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Whole-Genome Sequence of *Clostridium botulinum* A2B3 87, a Highly Virulent Strain Involved in a Fatal Case of Foodborne Botulism in Italy

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Here, we report the genome sequence of a rare bivalent strain of *Clostridium botulinum*, A2B3 87. The strain was isolated from a foodborne botulism case that occurred in Italy in 1995. The case was characterized by rapid evolution of the illness and failure of conventional treatments.

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Clostridium botulinum is an anaerobic spore-forming bacterium capable of synthesizing the botulinum neurotoxin (BoNT), a powerful and highly lethal poison (1). BoNTs are the only toxins which are tier 1 select agents (select agents and toxins lists are available at http://www.selectagents.gov). The intoxication by BoNT causes a neuromuscular paralysis produced by the block of peripheral cholinergic synapses. Botulism occurs from ingestion of preformed BoNT within contaminated food (foodborne) or by infections with bacterial spores and consequently toxin formation *in situ* (intestinal or wound) (1). The *C. botulinum* taxon has been divided into four groups (I to IV), as demonstrated by rRNA 16S gene comparison, amplified fragment length polymorphism (AFLP), and other techniques (2–4). On the basis of their serological activity, *Clostridium botulinum* strains are classified by 8 serotypes of BoNT (A to H), which are further divided into subtypes (A1 to A5, B1 to B7, E1 to E9, F1 to F7). Sixteen *C. botulinum* strains were sequenced and analyzed and several draft assemblies are available. These data show significant differences between the four groups (5–7).

A2B3 87 *C. botulinum* strain was isolated from a clinical case of foodborne botulism that occurred in a 76-year-old woman in Italy in 1995. The patient died 4 days after the hospital admission after being treated with the polyvalent antiserum and supported by respiratory aid. The bacterium was isolated in a sample of canned macrobiotic food based on "seitan," a traditional Eastern recipe (8). The strain was found to produce both A and B toxin serotypes (ratio 10/1) (8).

The A2B3 87 genome was sequenced with the Roche 454 GS FLX Titanium and Illumina MiSeq platforms. From the 454 sequencing, a ~25× coverage was obtained (103,330,725 total sequenced bases, 272,724 total reads), while MiSeq sequencing reached ~246× coverage (970,106,826 total sequenced bases, 3,350,829 total paired reads). Illumina reads were used to cover the gaps in the 454 sequencing assembly and to correct the homopolymers length inaccuracies produced by 454 sequencing (9). The final draft assembly, with a G+C content of 27.9%, consists of 13 contigs. The 11 representing the chromosomal sequence are 3,847,714 bp long, while the 2 that constitute the plasmids are 275,568 and 45,268 bp long. The chromosomal gaps are caused by unresolved repeated sequences: the nine copies of the rRNA genes operon (total length of ~43 kb) and the two copies of the beta-*N*-acetyl-glucosamidase gene.

The bigger plasmid contains the two (A and B) BoNT genes. The BoNT/A gene is an A2 subtype, with a similarity of 99.85% (2 amino acid different) with A2 Kyoto BoNT sequence (YP_002803127.1) (3). BoNT/B is a B3 subtype but shows a considerable number of amino acid mutations compared to the other B3s; the similarity with CDC 795 (EF028400.1) is 98.22% and there are 21 different amino acids. More studies are needed to really understand the role played by the amino acid substitutions in this BoNT sequence. Moreover, the smaller plasmid showed no homologies with any other plasmid sequenced to date.

Nucleotide sequence accession number. The genome sequence of *C. botulinum* A2B3 87 is available in DDBJ/EMBL/GenBank under the accession no. AUZB00000000.

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