AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Genome Sequences of Penicillin-Resistant *Bacillus anthracis* Strains

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ABSTRACT *Bacillus anthracis*, the etiologic agent of anthrax, is characteristically susceptible to penicillin despite containing two chromosomal β-lactamase genes. Few naturally occurring penicillin-resistant *B. anthracis* isolates have been reported. Here, we report the draft genome sequences for three penicillin-resistant *B. anthracis* strains, strain 32, UT308, and SK57.

Penicillin (PEN) is an effective treatment for anthrax, the disease caused by *Bacillus anthracis*; however, surveys indicate that PEN resistance occurs in 2 to 16% of strains (1). Expression of the *B. anthracis* β-lactamase genes (*bla1* and *bla2*) is not sufficient to confer resistance to β-lactam antibiotics (1, 2). An extracytoplasmic function sigma factor (SigP) and its cognate anti-sigma factor (RsiP) regulate β-lactamase expression in *B. anthracis* (3). β-Lactamase expression and PEN resistance in *B. anthracis* strain 32 and derivatives of this strain were described previously (1–6); however, the genomes for these strains were not reported previously. Strain 32 was isolated from a fatal anthrax case in Northampton, England, in 1974 (7, 8). A mutation resulting in a truncated RsiP was described as the basis of PEN resistance in strain 32 (3). Previously, novobiocin was used to create the attenuated strain, UT308, from strain 32 (3), with a PEN MIC of >32 μg/ml (9). Strain SK57, with a PEN MIC of >32 μg/ml (9), was also studied as a PEN-resistant isolate (9, 10). SK57 was isolated from England in November 1975; however, additional details about the source are not available. Mutations in the *sigP-rsiP* region of these strains and β-lactamase expression are described by Ross et al. (3) and Gargis et al. (11).

Genomic DNA was purified from colonies cultured overnight on Trypticase soy agar II with 5% sheep blood (BD BBL) at 35°C in ambient air from glycerol stocks. DNA was isolated following MasterPure complete DNA and RNA purification kit (Epicentre) instructions using a modified lysis buffer (20 mM Tris-HCl [pH 8.0], 2 mM EDTA, 1.2% Triton X-100) with 60 min of incubation at 37°C. Sequencing was performed on an Illumina MiSeq platform using the version 2 kit (2 × 250-bp paired ends) with TruSeq DNA LT libraries for UT308 and Nextera XT libraries for strains 32 and SK57. Sequencing adapters were removed, and reads were trimmed using Cutadapt (version 1.14, settings: -m 75 -u -4 -U -4 -a AGATCGGAAGAG -A AGATCGGAAGAG). *De novo* assembly was performed using the SPAdes genome assembler (version 3.10.0, settings: –k 71,81,91,101). Assembled genomes were submitted to the NCBI Prokaryotic Genome Annotation Pipeline for annotation. The results are summarized in Table 1. Single-nucleotide polymorphisms (SNPs) between the assembled genomes and the Ames Ancestor reference sequence (GenBank accession number AE017334) were identified using the Parsnp utility (version 1.2, with default parameters) (12), and large insertions and deletions were identified using the MUMmer utility (version 4.0.0beta2, with default parameters) (13).

Citation Gargis AS, Lascols C, McLaughlin HP, Conley AB, Hoffmaster AR, Sue D. 2019. Genome sequences of penicillin-resistant *Bacillus anthracis* strains. Microbiol Resour Announc 8:e01122-18. https://doi.org/10.1128/MRA.01122-18.

Editor David A. Baltrus, University of Arizona

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Received 10 August 2018
Accepted 27 November 2018
Published 10 January 2019

TABLE 1 Genome characteristics of three penicillin-resistant *B. anthracis* strains[a]

Strain/alternative name	GenBank accession no.	Total no. of reads	Chromosome				pXO1				pXO2			
			Size (bp)	No. of contigs	No. of CDSs	Coverage (×)	Size (bp)	No. of contigs	No. of CDSs	Coverage (×)	Size (bp)	No. of contigs	No. of CDSs	Coverage (×)
2000031103/strain 32	QPKO00000000	12,627,396	5,178,673	39	5,097	71	180,475	6	162	88	94,895	1	92	68
UT308	QPKP00000000	13,700,164	5,227,967	27	5,189	175	135,250	5	99	991	NA	NA	NA	NA
2007740863/SK57	QPKQ00000000	12,655,760	5,163,883	34	5,100	89	180,476	6	161	157	94,899	1	92	128

[a] CDSs, coding sequences; NA, information is not available, as pXO2 is absent in UT308. The NCBI RefSeq accession numbers are AE017334 (chromosome), AE017336 (plasmid pXO1), and AE017335 (plasmid pXO2).

Sequence analysis revealed that strains 32 and SK57 are closely related, with 159 SNPs separating the two strains. However, they have diverged and, relative to the Ames Ancestor reference sequence, there is a 13,945-bp deletion (positions 2124811 to 2138755) that is not found in strain 32 or UT308. This deletion results in the loss of 11 genes from the SK57 genome, including several amino acid transporters.

The three assembled genomes were each approximately 5.2 Mb long and had an average G+C content of 35.5%. Strains 32 and SK57 both contained pXO1 and pXO2. Plasmid pXO2 was absent from UT308, but a 135,293-bp contig corresponding to pXO1 was identified. In UT308, pXO1 is missing approximately 47 kb that includes the 44.8-kb pathogenicity island containing the three genes (*lef*, *pagA*, and *cya*) encoding the anthrax toxin proteins (14).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers QPKO00000000, QPKP00000000, and QPKQ00000000, and the first versions are described here. The raw reads were deposited in the Sequence Read Archive under the accession number SRP155512.

ACKNOWLEDGMENTS

This work was supported by the U.S. CDC Office of Public Health Preparedness and Response. We thank Theresa Koehler, University of Texas Health Science Center at Houston, for providing *B. anthracis* strain UT308. We thank Peter Turnbull for providing strain 32. We thank Bret K. Purcell, U.S. Army Medical Research Institute of Infectious Diseases, for providing strain SK57. We thank Chung Marston, CDC, for laboratory support. We thank Julia Bugrysheva, CDC, for valuable discussions and critical review of the announcement.

Bacillus anthracis is subject to the select agent regulations (15). The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention/The Agency for Toxic Substances and Disease Registry. The use of trade names is for identification only and does not imply endorsement by the U.S. Centers for Disease Control and Prevention, the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

REFERENCES

1. Chen Y, Succi J, Tenover FC, Koehler TM. 2003. Beta-lactamase genes of the penicillin-susceptible *Bacillus anthracis* Sterne strain. J Bacteriol 185:823–830. https://doi.org/10.1128/JB.185.3.823-830.2003.
2. Chen Y, Tenover FC, Koehler TM. 2004. Beta-lactamase gene expression in a penicillin-resistant *Bacillus anthracis* strain. Antimicrob Agents Chemother 48:4873–4877. https://doi.org/10.1128/AAC.48.12.4873-4877.2004.
3. Ross CL, Thomason KS, Koehler TM. 2009. An extracytoplasmic function sigma factor controls beta-lactamase gene expression in *Bacillus anthracis* and other *Bacillus cereus* group species. J Bacteriol 191:6683–6693. https://doi.org/10.1128/JB.00691-09.
4. Davis JJ, Boisvert S, Brettin T, Kenyon RW, Mao C, Olson R, Overbeek R, Santerre J, Shukla M, Wattam AR, Will R, Xia F, Stevens R. 2016. Antimicrobial resistance prediction in PATRIC and RAST. Sci Rep 6:27930. https://doi.org/10.1038/srep27930.
5. Lightfoot NF, Scott RJD, Turnbull PCB. 1990. Antimicrobial susceptibility of *Bacillus anthracis*. Salisbury Med Bull 68:95–98.
6. Mohammed MJ, Marston CK, Popovic T, Weyant RS, Tenover FC. 2002. Antimicrobial susceptibility testing of *Bacillus anthracis*: comparison of results obtained by using the National Committee for Clinical Laboratory Standards broth microdilution reference and Etest agar gradient diffusion methods. J Clin Microbiol 40:1902–1907. https://doi.org/10.1128/JCM.40.6.1902-1907.2002.
7. Severn M. 1976. A fatal case of pulmonary anthrax. Br Med J 1:748. https://doi.org/10.1136/bmj.1.6012.748.
8. Turnbull PC, Hutson RA, Ward MJ, Jones MN, Quinn CP, Finnie NJ, Duggleby CJ, Kramer JM, Melling J. 1992. *Bacillus anthracis* but not always anthrax. J Appl Bacteriol 72:21–28.
9. McLaughlin HP, Gargis AS, Michel P, Sue D, Weigel LM. 2017. Optical screening for rapid antimicrobial susceptibility testing and for observation of phenotypic diversity among strains of the genetically clonal species *Bacillus anthracis*. J Clin Microbiol 55:959–970. https://doi.org/10.1128/JCM.02209-16.
10. Gill SC, Rubino CM, Bassett J, Miller L, Ambrose PG, Bhavnani SM, Beaudry A, Li J, Stone KC, Critchley I, Janjic N, Heine HS. 2010. Pharmacokinetic-pharmacodynamic assessment of faropenem in a lethal murine *Bacillus anthracis* inhalation postexposure prophylaxis model. Antimicrob Agents Chemother 54:1678–1683. https://doi.org/10.1128/AAC.00737-08.
11. Gargis AS, McLaughlin HP, Conley AB, Lascols C, Michel PA, Gee JE, Marston CK, Kolton CB, Rodriguez-R LM, Hoffmaster AR, Weigel LM, Sue D. 2018. Analysis of whole-genome sequences for the prediction of penicillin resistance and β-lactamase activity in *Bacillus anthracis*. mSystems 3:e00154-18. https://doi.org/10.1128/mSystems.00154-18.
12. Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15:524. https://doi.org/10.1186/s13059-014-0524-x.
13. Marçais G, Delcher AL, Phillippy AM, Coston R, Salzberg SL, Zimin A. 2018. MUMmer4: a fast and versatile genome alignment system. PLoS Comput Biol 14:e1005944. https://doi.org/10.1371/journal.pcbi.1005944.
14. Okinaka RT, Cloud K, Hampton O, Hoffmaster AR, Hill KK, Keim P, Koehler TM, Lamke G, Kumano S, Mahillon J, Manter D, Martinez Y, Ricke D, Svensson R, Jackson PJ. 1999. Sequence and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. J Bacteriol 181:6509–6515.
15. Code of Federal Regulations. 2005. Title 42. Select agents and toxins regulations. 42 CFR 73. https://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=8a4be60456973b5ec6bef5dfeaffd49a&r=PART&n=42y1.0.1.6.61.