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Genome Announcement

Genome sequencing of two *Bacillus anthracis* strains: a virulent strain and a vaccinal strain

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ABSTRACT

Bacillus anthracis strain SPV842_15 was isolated from bovine fetus, while *B. anthracis* strain Brazilian vaccinal was recovered from a commercial vaccine. We report here the genome sequences of both strains. The SPV842_15 genome is composed of a single circular chromosome with a length of 5,228,664 base pairs, and comprises 5911 coding sequences. In turn, the Brazilian vaccinal genome remains in 201 contigs with 5733 coding sequences. Both genomes have an overall C + G content of 35.4%, and 11 genes encoding the ribosomal RNAs (rRNAs) 5S, 16S and 23S. Only the plasmid pX01 sequence, which carries genes for toxins synthesis, was detected and completely assembled for both strains. These plasmids have a length of 181,684 base pairs and a C + G content of 32.5%. These genomic data generate insights about vaccinal *B. anthracis* virulence.

Genome announcement

Bacillus anthracis is a Gram-positive bacterium that causes anthrax.[1] The anthrax veterinary vaccine is an attenuated non-encapsulated and toxigenic *B. anthracis* strain.[2] The attenuation is due to the loss of the pXO2 plasmid, which carries the genes required for the biosynthesis and degradation of an anti-phagocytic capsule.[3] On the other hand, the pXO1 plasmid carries the genes for the lethal factor, the edema factor, and a protective antigen,[4] and is present in both pathogenic and vaccinal *B. anthracis* strains.

Here we describe two genome sequences: (i) the genome of *B. anthracis* strain SPV842_15, which was isolated from Aberdeen Angus bovine fetuses, and (ii) the genome of a vaccine strain, which we named *B. anthracis* Brazilian vaccinal strain.

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Total DNA from the bacteria strains were extracted by lysis with cetyltrimethylammonium bromide (CTAB) and isolation by the addition of phenol:chloroform. DNA libraries were prepared with Nextera DNA Library Kit and high-throughput sequencing was performed on Illumina MiSeq system (Illumina, Inc.) to generate 150-bp paired end reads (v2 chemistry) according to the manufacturer's instructions.

Reads were imported into the Geneious software (version 9.1.5) and trimmed. Assembling of the genomes was then accomplished by de novo assembly using SPAdes 3.9.0[5] followed by template-assisted assembly to the reference *B. anthracis* Sterne strain (NC_005945) and *B. anthracis* Ames strain (NC_003997). NCBI Prokaryotic Genome Annotation Pipeline performed the annotations of the genomes.

The whole genome sequencing generated 11x and 23x coverage, for Brazilian vaccinal genome and SPV842_15 genome, respectively. Both genomes have an overall C + G content of 35.4%. The SPV842_15 genome is composed of a single circular chromosome with a length of 5,228,664 bps, and comprises 5911 Coding Sequences (CDSs). In turn, the Brazilian vaccinal genome remains in 201 contigs with 5733 CDSs. Each genome contains 11 genes encoding the ribosomal RNAs (rRNAs) 5S, 16S and 23S, and 95 genes encoding the transfer RNAs (tRNAs). In both strains, only the plasmid pX01 sequence was detected and completely assembled. These plasmids have a length of 181,684 bps.

The pXO2 plasmid was not detected in the SPV842_15 or in the Brazilian vaccinal strains. Therefore, we hypothesized that the vaccine non-encapsulated attenuated bacteria may have a pathogenic threshold.

The complete sequences of the pX01 plasmid from each *B. anthracis* genomes were assembled and the alignment showed a high identity level. There are entire identities in the whole pX01 plasmid sequences. According to the assembly, the pX02 plasmid is absent in the pathogenic *B. anthracis* SPV842_15 strain, and the pX01 plasmid is present with total identity to the pX01 plasmid from the Brazilian vaccinal strain. These genomic data generate insights about the pathogenicity of *B. anthracis*. Additional comparative genomic studies in these strains will help to understand the potential infection profile of *B. anthracis* vaccine, and provide information to understand the source dynamics of the *B. anthracis* SPV842_15 strain.

The genomes and pX01 plasmid sequences have been deposited in DDBJ/ENA/GenBank under the accession numbers CP019588.1 and CP019589.1 for *B. anthracis* SPV842_15 genome and plasmid, respectively; and MVOA00000000 for *B. anthracis* Brazilian vaccinal Shotgun genome.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Markey B, Leonard F, Archambault M, et al. *Clinical Veterinary Microbiology*. second ed. Elsevier; 2013, 901p.
2. Turnbull PC. Anthrax vaccines: past, present and future. *Vaccine*. 1991;9(8):533–539.
3. Makino SI, Uchida I, Terakado N, et al. Molecular characterization and protein analysis of the cap region, which is essential for encapsulation in *Bacillus anthracis*. *J Bacteriol*. 1989;171:722–730.
4. Koehler TM. *Bacillus anthracis* genetics and virulence gene regulation. *Curr Top Microbiol Immunol*. 2002;271: 143–164.
5. Nurk S, Bankevich A, Antipov D, et al. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol*. 2013;20:714–737.