



## Genome Sequence of *Bacillus thuringiensis* Strain Btm27, an Egyptian Isolate Highly Toxic to Cotton Leafworm

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*Bacillus thuringiensis* is a potent microbial control agent against insect pests. Here, we present the draft genome of the Egyptian strain Btm27 that shows high toxicity toward the cotton leafworm. The genome contains three insecticidal genes *cry1Ac9*, *cry2Ab1*, and *vip3V* that have been implicated in conferring toxicity toward lepidoptera.

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*acillus thuringiensis* has been successfully used as a biopesticide to control many agricultural pests and insect vectors of human disease (1). The entomopathogenicity of B. thuringiensis is attributed to the expression of a broad variety of species-specific toxic proteins, driven by evolutionary sequence divergence and recombination events (2). These include largely plasmid-borne (3) vegetative insecticidal proteins (Vip), sporulation associated crystal proteins (Cry), and cytolytic toxins (Cyt) (4). Besides its role as biopesticide, further promising biotechnological applications included the production of industrially important enzymes (5) for cytotoxic effects on cancer cells (6). As recently demonstrated by Alfazairy et al. (7), the sequenced Egyptian strain Btm27 is a potent control agent against cotton leafworm. Structural and functional genomic analyses of the strain will allow one to better characterize its insecticidal efficiency and biotechnological potential.

Total genomic DNA was extracted with QIAamp DNA minikit according to the manufacturer's protocol. Sequencing was performed on the Illumina MiSeq platform using a paired-end library with 300-bp read length. The draft genome was assembled with Spades 3.0 (8). The average G+C content of 35% and total length of 5,871,441 bp of the obtained Btm27 sequences are in accordance with the findings for other *B. thuringiensis* genomes (9, 10). All contigs were annotated using the PROKKA annotation pipeline (11) and a total of 5,050 coding sequences, 79 tRNAs, 11 rRNA operons, and four circular plasmids were identified. A BLASTn (9) analysis of the Btm27 contigs against the NCBI nonredundant (nr) database identified B. thuringiensis serovar kurstaki strain YBT-1520 as the closest relative. Draft sequences were further compared at the nucleotide and protein levels against a B. thuringiensis specific plasmid database on the Galaxy platform (10). The results suggest that the Btm27genome is organized into five replicons: a circular chromosome and four plasmids that show high similarity to plasmids pBMB293, pBMB8513, and pBMB400 in B. thuringiensis subspecies kurstaki strain YBT-1520, and pBMB65 in *B. thuringiensis* subspecies kurstaki strain HD-1. Utilizing BtToxinScanner, we identified three toxin genes in the

Btm27 genome and classified them as crv1Ac9, crv2Ab1, and vip (12). The predicted *vip* coding sequence was further compared to curated Vip proteins in Uniprot (13) and showed 100% identity to Vip3V, which has toxic activity against lepidopteran larvae (14). Interestingly, all three toxins have been demonstrated to be highly active against a range of lepidopteran insect pests (15, 16). The limited variety of insecticidal genes carried by Btm27 suggests that the strain is an ideal candidate for specified pest control preventing unwanted toxic effects on taxonomically unrelated insects. Genome annotation also revealed the presence of genes responsible for the expression of biotechnologically important degradative enzymes, such as chitinases and proteases that cover serine protease, neutral protease, and metalloprotease activities. The availability of the genome sequence of B. thuringiensis strain Btm27 lays the foundation for further structural and functional analyses to fully elucidate its biotechnological potential.

**Nucleotide sequence accession number.** This genome sequence has been deposited in GenBank under the accession no. JWJY00000000. Strain Btm27 has been deposited into the Bacillus Genetic Stock Center (BGSC) collection as 4AC2.

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## REFERENCES

- Bravo A, Gill SS, Soberón M. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49:423–435. http://dx.doi.org/10.1016/j.toxicon.2006.11.022.
- 2. De Maagd RA, Bravo A, Berry C, Crickmore N, Schnepf HE. 2003.

Structure, diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria. Annu Rev Genet 37:409–433. http://dx.doi.org/10.1146/annurev.genet.37.110801.143042.

- Ibrahim MA, Griko N, Junker M, Bulla LA. 2010. Bacillus thuringiensis: a genomics and proteomics perspective. Bioeng Bugs 1:31–50. http:// dx.doi.org/10.4161/bbug.1.1.10519.
- 4. Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P. 2011. *Bacillus thuringiensis*: a century of research, development and commercial applications. Plant Biotechnol J 9:283–300. http://dx.doi.org/10.1111/j.1467-7652.2011.00595.x.
- Vu KD, Yan S, Tyagi RD, Valéro JR, Surampalli RY. 2009. Induced production of chitinase to enhance entomotoxicity of *Bacillus thuringien*sis employing starch industry wastewater as a substrate. Bioresour Technol 100:5260–5269. http://dx.doi.org/10.1016/j.biortech.2009.03.084.
- Melo AL, Soccol VT, Soccol CR. 29 September 2014. *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. Crit Rev Biotechnol. http://dx.doi.org/10.3109/07388551.2014.960793.
- Alfazairy AA, El-Ahwany AM, Mohamed EA, Zaghloul HA, El-Helow ER. 2013. Microbial control of the cotton leafworm *Spodoptera littoralis* (Boisd.) by Egyptian *Bacillus thuringiensis* isolates. Folia Microbiol (Praha) 58:155–162. http://dx.doi.org/10.1007/s12223-012-0193-7.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to singlecell sequencing. J Comput Biol 19:455–477. http://dx.doi.org/10.1089/ cmb.2012.0021.
- 9. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local

alignment search tool. J Mol Biol 215:403–410. http://dx.doi.org/10.1016/ S0022-2836(05)80360-2.

- Goecks J, Nekrutenko A, Taylor J, Galaxy Team. 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol 11:R86. http://dx.doi.org/10.1186/gb-2010-11-8-r86.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bio-Informatics 30:2068–2069. http://dx.doi.org/10.1093/bioinformatics/ btu153.
- Ye W, Zhu L, Liu Y, Crickmore N, Peng D, Ruan L, Sun M. 2012. Mining new crystal protein genes from *Bacillus thuringiensis* on the basis of mixed plasmid-enriched genome sequencing and a computational pipeline. Appl Environ Microbiol 78:4795–4801. http://dx.doi.org/ 10.1128/AEM.00340-12.
- UniProt Consortium. 2014. Activities at the universal protein resource (UniProt). Nucleic Acids Res 42:D191–D198. http://dx.doi.org/10.1093/ nar/gkt1140.
- Doss VA, Kumar KA, Jayakumar R, Sekar V. 2002. Cloning and expression of the vegetative insecticidal protein (*vip3V*) gene of *Bacillus thuringiensis* in *Escherichia coli*. Protein Expr Purif 26:82–88. http://dx.doi.org/10.1016/S1046-5928(02)00515-6.
- Crickmore N, Zeigler DR, Feitelson J, Schnepf E, Van Rie J, Lereclus D, Baum J, Dean DH. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol Mol Biol Rev 62: 807–813.
- Palma L, de Escudero IR, Maeztu M, Caballero P, Muñoz D. 2013. Screening of *vip* genes from a Spanish *Bacillus thuringiensis* collection and characterization of two Vip3 proteins highly toxic to five lepidopteran crop pests. Biol Contr 66:141–149. http://dx.doi.org/10.1016/j.biocontrol.2013.05.003.