

Draft Genome Sequence of *Bacillus anthracis* UR-1, Isolated from a German Heroin User

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We report the draft genome sequence of *Bacillus anthracis* UR-1, isolated from a fatal case of injectional anthrax in a German heroin user. Analysis of the genome sequence of strain UR-1 may aid in describing phylogenetic relationships between virulent heroin-associated isolates of *B. anthracis* isolated in the United Kingdom, Germany, and other European countries.

he Gram-positive, spore-forming soil bacterium Bacillus anthracis is the causative agent of the zoonosis anthrax. Several virulence factors are required for successfully establishing infection in animal and human hosts. These include the binary toxins lethal toxin and edema toxin and a poly- γ -D-glutamic acid capsule for evading host immune response (4). In 2009 and 2010, the unorthodox manifestation of the disease termed injectional anthrax caused several cases among consumers of presumably endospore-contaminated heroin in the United Kingdom and Germany, resulting in 19 fatalities (2, 5). Now, after almost 2 years, additional cases of heroin-related injectional anthrax have emerged in Germany, France, Great Britain, and Denmark. In our ongoing efforts to elucidate the phylogenetic relationships between the isolates involved in the 2012 and 2009-2010 outbreaks, the genome of the strain from the first fatal case from Bavaria in June of 2012 (3) was seauenced.

Whole-genome shotgun (WGS) sequencing of *B. anthracis* UR-1 was performed with Ion Torrent sequencing technology (Ion Torrent Systems, Inc.). For the WGS library, 1,320,223 reads with a total of 186,883,347 bp were generated. About 93.3% of the reads containing 97.3% of the bases were assembled into 125 contigs using Newbler version 2.6 (Roche) to reach 33.4-fold coverage. The assembled contigs were submitted to the RAST annotation server for subsystem classification and functional annotation (1). Coding sequences (CDSs) were assigned using BLASTp with KEGG Orthology (KO). The G+C content was calculated using an in-house Perl script. The NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) was employed for gene annotation in preparation for submission to GenBank (http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html).

The total length of the draft genome shotgun sequence of *B. anthracis* UR-1 was 5,444,479 bp, and the mean G+C content was 35.09%. Its chromosomal sequence contained 113 contigs with 5.17 Mbp with 25.7-fold coverage, and the assembled genome consisted of 109 large contigs (>200 bp) with an average contig size of 51,363 bp. The plasmids pXO1 and pXO2 comprised 8 and 1 contigs with average sizes of 18.0 and 47.4 kbp and with 71.1- and 35.6-fold coverage, respectively. The overall coverage levels suggest a chromosome/pXO1/pXO2 molecular ratio in UR-1 roughly correlating to the 1:3:2 ratio in the Ames Florida strain

from the 2001 terrorist letter attack (6, 7). The genome contains 5,738 putative coding sequences (CDSs) (5,449, 189, and 100 on the chromosome, pXO1, and pXO2, respectively). The draft genome sequence contains four ribosomal RNA, two 5S rRNA, and 70 tRNA loci. For the CDSs, 69%, corresponding to 3,953 proteins, could be assigned to Cluster of Orthologous Groups (COG) families (8).

Availability of the *B. anthracis* UR-1 genome sequence will help to explain the taxonomic relationship between several isolates of the recent injectional anthrax cases in Europe and can be expected to provide novel insights into the epidemiology and microevolution of this group of organisms.

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number ALNY00000000. The version described in this paper is the first version, ALNY01000000.

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REFERENCES

- 1. Aziz RK, et al. 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9:75. doi:10.1186/1471-2164-9-75.
- Health Protection Scotland. 2011. An outbreak of anthrax among drug users in Scotland, December 2009 to December 2010: A report on behalf of the National Anthrax Outbreak Control Team. Health Protection Scotland, NHS National Services Scotland, Edinburgh, Scotland. Available at http://www.documents.hps.scot .nhs.uk/giz/anthrax-outbreak/anthrax-outbreak-report-2011-12.pdf.

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- 3. Holzmann T, et al. 2012. Fatal anthrax infection in a heroin user from southern Germany, June 2012. Euro Surveill. 17:20204.
- 4. Mock M. Fouet A. 2001. Anthrax. Annu. Rev. Microbiol. 55:647-671.
- 5. Price EP, et al. 2012. Molecular epidemiologic investigation of an anthrax outbreak among heroin users, Europe. Emerg. Infect. Dis. 18: 1307–1313.
- 6. Rasko DA, et al. 2011. Bacillus anthracis comparative genome analysis in

support of the Amerithrax investigation. Proc. Natl. Acad. Sci. U. S. A. 108:5027–5032.

- Read TD. 2002. Comparative genome sequencing for discovery of novel polymorphisms in *Bacillus anthracis*. Science 296:2028–2033.
- Tatusov RL, Galperin MY, Natale DA, Koonin EV. 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. 28:33–36.