AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Genome Sequence of the Early 20th-Century Extreme Halophile *Halobacterium* sp. Strain NRC-34001

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ABSTRACT *Halobacterium* sp. strain NRC-34001 is a red, extremely halophilic archaeon isolated in Canada in 1934. Single-molecule real-time sequencing revealed a 2.3-Mbp genome with a 2-Mbp chromosome and two plasmids (235 kb and 43 kb). The genome encodes all conserved core haloarchaeal groups (cHOGs) and a highly acidic proteome.

The red haloarchaeon *Halobacterium* sp. strain NRC-34001 was originally isolated as "*Serratia cutirubra*" (subsequently, *Pseudomonas*/"*Halobacter*"/*Halobacterium cutirubrum*) from Canadian salted buffalo hide by A. G. Lochhead (1890 to 1980) while investigating damaging red stains on the flesh side of salted hide ("red heat"), similar to fish spoilage (1–5). It was vital to the discovery of classical haloarchaeal traits such as hypotonic lysis, spheroplast formation, high-GC content genome, compartmentalization, and endemic halophage infection, all prior to the classification of *Halobacterium* species as members of the Archaea in the third domain of life (6–10).

The strain in this study, obtained from the American Type Culture Collection (ATCC 33170), was grown in CM^+ (complete medium plus trace metals) at 37°C with shaking at 220 rpm (New Brunswick Innova 4230 instrument; New Brunswick, NJ, USA), as previously described (11, 12). Cells were lysed by osmotic shock, and nucleic acids were isolated using standard phenol extraction-based methods (12–15).

Single-molecule real-time (SMRT) sequencing was performed using the PacBio Sequel platform (Menlo Park, CA). A SMRTbell sequencing library was prepared from 3.9 μg genomic DNA, without further shearing, using the SMRTbell Express template prep kit 2.0. The library was purified by removing SMRTbell reads of $<$15 kb using the BluePippin size selection system (Sage Science, Beverly, MA) and sequenced on a single SMRT cell (Sequel Binding kit 3.0, Sequel Sequencing Plate 3.0) with a 20-h collection time. Sequencing reads were filtered and assembled *de novo* using the Microbial Assembly pipeline (SMRT Link 9.0.0.92188), run with default parameters. A total of 28,995 subreads aligned to the draft assembly, with a mean subread length of 15,890 bp, and the polished assembly resolved automatically into three circular contigs, with 200$\times$ mean coverage. The GC-rich genome was found to consist of a large circular chromosome (2,012,898 bp; GC content, 67.9%) and 2 plasmids, pHcu_235 (235,323 bp; GC content, 59.8%) and pHcu_43 (42,817 bp, GC content, 57.8%).

Genome annotation was performed at NCBI using the Prokaryotic Genome Annotation Pipeline (PGAP) build 3190, and the taxonomy was determined by NCBI Taxonomy (16, 17). The genome includes 1 rRNA operon and 47 tRNA genes and encodes 2,341 proteins with a calculated mean isoelectric point (pI) value of 4.9, a highly acidic proteome characteristic of haloarchaea (18–20). Based on additional in-house analysis using GeneMark.hmm 2, FeatureExtract, Sequence Manipulation Suite, and EMBOSS (21–24), all 799 conserved core haloarchaeal groups (cHOGs), 6 Orc/Cdc6 proteins, 3 TATA-binding proteins, and 4 TFB proteins (25–28) were found, as well as genes coding for bacteriorhodopsin, halorhodopsin, and sensory rhodopsins 1 and 2 (29, 30). The genome also includes a complete buoyant gas vesicle

Editor Kenneth M. Stedman, Portland State University

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The authors declare a conflict of interest.

Received 7 December 2021
Accepted 13 December 2021
Published 13 January 2022

nanoparticle (GVNP) gene cluster, although the strain has not been shown to produce GVNPs (31, 32).

Methylation patterns were determined using the Base Modification and Motif Analysis application within the same SMRT Link environment using default settings (minimum Qmod score = 100). A single methylated DNA motif was identified (^{m4}CTAG, the product of M.Hsp34001I, which is commonly found in *Halobacterium* species) (33).

Data availability. The *Halobacterium* sp. NRC-34001 genome sequence has been deposited at GenBank (accession numbers CP085884.1, CP085882.1, CP085883.1). The raw data are available in the NCBI Sequence Read Archive under the accession number SRR16600243 and the BioProject accession number PRJNA412908.

ACKNOWLEDGMENTS

Work in the DasSarma laboratory is supported by NASA Exobiology grant 80NSSC17K0263 and NIH grant AI139808. We thank Joan Slonczewski, at Kenyon College, Gambier, OH, whose students (J.M.G. and K.S.K.) were supported in the DasSarma laboratory through a NASA Astrobiology Program Early Career Collaboration Award.

B.P.A. and R.J.R. work for New England Biolabs, a company that sells research reagents, including restriction enzymes and DNA methyltransferases, to the scientific community.

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