. Complete genome sequence of *Enterobacter asburiae* strain AEB30, determined using Illumina and PacBio sequencing. Microbiol Resour Announc 10:e00562-21. https://doi.org/10.1128/MRA.00562-21.

**Editor** Catherine Putonti, Loyola University Chicago



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**Received** 8 June 2021
**Accepted** 16 July 2021
**Published** 5 August 2021

chromosome of *E. asburiae* AEB30, as indicated by PHASTER in March 2020 (http://phaster.ca) (11, 12). Insertion sequences (IS) in the genome of *E. asburiae* AEB30 were determined using ISfinder (https://www-is.biotoul.fr/) (13), and 165 IS were found (E value $<$ 10).

**Data availability.** This whole-genome sequence has been deposited in DDBJ/ENA/GenBank under accession number CP046618.1, BioProject accession number PRJNA594005, and BioSample accession number SAMN13499302. The Illumina and PacBio raw data are available via the SRA under accession numbers SRR11188287 and SRR10600436, respectively.

## ACKNOWLEDGMENT

This work was supported by the United States Department of Agriculture, Agricultural Research Service, National Program 108: Food Safety (animal and plant products).

## REFERENCES

1. Mezzatesta ML, Gona F, Stefani S. 2012. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiol 7:887–902. https://doi.org/10.2217/fmb.12.61.
2. Brenner DJ, McWhorter AC, Kai A, Steigerwalt AG, Farmer JJ, III. 1986. *Enterobacter asburiae* sp. nov., a new species found in clinical specimens, and reassignment of *Erwinia dissolvens* and *Erwinia nimipressuralis* to the genus *Enterobacter* as *Enterobacter dissolvens* comb. nov. and *Enterobacter nimipressuralis* comb. nov. J Clin Microbiol 23:1114–1120. https://doi.org/10.1128/jcm.23.6.1114-1120.1986.
3. Cooley MB, Miller WG, Mandrell RE. 2003. Colonization of *Arabidopsis thaliana* with *Salmonella enterica* and enterohemorrhagic *Escherichia coli* O157:H7 and competition by *Enterobacter asburiae*. Appl Environ Microbiol 69:4915–4926. https://doi.org/10.1128/AEM.69.8.4915-4926.2003.
4. Ye J, Kostrzynska M, Dunfield K, Warriner K. 2010. Control of *Salmonella* on sprouting mung bean and alfalfa seeds by using a biocontrol preparation based on antagonistic bacteria and lytic bacteriophages. J Food Prot 73:9–17. https://doi.org/10.4315/0362-028x-73.1.9.
5. Ye J, Kostrzynska M, Dunfield K, Warriner K. 2009. Evaluation of a biocontrol preparation consisting of *Enterobacter asburiae* JX1 and a lytic bacteriophage cocktail to suppress the growth of *Salmonella javiana* associated with tomatoes. J Food Prot 72:2284–2292. https://doi.org/10.4315/0362-028x-72.11.2284.
6. McGarvey JA, Tran TD, Hnasko R, Gorski L. 2019. Use of phyllosphere-associated lactic acid bacteria as biocontrol agents to reduce *Salmonella enterica* serovar Poona growth on cantaloupe melons. J Food Prot 82:2148–2153. https://doi.org/10.4315/0362-028X.JFP-19-246.
7. Miller WG, On SLW, Wang G, Fontanoz S, Lastovica AJ, Mandrell RE. 2005. Extended multilocus sequence typing system for *Campylobacter coli*, *C. lari*, *C. upsaliensis*, and *C. helveticus*. J Clin Microbiol 43:2315–2329. https://doi.org/10.1128/JCM.43.5.2315-2329.2005.
8. PacBio. 2015. Procedure and checklist: 20 kb template preparation using BluePippin size-selection system.
9. Parker CT, Cooper KK, Huynh S, Smith TP, Bono JL, Cooley M. 2018. Genome sequences of eight Shiga toxin-producing *Escherichia coli* strains isolated from a produce-growing region in California. Microbiol Resour Announc 7:e00807-18. https://doi.org/10.1128/MRA.00807-18.
10. 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.
11. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. Nucleic Acids Res 39:W347–W352. https://doi.org/10.1093/nar/gkr485.
12. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.
13. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. 2006. ISfinder: the reference centre for bacterial insertion sequences. Nucleic Acids Res 34:D32–D36. https://doi.org/10.1093/nar/gkj014.