



Complete genome sequence of *Bacillus thuringiensis* BR145, a strain with insecticidal activity against Lepidoptera pests

Ana Paula Scaramal Ricietto¹, Kátia Cristiane Brumatti Gonçalves², Renan José Casarotto Appel², Daniel Ricardo Sosa-Gómez³, Gislayne Trindade Vilas-Bôas² and Laurival Antonio Vilas-Boas² 

¹Universidade Norte do Paraná, Londrina, PR, Brazil.

²Universidade Estadual de Londrina, Departamento de Biologia Geral, Londrina, PR, Brazil.

³Empresa Brasileira de Pesquisa Agropecuária (Embrapa Soja), Centro Nacional de Pesquisa de Soja, Londrina, PR, Brazil.

Abstract

Bacillus thuringiensis BR145 isolated from a soybean field in Southern Brazil showed toxicity against two important insect pests from soybean crop, *Helicoverpa armigera*, and *Chrysodeixis includens*, with LC₅₀ 0.294 µg.cm⁻² and 0.277 µg.cm⁻², respectively. We analyzed the genome of this strain through sequences obtained by Next Generation DNA Sequencing and *de novo* assembly. The analysis of the genome revealed insecticidal genes *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1Ia*, *cry2Ab*, *cyt1*, and *vip3Aa*, suggesting the use of this strain in new strategies of biological control.

Keywords: *Bacillus thuringiensis*, insecticidal genes, virulence factors, *Helicoverpa armigera*, *Chrysodeixis includens*.

Received: September 15, 2021; Accepted: February 03, 2022.

Bacillus thuringiensis is a Gram-positive bacterium with entomopathogenic activity associated with Cry, Cyt, and Vip proteins, synthesized in the sporulation phase and during vegetative growth. Besides these toxins, *B. thuringiensis* produces virulence factors, which potentiate their pathogenicity, including phospholipases, metalloproteases, hemolysins, enterotoxins, cytotoxins, and others factors (Vilas-Bôas *et al.*, 2007; Palma *et al.*, 2014). Several toxins produced by *B. thuringiensis* strains were described with toxicity to insect larvae of Lepidoptera, Coleoptera, Diptera, and against species of other phyla (Vilas-Bôas *et al.*, 2012) and recently the classification of these toxins was revised (Crickmore *et al.*, 2020). Therefore, *B. thuringiensis*-based biopesticides have been used as alternative insect pest control and represent about 98% of formulated sprayable bacterial microbial pesticides (Lacey *et al.*, 2015).

Brazil has emerged as the largest producer and exporter of soybean. Therefore, key soybean pests such as soybean looper *Chrysodeixis includens* Walker, 1858 (Lepidoptera: Noctuidae), one of the most important soybean pests in Brazil (Yano *et al.*, 2016), have a profound impact on insecticide use, as well as the polyphagous pest *Helicoverpa armigera* Hübner, 1805 (Lepidoptera: Noctuidae), which eventually can reach pest status and cause damage, also in cotton and corn (Bueno and Sosa-Gómez, 2014; Pomari-Fernandes *et al.*, 2015). Since the use of safe and more selective insecticides is increasing in the world and is an important demand of the public and farmers, alternative methods of control of these insect pests must be developed. For these reasons, in this study, we performed a characterization of *B. thuringiensis* BR145, a novel strain with toxicity against Lepidoptera pests.

This strain was isolated in a Brazilian soybean field (Ricietto *et al.*, 2013) and showed entomopathogenic activity in assays with larvae of *Ecdytolopha aurantiana* Lima, 1927 (Lepidoptera: Tortricidae) (Zorzetti *et al.*, 2017a) and *Elasmopalpus lignosellus* Zeller, 1848 (Lepidoptera: Pyralidae) (Zorzetti *et al.*, 2017b). Bioassays were performed using lyophilized spores and crystal suspensions against larvae of *H. armigera* and soybean looper *C. includens*. Dilutions of lyophilized *B. thuringiensis* were applied uniformly to the diet surface and allowed to dry. Surface treatments provide doses ranging from 0.02 to 1.05 µg/cm². One neonate larva was placed on the treated surface in each cell of a bioassay tray (128 cells). The trays were sealed with self-adhesive plastic sheets (BIO-CV-16; CD International Inc., Pitman, NJ) and held for at 25 ± 1.5 °C. Mortality data were obtained after seven days of exposure. Lethal doses and parameters associated were calculated with Polo software (LeOra Software, 1987).

The strain showed insecticidal activity against *H. armigera* (LC₅₀ of 0.294 µg.cm⁻²) and to *C. includens* from both origins, with LC₅₀ 0.277 µg.cm⁻², and 0.398 µg.cm⁻², respectively (Table 1). This LC₅₀ of in both species is comparable to previous bioactive *B. thuringiensis* isolates with potential use in microbial control (Ignoffo *et al.*, 1977; Pinheiro and Valicente, 2021).

Genomic DNA was isolated from strain BR145 using Wizard® Genomic DNA Purification kit (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions and the DNA library was prepared with Illumina DNA Prep. Whole-genome sequencing was performed by Illumina HiSeq sequencing and the paired-end sequence strategy was chosen, which generated a total of 3,042,174 reads of high quality. The analysis methods were performed according to Zorzetti *et al.* (2015). The genome was assembled *de novo* with SPAdes version 3.9.0 (Gurevich *et al.*, 2013). The final draft genome consisted of 235 contigs (length > 1000 bp), with a total size

Send correspondence to Laurival Antonio Vilas-Boas. Universidade Estadual de Londrina, Departamento de Biologia Geral, Rodovia Celso Garcia Cid/Pr 445 Km 380, 86057-970, Londrina, PR, Brazil. E-mail: lavboas@uel.br.

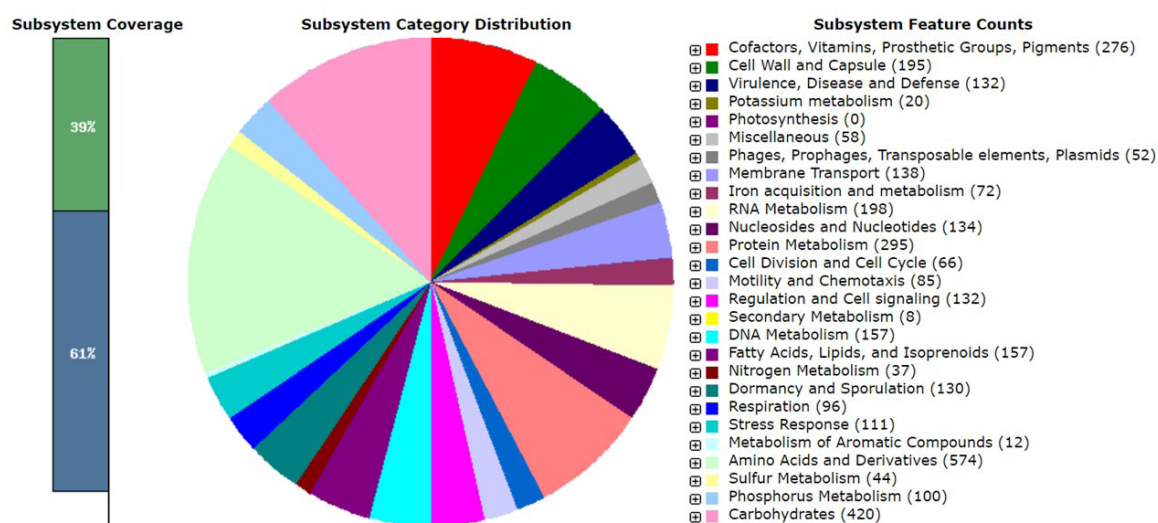
Table 1 – Concentration/mortality responses of neonate larva to lyophilized *Bacillus thuringiensis* BR145 applied on artificial diet.

Insect	Location	n	Slope ± (SE)	LC50 µg.cm2 (FL 0.05)	LC50 µg.cm2 (FL 0.05)	X2
<i>H. armigera</i>	Londrina, PR	224	2.171±0.409	0,294 (0,187- 0,402)	3.470 (1.890-12.088)	0.524
<i>C. includens</i>	Araguari, MG	192	2.362±0.478	0,368 (0.280-0.479)	3.557 (1.820-16.084)	3.833
<i>C. includens</i>	Campo Verde, MT	192	1.856±	0,277 (0.181-0.452)	4.967 (1.741-106.214)	1.522

^a n =number of insects tested.

^b Lethal concentration (LC); fiducial limits (FL) in µg.cm² of diet.

^c SE= standard error.

**Figure 1** – Subsystem coverage and category described by RAST server program in the genome sequence of *B. thuringiensis* BR145.

of 6,350,733 bp, N50 value of 84,578, and a G+C content of 34.78%. The RAST server program (Aziz *et al.*, 2008) proposed that this strain contains 6,647 coding sequences and 224 RNA genes in 494 subsystems (Figure 1).

Sequences that indicate insecticidal genes were identified using Blast tools. Five cry genes were found: *cryIAa*, *cryIAb*, *cryIAc*, *cryIIa*, and *cry2Ab*, as well as *cytI* and *vip3Aa* genes. All cry genes and the *vip3Aa* gene were found in plasmid sequences. Genes associated with virulence factors, such as phospholipases, hemolysins, metalloproteases, and enterotoxins were also located in this genome. The data can be found in genome annotation. Complete genome sequences of several *B. thuringiensis* strains are available on the NCBI Genome website (<https://www.ncbi.nlm.nih.gov/genome/genomes/486/>). Comparative analysis using BR145 contigs against the nonredundant database identified *B. thuringiensis* serovar *kurstaki* as the closest relative. The complete genome sequence of *B. thuringiensis* BR145 strain has been deposited at GenBank and is available on the NCBI website ([https://www.ncbi.nlm.nih.gov/nuccore?term=NZ_PDVK01000001:NZ_PDVK01000235\[PACC\]](https://www.ncbi.nlm.nih.gov/nuccore?term=NZ_PDVK01000001:NZ_PDVK01000235[PACC]))

The analysis of the genome sequence and the bioassay results allowed the characterization of *B. thuringiensis* BR145 as a new alternative to be used against a wide range of lepidopteran pests with economic importance, including *H. armigera* and *C. includens*, two important pests causing damages in soybean culture in Brazil.

Acknowledgments

We thank Fabio Paro from Entomology Laboratory (EMBRAPA Soja, Brazil) for performing bioassays against *H. armigera* and *C. includens*. This work received support from the Coordenação de Aperfeiçoamento de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil. Ricietto, A.P.S., Gonçalves, K.C.B and Appel, R.J.C were supported by fellowships from CAPES and CNPq.

Conflict of Interest

There are no conflicts of interest.

Author Contributions

APSR Investigation, Methodology, Writing-original draft; KCBG 121 Methodology; RJCA Methodology; DRSG Data curation Writing-original 122 draft; GTVB Data curation, Writing-original draft, Writing-review & editing. 123 Supervision; LAVB Data curation, Writing-original draft, Writing-review & 124 editing. All authors read and approved the final version.

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Associate Editor: Rogerio Margis

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