

Complete Genome Sequence of *Bacillus thuringiensis* subsp. *chinensis* Strain CT-43[∇]

Jin He,^{1*} Jieping Wang,¹ Wen Yin,¹ Xiaohu Shao,¹ Huajun Zheng,² Mingshun Li,¹
Youwen Zhao,¹ Ming Sun,¹ Shengyue Wang,² and Ziniu Yu^{1*}

State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, No. 1 Shizishan St., Wuhan, Hubei 430070, People's Republic of China,¹ and Chinese National Human Center at Shanghai, 250 Bi Bo Road, Shanghai 201203, People's Republic of China²

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***Bacillus thuringiensis* has been widely used as an agricultural biopesticide for a long time. As a producing strain, *B. thuringiensis* subsp. *chinensis* strain CT-43 is highly toxic to lepidopterous and dipterous insects. It can form various parasporal crystals consisting of Cry1Aa3, Cry1Ba1, Cry1Ia14, Cry2Aa9, and Cry2Ab1. During fermentation, it simultaneously generates vegetative insecticidal protein Vip3Aa10 and the insecticidal nucleotide analogue thuringiensin. Here, we report the finished, annotated genome sequence of *B. thuringiensis* strain CT-43.**

Bacillus thuringiensis strains can produce insecticidal crystal proteins (ICPs) and have specific insecticidal activity against many kinds of insects (7, 8, 10). *B. thuringiensis* strain CT-43, which was isolated from China by our laboratory, is the producing strain for the microbial preparation of “double toxins.” It is not motile and has no flagella (11), and according to the flagellum antigen serotype and biochemical characteristics, we classified it as a new subspecies, *B. thuringiensis* subsp. *chinensis* (5). *B. thuringiensis* CT-43 contains 140-kDa, 130-kDa, and 68-kDa ICPs and is highly toxic to lepidopterous and dipterous insects (9). More importantly, it can produce high levels of the nucleotide analogue thuringiensin (4), which has insecticidal activities against a wide range of insects (6).

The *B. thuringiensis* CT-43 genome was sequenced at the National Human Center in Shanghai, China, using the Genome Sequencer FLX system (454). A total of 227 large contigs (>500 bp) were assembled based on 379,325 reads with a 395-bp average read length and a 25-fold coverage of the

genome. We arranged the large contigs by using the *Bacillus cereus* ATCC 14579 genome as the reference sequence (3), and the gaps between the large contigs were then closed by PCR amplification, primer walking, or subcloning in T vector. The gaps between the large contigs without clear context were confirmed and closed by multi-PCR. The Phred/Phrap/Consed software package (1) was used for sequence assembly and quality assessment. The complete genome of *B. thuringiensis* CT-43 achieves an error rate of less than 1 in a range of a 10-kb sequence and can be accessed by GenBank accession numbers CP001907.1 to CP001917.1.

Gene predictions and annotation were performed as described previously (2). The 6.15-Mb genome of *B. thuringiensis* CT-43 contains 11 replicons, a circular chromosome (5,486,830 bp) encoding 5,596 predicted open reading frames (ORFs), including 5,489 coding sequences (CDSs) and 10 circular plasmids (from 6,880 to 281,231 bp; named pCT6880, pCT8252, pCT8513, pCT9547, pCT14, pCT51, pCT72, pCT83,

TABLE 1. The sequence features of 10 plasmids from the *Bacillus thuringiensis* strain CT-43 genome

Plasmid name	Full length (bp)	No. of CDSs	No. of bp in the CDSs	CDS density (kb) ^a	Avg length of CDSs	Coding %	GC %	Accession no.
pCT281	281,231	271	197,631	0.963	729	70.2	33.01	CP001910.1
pCT127	127,885	141	103,485	1.102	733	80.9	32.09	CP001908.1
pCT83	83,590	96	61,929	1.148	645	74	33.15	CP001915.1
pCT72	72,074	86	58,596	1.193	681	81.2	32.04	CP001913.1
pCT51	51,488	72	44,817	1.398	622	87	34.97	CP001911.1
pCT14	14,860	22	10,638	1.480	483	71.5	31.47	CP001909.1
pCT9547	9,547	5	6,462	0.523	1,292	67.6	33.09	CP001917.1
pCT8513	8,513	10	5,133	1.174	513	60.2	30.79	CP001916.1
pCT8252	8,252	8	4,068	0.969	508	49.2	32.42	CP001914.1
pCT6880	6,880	6	4,128	0.872	688	60	31.76	CP001912.1

^a Full length of a plasmid/no. of CDSs in the plasmid.

* Corresponding author. Mailing address: College of Life Science and Technology, Huazhong Agricultural University, No. 1 Shizishan St., Wuhan, Hubei 430070, People's Republic of China. Phone: 86 (27) 8728 0802. Fax: 86 (27) 8728 0670. E-mail for Jin He: hejinhzau@yahoo.com.cn. E-mail for Ziniu Yu: yzn41@yahoo.com.cn.

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pCT127 and pCT281, according to their sizes), and carries a total of 737 predicted ORFs (Table 1). The G+C content of the chromosome is 35.38%, while those of the plasmids are from 30.79% to 34.97% (Table 1). The genome encodes 104 tRNA and 13 rRNA operons.

The biggest plasmid, pCT281, harbors four ICP genes, *cry1Aa3* (CT43_P281270), *cry1Ia14* (CT43_P281271), *cry2Aa9* (CT43_P281278), and *cry2Ab1* (CT43_P281265), and one vegetative insecticidal protein gene, *vip3Aa10* (CT43_P281262). These toxic genes are close to each other and constitute a pathogenicity island. The second-biggest plasmid, pCT127, contains another ICP gene, *cry1Ba1* (CT43_P127021), and a gene cluster for thuringiensin biosynthesis (from CT43_P127037 to CT43_P127041).

We deposited the complete sequence in GenBank on 3 February 2010, so this is the first genome sequence of *B. thuringiensis* harboring crystal protein genes (12). Certainly, the genome sequence provides a better-defined background for the genetic improvements and regulation studies of the toxic genes, especially ICPs and thuringiensin synthesis genes.

Nucleotide sequence accession numbers. The sequence of the *Bacillus thuringiensis* subsp. *chinensis* strain CT-43 has been deposited in GenBank. The accession number for the chromosome is CP001907.1, and those for plasmids are listed in Table 1.

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