

Complete Genome Sequence of *Bacillus thuringiensis* Serovar Sichuansis Strain MC28

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Bacillus thuringiensis is an important microbial insecticide used in the control of agricultural pests. Here we report the finished, annotated genome sequence of *Bacillus thuringiensis* serovar Sichuansis strain MC28, which can form parasporal crystals consisting of Cry4Cc1, Cry30Fa1, Cry53Ab1, Cry54Aa1, Cry54Ab1, Cry68Aa1, Cry69Aa1, Cry69Aa2, Cry70Ba1, Cyt1Da1, and Cyt2Aa3. It is also highly toxic to lepidopterous and dipterous insects.

Bacillus thuringiensis strain MC28 was isolated from Mu Chuan virgin forest in China's Sichuan Province. It produces spherical parasporal crystals during the stationary phase of its growth cycle, and it is highly toxic to lepidopterous and dipterous insects (4, 5).

Whole-genome sequencing of MC28 was performed at the Beijing Genomics Institute (BGI, Shenzhen, China) using Solexa paired-end sequencing technology. A total of 18,324,961 filtered paired-end reads were obtained and 451-fold coverage of the genome was achieved using an Illumina Solexa GA II. About 97.13% of the reads were assembled into 113 scaffolds using the SOAPdenovo alignment tool. Gaps within and between the scaffolds were confirmed and closed using primer walks, long-distance PCR amplification, and the construction of a fosmid library using an ABI 3730 capillary sequencer. Gene predictions and annotations were performed as described previously (1). tRNA and rRNA were identified using tRNAscan-SE and RNAmmer, respectively (2, 3).

The 6.68-Mb genome of MC28 is found to contain 8 replicons: a circular chromosome (5,414,461 bp), containing 5,279 predicted open reading frames (ORFs), and 7 circular plasmids, pMC8, pMC54, pMC95, pMC183, pMC189, pMC319, and pMC429. These plasmids contain a total of 1,278 predicted ORFs (Table 1). The G+C content of the chromosome is 35.41%, and the G+C contents of the plasmids range from 32.11% to 34.78% (Table 1). The MC28 genome contains 74 tRNA and 45 rRNA operons.

Three plasmids from MC28 contain insecticidal crystal genes. Plasmid pMC189 is found to harbor seven insecticidal crystal genes: *cry30Fa1* (MC28_E074), *cry53Ab1* (MC28_E095), *cry54Aa1* (MC28_E038), *cry54Ab1* (MC28_E085), *cry68Aa1* (MC28_E064), *cyt1Da1* (MC28_E051), and *cyt2Aa3* (MC28_E053). Plasmid pMC95 is found to harbor three *cry* genes: *cry4Cc1* (MC28_C067), *cry69Aa1* (MC28_C001), and *cry70Ba1* (MC28_C076). Plasmid pMC183 is found to contain only one *cry* gene, *cry69Aa2* (MC28_D165).

In brief, the MC28 genome not only enriches the genome database of *Bacillus thuringiensis* but also facilitates understanding of toxic gene regulation and evolutionary relationships among *B. cereus* group organisms.

Nucleotide sequence accession numbers. The sequence of the *Bacillus thuringiensis* strain MC28 has been deposited in GenBank. The accession number for the chromosome is CP003687, and the accession numbers for plasmids pMC8, pMC54, pMC95, pMC183, pMC189, pMC318, and pMC429 are CP003688, CP003689,

TABLE 1 Sequence features of 7 plasmids from strain MC28

Plasmid name	Length (bp)	G+C content (%)	No. of genes	Total gene length (bp)	Ratio of total gene length to plasmid length (%)	GenBank accession no.
pMC8	7,826	32.11	10	5,028	64.25	CP003688
pMC54	54,484	34.73	72	44,338	81.38	CP003689
pMC95	95,433	34.02	101	75,485	79.10	CP003690
pMC183	183,210	32.85	210	150,657	82.23	CP003691
pMC189	189,702	33.41	185	132,265	69.72	CP003692
pMC319	319,710	32.48	289	229,127	71.67	CP003693
pMC429	429,674	32.57	411	298,468	69.46	CP003694

CP003690, CP003691, CP003692, CP003693, and CP003694, respectively.

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REFERENCES

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
- Lagesen K, et al. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res.* 35:3100–3108.
- Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
- Tan F, et al. 2010. Rapid cloning, identification, and application of one novel crystal protein gene *cry30Fa1* from *Bacillus thuringiensis*. *FEMS Microbiol. Lett.* 302:46–51.
- Tan F, et al. 2009. Cloning and characterization of two novel crystal protein genes, *cry54Aa1* and *cry30Fa1*, from *Bacillus thuringiensis* strain BtMC28. *Curr. Microbiol.* 58:654–659.

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