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GENOME SEQUENCES

Draft Genome Sequence of *Clostridium botulinum* Subtype *bont*/A5(B2′)

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ABSTRACT Here, we report the draft genome sequence of *Clostridium botulinum* strain CDC76130, which harbors a rare botulinum toxin gene (*bont*) complex arrangement of *bont*/A5 and truncated *bont*/B2 within the same *ha* toxin gene cluster.

Clostridium botulinum is a Gram-positive, spore-forming, anaerobic bacterium that is capable of producing neurotoxins known as botulinum neurotoxins (BoNTs), some of the most potent and threatening toxins to human and animal health (1). According to their ability to neutralize serotype-specific antisera, BoNTs are classified into seven distinct serotypes (A to G) and subdivided in more than 40 subtypes, which are determined by at least 2.6% differences in the amino acid sequences of the BoNT gene (*bont*) (2).

The *bont* genes are associated with protein accessory genes, arranged in two possible clusters, *ha* or *orfX* (3). Depending on the BoNT serotype or subtype, the gene cluster may vary in sequence and organization, for example, *bont*/B and *bont*/A5 are always found within a *ha* cluster, and *bont*/A2 to *bont*/A4, *bont*/A6 to *bont*/A8, and *bont*/F are located in an *orfX* cluster. Additionally, *bont*/A1 and bivalent strains are uniquely capable of harboring either of the two *bont* cluster types (4).

During a routine outbreak investigation of an infant botulism case from the Midwest region of the United States, CDC76130 was isolated from a fecal sample (5). A single colony was inoculated into Trypticase-peptone-glucose-yeast (TPGY) broth and grown anaerobically for 16 h prior to genomic DNA extraction. DNA extraction was performed using a modified MasterPure complete DNA and RNA purification kit (Lucigen, Middleton, WI) (6). The protocol utilized in this study was approved by the Centers for Disease Control and Prevention (CDC) Institutional Review Board (protocol approval number 6911).

Libraries were generated with a DNA preparation kit and sequenced using a MiniSeq system (2 × 150-bp chemistry) (Illumina, San Diego, CA), according to the manufacturer's instructions. Quality assurance and control and assembly of a total of 4,539,446 paired-end reads were performed using the SneakerNet pipeline v0.20.1 (7). The quality of the assembly was estimated with QUAST v5.1.0rc1 (8), and the draft genome assembly yielded a total read length of 3,931,884 bp (N_{50}, 435,765 bp), assembled into 30 contigs, with an average coverage depth of 134× and a GC content of 28.02%. Default settings were used for all software unless otherwise noted.

Traditional seven-gene multilocus sequence typing (MLST) (9, 10) revealed that isolate CDC76130 harbored a new *mdh* allele. The new allele and new sequence type (ST), ST-153, were submitted to the *C. botulinum* MLST database (http://pubmlst.org/cbotulinum). Using the CLC Genomics Workbench v20.0.2 (Qiagen, Denmark) Map Reads to Reference tool with default parameters, subtype *bont*/A5 and a truncated *bont*/B2 were identified. The genome was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v5.3 (11), which resulted in the identification of 3,672 genes, including 3,609 coding sequences (CDSs) and 63 total RNA genes.

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Sequence analysis of the *bont* gene cluster revealed a *ha* gene cluster arrangement very similar to the ones identified for *C. botulinum* strains IBCA94-0216 (12) and H04402 065 (13), with the same deletion between the *ha-33* gene and the *botR* gene and two copies of IS*3* transposases preceding the truncated, nonfunctional *bont*/B2.

Data availability. The draft genome assembly is available in NCBI GenBank under the accession number JAKETJ000000000, and the raw reads have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRR17486303.

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