

Genome Sequence of *Bibersteinia trehalosi* Strain Y31 Isolated from the Pneumonic Lung of a Bighorn Sheep

Abirami Kugadas,^{a*} Jodi L. Humann,^b Sebastián Aguilar Pierlé,^{a*} Subramaniam Srikumaran,^a Kelly A. Brayton^a

Program in Genomics, Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, Washington, USA^a; Department of Horticulture, Washington State University, Pullman, Washington, USA^b

* Present address: Abirami Kugadas, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; Sebastián Aguilar Pierlé, Institut Pasteur, Paris, France.

Here, we report the genome sequence for *Bibersteinia trehalosi* strain Y31, isolated from the lungs of a bighorn sheep (*Ovis canadensis*) that had succumbed to pneumonia, which exhibits proximity-dependent inhibition (PDI) of *Mannheimia haemolytica*. The sequence will be used to understand the mechanism of PDI for these organisms.

Received 1 June 2016 Accepted 3 June 2016 Published 21 July 2016

Citation Kugadas A, Humann JL, Pierlé SA, Srikumaran S, Brayton KA. 2016. Genome sequence of *Bibersteinia trehalosi* strain Y31 isolated from the pneumonic lung of a bighorn sheep. *Genome Announc* 4(4):e00722-16. doi:10.1128/genomeA.00722-16.

Copyright © 2016 Kugadas 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 Kelly A. Brayton, kbrayton@vetmed.wsu.edu.

The gammaproteobacterial species *Pasteurella haemolytica* was subdivided into biotypes A and T based on the ability to ferment either arabinose or trehalose. In 1990, the T biotypes were reclassified and named *Pasteurella trehalosi* (1). In a recent reorganization, *P. trehalosi* was once again reclassified and given the name *Bibersteinia trehalosi*, in honor of Ernst Biberstein, who did much of the characterization work on the organism (2). *Bibersteinia trehalosi* is a Gram-negative, nonmotile, and rod-shaped or pleomorphic bacterium that is associated with pneumonia in bighorn sheep (BHS) (3). *Mannheimia haemolytica* strains that produce leukotoxin consistently cause fatal pneumonia in BHS under experimental conditions, but surprisingly, *M. haemolytica* is isolated less frequently than *B. trehalosi* from pneumonic lung tissue. We previously reported that a broad range of *B. trehalosi* strains are able to inhibit the growth of *M. haemolytica* in a proximity-dependent inhibition (PDI) manner, and this inhibition contributes to the infrequent detection of *M. haemolytica* from pneumonic lungs (4, 5). To identify the genomic component that provides this inhibitory effect, we selected an inhibitory strain of *B. trehalosi*, Y31, isolated from the pneumonic lung of a BHS (4), for complete genome sequencing.

B. trehalosi Y31 was grown in brain heart infusion medium (Remel, Lenexa, KS), and genomic DNA was isolated using the QIAamp DNA minikit (Qiagen, Valencia, CA). The genome was sequenced on a PacBio RS instrument (Pacific Biosciences, Menlo Park, CA) using single-molecule real-time (SMRT) sequencing technology (6), yielding 86× coverage. The sequences from 12 SMRT cells were assembled with SMRT Pipe version 2.0 (Pacific Biosciences) using the HGAP protocol. The assembly yielded 17 contigs, with an N_{50} length of 351,951 bases. Optical mapping (OpGen, Gaithersburg, MD) was used to order the contigs, and gap-spanning PCR was used to link the contigs. Finally, two contigs were produced that could not be definitively connected; however, optical mapping suggests the gaps are 2,872 and 31,034 bp. The genome sequence includes 2,334,734 bp. The G+C content was 41%. The genome sequence was subjected to autoannotation

at NCBI using the Prokaryotic Genome Annotation Pipeline (7) and yielded 2,240 genes, 5 rRNA operons, 56 tRNA genes, and one noncoding RNA (ncRNA).

PDI is often effected by a bacteriocin that may be encoded on a plasmid or on the chromosome (8). This strain contained no plasmids; thus, the gene(s) responsible for PDI is chromosomally located. In addition to the effector molecule, a transporter protein would be necessary to secrete/transport the effector to the target cell. We examined the genome for transporters and detected 274, with most belonging to the ABC superfamily of transport proteins.

Nucleotide sequence accession number. The genome sequence has been deposited at GenBank under accession no. [JACI000000000](https://www.ncbi.nlm.nih.gov/nuccore/JACI000000000).

ACKNOWLEDGMENTS

We thank Mark Wildung and Derek Pouchnik for expert advice and assistance with sequencing.

FUNDING INFORMATION

This work, including the efforts of Subramaniam Srikumaran, was funded by Wild Sheep Foundation (WSF#12-18) and its State Chapters, and the United States Forest Service. This work, including the efforts of Abirami Kugadas, was funded by Morris Animal Foundation (MAF) (D13ZO-418).

REFERENCES

1. Sneath PH, Stevens M. 1990. *Actinobacillus rossii* sp. nov., *Actinobacillus seminis* sp. nov., nom. rev., *Pasteurella bettii* sp. nov., *Pasteurella lymphangitidis* sp. nov., *Pasteurella mairi* sp. nov., and *Pasteurella trehalosi* sp. nov. *Int J Syst Bacteriol* 40:148–153. <http://dx.doi.org/10.1099/00207713-40-2-148>.
2. Blackall PJ, Bojesen AM, Christensen H, Bisgaard M. 2007. Reclassification of [*Pasteurella*] *trehalosi* as *Bibersteinia trehalosi* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 57:666–674. <http://dx.doi.org/10.1099/ijs.0.64521-0>.
3. Gilmour NJL, Gilmour JS. 1989. Pasteurellosis of sheep, p 223–261. In Adlam CF, Rutter JM (ed), *Pasteurella* and Pasteurellosis. Academic Press, London, United Kingdom.

4. Dassanayake RP, Call DR, Sawant AA, Casavant NC, Weiser GC, Knowles DP, Srikumaran S. 2010. *Bibersteinia trehalosi* inhibits the growth of *Mannheimia haemolytica* by a proximity-dependent mechanism. *Appl Environ Microbiol* 76:1008–1013. <http://dx.doi.org/10.1128/AEM.02086-09>.
5. Kugadas A, Poindexter J, Lee ML, Bavananthasivam J, Call DR, Brayton KA, Srikumaran S. 2014. Growth of *Mannheimia haemolytica*: inhibitory agents and putative mechanism of inhibition. *Vet Microbiol* 174:155–162. <http://dx.doi.org/10.1016/j.vetmic.2014.08.003>.
6. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, Peluso P, Rank D, Baybayan P, Bettman B, Bibillo A, Bjornson K, Chaudhuri B, Christians F, Cicero R, Clark S, Dalal R, Dewinter A, Dixon J, Foquet M, Gaertner A, Hardenbol P, Heiner C, Hester K, Holden D, Kearns G, Kong X, Kuse R, Lacroix Y, Lin S, Lundquist P, Ma C, Marks P, Maxham M, Murphy D, Park I, Pham T, Phillips M, Roy J, Sebra R, Shen G, Sorenson J, Tomaney A, Travers K, Trulson M, Vieceli J, Wegener J, Wu D, Yang A, Zaccarin D. 2009. Real-time DNA sequencing from single polymerase molecules. *Science* 323:133–138. <http://dx.doi.org/10.1126/science.1162986>.
7. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of Standard Operating Procedures (SOPs) for (meta)genomic annotation. *Omics* 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
8. Eberhart LJ, Deringer JR, Brayton KA, Sawant AA, Besser TE, Call DR. 2012. Characterization of a novel microcin that kills enterohemorrhagic *Escherichia coli* O157:H7 and O26. *Appl Environ Microbiol* 78:6592–6599. <http://dx.doi.org/10.1128/AEM.01067-12>.