Complete genome sequence of *Bacillus cereus* NC7401, a high producer of the emetic toxin, cereulide.

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Abstract.

We report the complete and annotated genome sequence of *Bacillus cereus* NC7401, a representative of a specific group of emetic strains. The emetic toxin, cereulide, is produced by a non-ribosomal protein synthesis (NRPS) system that is encoded by a gene cluster on a large resident plasmid, pNCcld.

Bacillus cereus is a ubiquitous spore-forming bacterium, isolated from foodborne illnesses and other infections (4, 9, 11, 19). Food poisoning caused by *B. cereus* is divided into two types according to symptoms: vomiting type or diarrhea type. The vomiting type is often life threatening (6, 13, 17). The toxin, cereulide, is responsible for the emetic foodborne diseases (1, 2).

B. cereus, *B. anthracis* and *B. thuringiensis* are thought to be descended from a common *Bacillus* ancestor species that adapted to animal hosts (10, 14). *B. cereus* species include a large variety of strains that constitute different clusters, one of which is phylogenetically close to the *B. anthracis* group. Our multi locus sequence typing (MLST) study also revealed that most cereulide-producing strains could be allocated to the known sequence type of exclusively emetic *B. cereus* strains (18). Herein, we report the complete and annotated genome sequence of *B. cereus* NC7401 as a representative of this group.

The complete genome sequence of NC7401 was determined by a whole-genome shotgun strategy using the Sanger method. Genomic libraries containing 2-kb and 10-kb inserts were constructed and sequenced. 112,896 sequences were generated, giving 10.4-fold coverage from both ends of the genomic clones. Sequence reads were assembled using the Phred-Phrap-Consed program (7, 8). Direct sequencing of the clones was used to close gaps between contigs. tRNAscan-SE (12) was used to predict the tRNA. Genome Gambler 1.51 (16), GLIMMER 2.0 (5), and CRITICA (3) were used to identify potential open reading frames (ORFs) larger than 30 codons. All predicted proteins were searched against a non-redundant protein database (nr, NCBI) using BLASTP.

The complete genome of *B. cereus* NC7401 comprises a single circular chromosome of 5,221,581 bp, with a G+C content of 35.6%. The 14 rRNA operons and 104 tRNA genes are mainly located around the putative origin of replication. NC7401 harbors five plasmids (270, 48, 5, 4, and 3 kb). The chromosome of NC7401 contains 5,415 protein-coding genes, of which 3,832 are highly conserved among closely related strains in the *Bacillus cereus sensu stricto* group (NC7401, *B. cereus* ATCC 14579, *B. cereus* ATCC 10987, and *B. anthracis* Ames). The average levels of amino acid sequence identity of the 3,832 orthologs between NC7401 and the other three strains were 97.0% (ATCC 10987), 96.3% (Ames), and 94.1% (ATCC 14579), showing that NC7401 is more closely related to *B. anthracis* Ames than *B. cereus* ATCC 14579.

The cereulide-synthesizing gene cluster is encoded by the large plasmid, pNCcld. The number of assembled sequence reads indicated that there is only one copy of pNCcld in NC7401. The structure of pNCcld is almost identical to the large plasmid pCER270 (270 kb, accession no. DQ889676). pCER270 is harbored by *B. cereus* AH187 (also known as *B. cereus* F4810/72) that was isolated from an emetic food poisoning patient (15).

Nucleotide sequence accession numbers.

This whole genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession numbers of AP007209 (chromosome), AP007210 (the largest plasmid, pNCcld), AP007211 (pNC1), AP007212 (pNC2), AP007213 (pNC3), and AP007214 (pNC4).

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