



Draft Genome Sequences of Two Bacillus anthracis Strains from Etosha National Park, Namibia

Karoline Valseth,^{a,b} Camilla L. Nesbø,^{a,c} W. Ryan Easterday,^a Wendy C. Turner,^d Jaran S. Olsen,^b Nils C. Stenseth,^a ^(D) Thomas H. A. Haverkamp^a

Centre for Ecological and Evolutionary Synthesis (CEES), Department of Biosciences, University of Oslo, Blindern, Oslo, Norway^a; Norwegian Defence Research Establishment, Kjeller, Norway^b; Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada^c; State University of New York, Albany, New York, USA^d

Bacillus anthracis strains K1 and K2 were isolated from two plains zebra anthrax carcasses in Etosha National Park, Namibia. These are draft genomes obtained by Illumina MiSeq sequencing of isolates collected from culture of blood-soaked soil from each carcass.

Received 24 June 2016 Accepted 29 June 2016 Published 25 August 2016

Citation Valseth K, Nesbø CL, Easterday WR, Turner WC, Olsen JS, Stenseth NC, Haverkamp THA. 2016. Draft genome sequences of two *Bacillus anthracis* strains from Etosha National Park, Namibia. Genome Announc 4(4):e00861-16. doi:10.1128/genomeA.00861-16.

Copyright © 2016 Valseth et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Thomas H. A. Haverkamp, thhaverk@ibv.uio.no.

B*acillus anthracis* is a Gram-positive, rod-shaped bacterium which sporulates to survive the transmission between hosts (1). It belongs to the genetically similar *B. cereus* group, and is the causative agent of anthrax (2). Anthrax is endemic to Etosha National Park, Namibia, which has yearly outbreaks of the mortal infection arising from spore-contaminated soil of carcass sites from previous years (3).

Two B. anthracis isolates were obtained by culturing serial dilutions on polymyxin-lysozyme-EDTA-thallous acetate (PLET) agar culture medium following previously described protocols (4, 5) from contaminated soil beneath two plains zebra (Equus quagga) carcasses (Ca1 and Ca2) (located on 3 March 2014coordinates, Ca1: S19.031/E015.548; Ca2: S19.037/E015.553). One colony per carcass was picked from the PLET plates and regrown as a lawn on a fresh PLET plate, then colonies were scraped of the PLET agar with a spatula and dispensed in 300 μ L phosphate buffered saline (PBS) buffer (1 tablet from Sigma-Aldrich, USA, dissolved in 200 mL dH₂O, autoclaved at 121°C for 20 min) before isolation. DNA isolation was performed using a FastDNA spin kit for soil (MP Biomedicals, CA, USA) following the manufacturer's protocol with these alterations: tubes were homogenized with a MiniBead Beater, model 607 (BioSpec Products, OK, USA) for 80 s at 3,450 oscillations/min, before being centrifuged at 14,000 rpm (rpm) for 10 min. Six hundred fifty μ L of the supernatant was removed in step 10 in protocol. In step 13 of the protocol, spin filters were centrifuged for 3 min without emptying the catch tube. DES buffer was heated to 55°C in Dri-Bath, type 17600 (Thermolyn, IA, USA) before addition. Then, tubes were put on a heat block for 5 min at 55°C. Finally, the DNA extracts were sterilized using an Ultrafree Durapore PVDF 0.1 μ M spin filter (Millipore, Darmstadt, Germany) by centrifugation at 11,000 rpm for 3 min.

The bacterial DNA isolates were whole-genome sequenced at the Norwegian Sequencing Centre (NSC) using the TruSeq Nano reagents (Illumina, Inc., San Diego, CA, USA) and Illumina MiSeq with the following parameters: paired-end, insert size of 500 bp and a read length of 300 bp. Sequences were cleaned with AdapterRemoval 1.5.4 (6) and assembled in CLC workbench v8 using the AdapterRemoval 1.5.4 output files for singleton, pair1, and pair2. The genomes were annotated using the NCBI PGAP pipeline. The sequenced *B. anthracis* isolates from Ca1 and Ca2 were named K1 and K2, respectively, after the Norwegian word for carcass, "kadaver." The K1 draft genome consisted of 38 contigs covering of 5,456,561 bp, with a G+C content of 35.1%, 5,837 genes, and 5,549 coding sequences (CDSs). The K2 draft genome is very similar with 38 contigs covering 5,461,141 bp, G+C content of 35.1%, 5,856 genes, and 5,549 CDSs.

Accession number(s). Both whole-genome shotgun projects have been deposited in DDBJ/ENA/GenBank under the accession no. LBBZ00000000 (K1) and LBCA00000000 (K2).

ACKNOWLEDGMENTS

We thank the Ministry of Environment and Tourism (MET) in Namibia for permission to conduct this research (MET research permit 1857/2013); Mari Espelund for help and input for optimization of DNA extractions from soil; Claudine C. Cloete and Zoe Barandongo for help with sample collecting and DNA extractions; the Etosha Ecological Institute for providing laboratory facilities; and Institutt for Biovitenskap (IBV) student funding at University of Oslo (UiO) for providing travel money.

This work was supported by a Research Council of Norway award (project 180444/V40) to C.L.N. and NSF OISE-1103054 to W.C.T.

FUNDING INFORMATION

This work, including the efforts of Camilla L. Nesbø, was funded by Research Council of Norway (180444/V40). This work, including the efforts of Wendy C. Turner, was funded by National Science Foundation (NSF) (OISE-1103054).

This work was supported by a Research Council of Norway award (project no. 180444/V40) to C.L.N. and NSF OISE-1103054 to W.C.T.

REFERENCES

- Sharp RJ, Roberts AG. 2006. Anthrax: the challenges for decontamination. J Chem Technol Biotechnol 81:1612–1625. http://dx.doi.org/10.1002/ jctb.1591.
- Helgason E, Økstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolstø A-B. 2000. Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis—one species on the basis of genetic evidence. Appl Environ Microbiol 66:2627–2630. http://dx.doi.org/10.1128/AEM.66.6.2627 -2630.2000.
- 3. Turner WC, Kausrud KL, Beyer W, Easterday WR, Barandongo ZR, Blaschke E, Cloete CC, Lazak J, Van Ert MN, Ganz HH, Turnbull PC, Stenseth NC, Getz WM. 2016. Lethal exposure: an integrated approach to

pathogen transmission via environmental reservoirs. Sci Rep 6:27311. http://dx.doi.org/10.1038/srep27311.

- 4. OIE 2008. Anthrax in humans and animals, 4th ed. World Organisation for Animal Health, Paris, France: http://www.who.int/csr/resources /publications/anthrax_webs.pdf
- Turner WC, Kausrud KL, Krishnappa YS, Cromsigt JP, Ganz HH, Mapaure I, Cloete CC, Havarua Z, Küsters M, Getz WM, Stenseth NC. 2014. Fatal attraction: vegetation responses to nutrient inputs attract herbivores to infectious anthrax carcass sites. Proc R Soc Lond B Biol Sci 281:20141785. http://dx.doi.org/10.1098/rspb.2014.1785.
- Lindgreen S. 2012. AdapterRemoval: easy cleaning of next-generation sequencing reads. BMC Res Notes 5:337. http://dx.doi.org/10.1186/1756 -0500-5-337.