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GENOME SEQUENCES

Complete Genome Sequence of *Bacillus toyonensis* Strain HA0190, Isolated from a Commercial Hydroxyapatite Product Extensively Used as a Synthetic Bone Graft Substitute

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ABSTRACT *Bacillus toyonensis* is a member of the *Bacillus cereus* group and is used as a probiotic in animal feeds and biological applications. We report the 5.8-Mbp genome sequence of strain HA0190, an isolate from a commercial hydroxyapatite nanoparticle product. The genome contains a circular chromosome and two plasmids, pBT001 and pBT002.

Bacillus toyonensis is a Gram-positive bacterium found in soil, crops, and deep sea and is used as a feed additive ingredient and to aid plant growth under salt stress (1, 2). The safety of probiotic *B. toyonensis* strain BCT-7112^T is supported by observations of non-detectable expression of two cytotoxin genes (*hb1* and *nhe*), absence of the cytotoxin K gene (*cytK*), and incomplete genes necessary for cereulide synthetase expression (3, 4). Hydroxyapatite (HA) has been used as a synthetic bone graft substitute because of its functional property of regenerating bone growth. Bacterial adherence to the surface of HA in dental materials and orthopedics can cause serious medical complications (5, 6).

A 100-μL aliquot of HA suspension was inoculated in 10 mL of tryptic soy (TS) broth (BD, Sparks, MD) and cultured at 37°C for 24 h. The culture was streaked on TS agar containing 5% sheep's blood (Remel, Lenexa, KS) and incubated at 37°C for 24 h. A Gram-positive, rod-shaped bacterium was isolated and identified as a *Bacillus cereus* group member using matrix-assisted laser desorption ionization–time of flight mass spectrometry following the manufacturer's protocols (Bruker, Billerica, MA). 16S rRNA sequence analysis of the genome further identified the isolate as *B. toyonensis*, showing 99.93% identity to BCT-7112^T (GenBank ID CP006863.1).

Genomic DNA for all sequencing was extracted using the DNeasy blood and tissue kit following manufacturer's instructions (Qiagen, Germantown, MD) and quantified using Qubit fluorometry (Thermo Fisher, Waltham, MA). A short-read genomic library was constructed using the Nextera XT library prep kit and sequenced using the MiSeq v2 reagent kit (2 × 250 bp) (Illumina, San Diego, CA). For long reads, a genomic library was constructed using the NEBNext kit (New England BioLabs, Ipswich, MA) and ligation sequencing kit SQK-LAK109 and was sequenced using a Nanopore MinIon (FLO-MIN107; Oxford Nanopore, Oxford, UK). Default parameters were used for all software. After quality checks and trimming using Fastq Utilities Services in PATRIC (v. 3.6.12), short reads (n = 6,374,515) and long reads (n = 24,000; N_{50} value of 7,080 bp) were assembled with 331× short-read depth and 17× long-read depth using Unicycler (v. 0.4.8) in PATRIC (7). The complete genome of HA0190 is 5,798,677 bp, including one chromosome of 5,235,892 bp and two plasmids, pBT001 (284,696 bp) and pBT002 (278,089 bp), with G+C contents of 35.4, 32.68, and 32.6%, respectively. The genome, annotated using the NCBI Prokaryotic Genome Pipeline (v. 5.3), contains 5,743 genes with functional assignments, 212 pseudogenes, 106 tRNAs,

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39 rRNAs, and 126 repeat regions (8). HA0190 shares an average nucleotide identity of 98.34% with the genome of *B. toyonensis* BCT-7112^T (CP006863.1), determined by Similar Genome Finder in PATRIC (8). pBT001 shows 97.6% identity (coverage, 44%) with *B. toyonensis* JAS03/3 plasmid p355 (CP036055.1), and pBT002 shows 99.4% identity (coverage, 64%) with JAS03/3 plasmid p271 (CP036054.1). HA0190 lacks *cytK*, as observed in BCT-7112^T, and differs in *hbl* and *nhe* sequences, with identities of 83.4% and 62.1%, respectively.

Data availability. Genome sequences were deposited in GenBank under accession numbers CP087103, CP087104, and CP087105. Raw reads were deposited in the Sequence Read Archive under accession numbers SRR16194856 and SRR17027411.

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