Draft Whole-Genome Sequences of Ciprofloxacin-Resistant Derivatives of a \textit{Bacillus anthracis} ΔANR Strain Lacking pXO1 and pXO2 Plasmids

Tracey D. Biggs, Mark A. Karavis, Jacquelyn V. Harris, Jessica M. Hill, Robert C. Bernhards, Lance B. Price, Paul Keim, Bruce G. Goodwin, Shanmuga Sozhamannan

ABSTRACT

Mutants of an attenuated \textit{Bacillus anthracis} (ΔANR) strain conferring increasing levels of ciprofloxacin resistance have been described. Here, we report the draft genome sequences of the parent strain (ΔANR pXO1\(^{-}\), pXO2\(^{-}\)) and its derivatives conferring low (step 1; 0.5 \(\mu\)g/ml), medium (step 2; 8 to 16 \(\mu\)g/ml), and high (step 3; 32 to 64 \(\mu\)g/ml) levels of ciprofloxacin resistance.

\textit{Bacillus anthracis} is a spore-forming, Gram-positive bacterium that causes anthrax disease. Natural resistance to the two commonly used postexposure prophylactic antibiotics, doxycycline (DOX) and ciprofloxacin (CIP), is rare. To understand the mechanism of ciprofloxacin resistance, mutants of an attenuated \textit{Bacillus anthracis} strain with high-level CIP resistance (\(\geq 32\) \(\mu\)g/ml) were isolated using a three-step \textit{in vitro} selection protocol (1). Step 1 mutants were resistant to 0.5 \(\mu\)g/ml of CIP and had one of two \textit{gyrA} quinolone resistance-determining region (QRDR) mutations. Step 2 mutants, derived from step 1 isolates, were resistant to 8 to 16 \(\mu\)g/ml of CIP and had one of three \textit{parC} QRDR mutations. Step 3 mutants, derived from step 2 isolates, were resistant to 32 to 64 \(\mu\)g/ml of CIP. Mutants with a MIC of 64 \(\mu\)g/ml had one of two additional mutations within the \textit{gyrA} QRDR or one of two mutations within the \textit{gyrB} QRDR. Mutants for which the MIC was 32 \(\mu\)g/ml had no additional target modifications but showed evidence of enhanced ciprofloxacin efflux. Indeed, mutations in a TetR-type transcriptional regulator upstream of a gene encoding an efflux pump protein were shown to be present in some of these mutants (2). To establish the link between the high-level CIP resistance phenotype and the unknown non-QRDR genetic changes, we sequenced the genomes of these mutants. Here, we report the whole-genome sequences of the parent and its derivatives.

For DNA preparation, all strains were plated from glycerol stocks onto tryptic soy agar plates and incubated overnight at 37°C. One colony was transferred to 15 ml of tryptic soy broth and grown overnight at 37°C with shaking at 250 rpm. The culture tube was centrifuged, and the pellet was used for DNA extraction using the Epicentre MasterPure Gram-positive DNA purification kit (catalog no. MGP04100) according to the manufacturer’s instructions. DNA samples were quantified using the Invitrogen Quant-IT PicoGreen double-stranded DNA (dsDNA) kit and a Lambda DNA standard. The concentration and purity of 2-\(\mu\)l DNA samples were determined from absorption ratios at both 260/280 and 230/260 nm using the NanoDrop 2000 instrument.

Citation Biggs TD, Karavis MA, Harris JV, Hill JM, Bernhards RC, Price LB, Keim P, Goodwin BG, Sozhamannan S. 2020. Draft whole-genome sequences of ciprofloxacin-resistant derivatives of a \textit{Bacillus anthracis} ΔANR strain lacking pXO1 and pXO2 plasmids. Microbiol Resour Announc 9:e00056-20. https://doi.org/10.1128/MRA.00056-20.

Editor Irene L. G. Newton, Indiana University, Bloomington

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Shanmuga Sozhamannan, shanmuga.sozhamannan.ctr@mail.mil.

Received 10 February 2020
Accepted 19 March 2020
Published 9 April 2020
Sequencing libraries were prepared using the Illumina Nextera XT kit and sequenced on an Illumina MiSeq (v2 mid-output 2 × 151-bp) instrument. MiSeq quality filters were used on quality-controlled (QC) reads, and the average Phred score was Q30. Adapter sequences were removed with Cutadapt (3) and assembled using Velvet v1.2.10 (4). The genome was annotated with the NCBI Prokaryotic Genome Annotation Pipeline v4.10 (5), and reference mapping was done with Bowtie 2 v2.3.5.1 (6). Default parameters were used for all software with the exception of Velvet (k-mer size, 2/3 of the average read length after adapter removal; -exp_cov auto) and Bowtie (n-ceil "0.0.30"; –very-sensitive-local).

Data availability. The accession numbers and sequence statistics of the 20 genomes are listed in Table 1.

ACKNOWLEDGMENTS

Funding for this study was provided by DBPAO/JPEO under the TARMAC initiative. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the JPEO, CBRND-EB, DBPAO, the Departments of the Army, Navy, or Defense, or the U.S. Government.

REFERENCES

1. Price LB, Vogler A, Pearson T, Busch JD, Schupp JM, Keim P. 2003. In vitro selection and characterization of Bacillus anthracis mutants with high-level resistance to ciprofloxacin. Antimicrob Agents Chemother 47:2362–2365. https://doi.org/10.1128/aac.47.7.2362-2365.2003.
2. Chen PE, Willner KM, Butani A, Dorsey S, George M, Stewart A, Lentz SM, Cook CE, Akmal A, Price LB, Keim PS, Mateczun A, Brahmbhatt TN, Bishop-Lilly KA, Zwick ME, Read TD, Sozhamannan S. 2010. Rapid identification of genetic modifications in Bacillus anthracis using whole genome draft sequences generated by 454 pyrosequencing. PLoS One 5:e12397. https://doi.org/10.1371/journal.pone.0012397.
3. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBO J 30:1766–1762. https://doi.org/10.1038/nmeth.1923.
4. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:822–829. https://doi.org/10.1101/gr.074492.107.
5. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:D561–D564. https://doi.org/10.1093/nar/gkw369.
6. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359. https://doi.org/10.1038/nmeth.1923.