

Draft Genome Sequence of *Bacillus thuringiensis* Serovar Tolworthi Strain Na205-3, an Isolate Toxic for *Helicoverpa armigera*

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We report here the complete annotated 6,510,053-bp draft genome sequence of *Bacillus thuringiensis* serovar tolworthi strain Na205-3, which is toxic for *Helicoverpa armigera*. This strain potentially contains nine insecticidal toxin genes homologous to *cry1Aa12*, *cry1Ab1*, *cry1Ab8*, *cry1Ba1*, *cry1Af1*, *cry1Ia10*, *vip1Bb1*, *vip2Ba2*, and *vip3Aa6*.

Received 18 February 2014 Accepted 21 February 2014 Published 13 March 2014

Citation Palma L, Muñoz D, Murillo J, Caballero P. 2014. Draft genome sequence of *Bacillus thuringiensis* serovar tolworthi strain Na205-3, an isolate toxic for *Helicoverpa armigera*. *Genome Announc.* 2(2):e00187-14. doi:10.1128/genomeA.00187-14.

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Bacillus thuringiensis (*Bacillales*: *Bacillaceae*), one of the best-characterized entomopathogenic bacteria, carries plasmids bearing a variety of genes coding for proteins with valuable insecticidal characteristics (1). These proteins can be classified into distinct protein groups according to their amino acid identities and protein structures: Cry and Cyt proteins (δ -endotoxins) (2); vegetative insecticidal proteins Vip1/Vip2 (binary toxin) (3), Vip3 (4), and Vip4 (http://www.lifesci.sussex.ac.uk/Home/Neil_Crickmore/Bt/); Bin-like and Mtx-like proteins (1, 5); the secreted insecticidal proteins Sip (6); accessory proteins P19 and P20 (7); and enhancin-like proteins (8). In this work, we report the draft genome sequence of *Bacillus thuringiensis* serovar tolworthi strain Na205-3, which is toxic against *Helicoverpa armigera* (Lepidoptera: Noctuidae) (9). Purified total DNA from strain Na205-3 was sequenced at the Beijing Genomics Institute (BGI) (Shenzhen, China) using high-throughput Illumina sequencing technology. The reads were assembled using SOAPdenovo (version 1.05) and produced 169 contigs totaling 6,510,053 bp, with a maximum scaffold size of 427,627 bp, an N_{50} length of 161,129 bp, and 34.7% G+C content. Genome annotation was added by the NCBI Prokaryotic Genome Annotation Pipeline (released in 2013), although it was also analyzed with BLAST (10) using a custom insecticidal toxin database, the RAST server (11), and the BtToxin_scanner (12). The custom insecticidal toxin database was constructed with amino acid sequences of Cry and Cyt proteins, Bin-like and Mtx-like proteins, Vip1/Vip2, Vip3, and Sip1A proteins (13), accessory proteins P19 and P20, and the enhancin-like Bel protein. The Na205-3 draft genome sequence carries nine insecticidal toxin genes homologous to *cry1Aa12*, *cry1Ab1*, *cry1Ab8*, *cry1Ba1*, *cry1Af1*, *cry1Ia10*, *vip1Bb1*, *vip2Ba2*, and *vip3Aa6*, a putative *etx_mtx* toxin gene, and 1 *bel* (enhancin-like) gene. This ample repertoire of insecticidal genes implies that strain Na205-3 might have a wider host range than was previously thought.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [AYXQ01000000](https://www.ncbi.nlm.nih.gov/nuccore/AYXQ01000000). The version described in this paper is version AYXQ01000000.

ACKNOWLEDGMENTS

This research was supported by the Spanish Ministry of Science and Innovation (grant AGL2009-13340-C02).

We thank Colin Berry and Steffan Adams (Cardiff University, United Kingdom) for providing remote access to the Assembler server to perform bioinformatics analyses.

REFERENCES

1. 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>.
2. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* 62:775–806.
3. Warren GW, Koziel MG, Mullins MA, Nye GJ, Carr B, Desai NM, Kostichka K, Duck NB, Estruch JJ. 1998. Auxiliary proteins for enhancing the insecticidal activity of pesticidal proteins. US patent 5,770,696.
4. Estruch JJ, Yu CG. 1998. Plant pest control. Patent WO 9844137.
5. Berry C. 2012. The bacterium, *Lysinibacillus sphaericus*, as an insect pathogen. *J. Invertebr. Pathol.* 109:1–10. <http://dx.doi.org/10.1016/j.jip.2011.11.008>.
6. Donovan WP, Donovan JC, Engleman JT. 2001. Gene knockout demonstrates that *vip3A* contributes to the pathogenesis of *Bacillus thuringiensis* toward *Agrotis ipsilon* and *Spodoptera exigua*. *J. Invertebr. Pathol.* 78:45–51. <http://dx.doi.org/10.1006/jip.2001.5037>.
7. Manasherob R, Zaritsky A, Ben-Dov E, Saxena D, Barak Z, Einav M. 2001. Effect of accessory proteins P19 and P20 on cytolytic activity of Cyt1Aa from *Bacillus thuringiensis* subsp. *israelensis* in *Escherichia coli*. *Curr. Microbiol.* 43:355–364. <http://dx.doi.org/10.1007/s002840010316>.
8. Fang S, Wang L, Guo W, Zhang X, Peng D, Luo C, Yu Z, Sun M. 2009. *Bacillus thuringiensis* Bel protein enhances the toxicity of Cry1Ac protein to *Helicoverpa armigera* larvae by degrading insect intestinal mucin. *Appl. Environ. Microbiol.* 75:5237–5243. <http://dx.doi.org/10.1128/AEM.00532-09>.
9. Martínez C, Caballero P. 2002. Contents of *cry* genes and insecticidal toxicity of *Bacillus thuringiensis* strains from terrestrial and aquatic habitats. *J. Appl. Microbiol.* 92:745–752. <http://dx.doi.org/10.1046/j.1365-2672.2002.01579.x>.
10. 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/10.16/S0022-2836\(05\)80360-2](http://dx.doi.org/10.16/S0022-2836(05)80360-2).
11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsm

- K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
12. 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>.
13. Donovan WP, Engleman JT, Donovan JC, Baum JA, Bunkers GJ, Chi DJ, Clinton WP, English L, Heck GR, Ilagan OM, Krasomil-Osterfeld KC, Pitkin JW, Roberts JK, Walters MR. 2006. Discovery and characterization of Sip1A: a novel secreted protein from *Bacillus thuringiensis* with activity against coleopteran larvae. *Appl. Microbiol. Biotechnol.* 72: 713–719. <http://dx.doi.org/10.1007/s00253-006-0332-7>.