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BIOLOGICAL CONTROL

Identification of *Bacillus thuringiensis* Strains for the Management of Lepidopteran Pests

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Abstract

Bacillus thuringiensis (*Bt*)-based bioinsecticides and transgenic plants expressing proteins with insecticidal activity (Cry and Vip) have been successfully used in several integrated pest management programs worldwide. Lepidoptera comprise some of the most economically important insect pests of the major agricultural crops. In this study, the toxicity of 150 *Bt* strains was evaluated against *Helicoverpa armigera* (Hübner) larvae. Eight strains (426, 520B, 1636, 1641, 1644, 1648, 1657 and 1658) showed high insecticide activity against *H. armigera* and were therefore tested against *Anticarsia gemmatalis* (Hübner), *Spodoptera cosmioides* (Walker), *Chrysodeixis includens* (Walker), and *Diatraea saccharalis* (Fabricius) larvae. Our results showed that most of the *Bt* strains were also toxic to these lepidopteran species. The biochemical and molecular analyses of these strains revealed that they had a similar protein profile; however, their *cry* and *vip* gene contents were variable. In addition, the median lethal concentration (LC_{50}) of the selected strains indicated that the strains 1636, 1641, and 1658 were the most effective against *H. armigera*, showing LC_{50} values of 185.02, 159.44, and 192.98 ng/cm^2, respectively. Our results suggest that the selected *Bt* strains have great potential to control the lepidopteran pests *H. armigera*, *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includes*.

Keywords Insect pest control · entomopathogenic bacteria · *cry* gene · *vip* gene · bioinsecticide

Introduction

Helicoverpa armigera (Lepidoptera: Noctuidae) (Hübner) is a polyphagous pest of many economically important crops and is widely distributed around the world. After the first reports of *H. armigera* in Brazil in 2013 (Czepak et al. 2013; Tay et al. 2013), this insect pest has successfully spread to several countries of South and Central America (Murúa et al. 2014; Kriticos et al. 2015; Arneodo et al. 2015; Arnemann et al. 2016), increasing its geographical distribution that comprised Europe, Africa, Asia, and Australasia (Tay et al. 2013).

The application of synthetic insecticides has been one of the most common methods used to control *H. armigera*; however, multiple cases of resistance to insecticides have been reported in *H. armigera* populations due to heavy selection pressure (www.pesticideresistance.org) (Mota-Sanchez and Wise 2021). In addition, the use of synthetic insecticides may result in negative environmental impacts, including off-target effects on beneficial insects (Brühl and Zaller 2019; Siviter and Muth 2020).

Besides *H. armigera*, other lepidopteran insects such as *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae), *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidaea), *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidaea), and *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) are relevant pests of soybean, cotton and maize. These insects compromise the productivity of the main Brazilian agricultural crops owing to feeding damage as well as increase the production costs associated with pest management (Lemes et al. 2014; Girón-Pérez et al. 2014; Bacalhau et al. 2020). Therefore, alternative strategies that can be deployed not only in the management of *H. armigera* but also other lepidopteran species are highly desirable to reduce crop losses.

Bacillus thuringiensis (Berliner) (Bacillales: Bacillaceae) (*Bt*) is an aerobic Gram-positive bacterium able to produce insecticidal crystal proteins (ICPs) during the sporulation

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phase (Schnepf et al. 1998). The pesticidal proteins synthesized as crystalline parasporal inclusions comprise two classes, Crystal (Cry) and Cytolytic (Cyt) proteins, which are classified in different subclasses based on their amino acid sequence homology and structural similarity (Crickmore et al. 2020). In addition, *Bt* can synthesize another type of pesticidal protein that has been designated as vegetative insecticidal protein (Vip). It is produced during the vegetative growth phase and subsequently secreted into the culture medium (Estruch et al. 1996). The vegetative insecticidal proteins include the classes Vip (Vip3), Vpa (Vip2), and Vpb (Vip1 and Vip4) that do not display sequence or structural homology with the Cry or Cyt proteins (Crickmore et al. 2020). These pesticidal proteins have demonstrated activity toward different insect species, especially lepidopterans (Estruch et al. 1996; Warren 1997; Palma et al. 2013; Chakroun et al. 2016).

The high effectiveness of the *Bt* pesticidal proteins against the target insect species associated with the fact that these pesticidal proteins usually do not show adverse effects on non-target organisms make them an attractive strategy to suppress insect pest populations (Koch et al. 2015). *Bt* bioinsecticides and *Bt* transgenic plants have been extensively used as alternative insect control approaches, thereby promoting more sustainable agriculture (Bravo et al. 2011; Bacalhau et al. 2020). This is reflected by the increasing area cultivated with *Bt* transgenic plants worldwide and the substantial adoption of *Bt* bioinsecticides to improve the control of insect pest populations that represent a threat to agriculture (Lacey et al. 2015; ISAAA GM Approval Database. http://www.isaaa.org/gmapprovaldatabase 2018).

The present study aimed to identify *Bt* strains that can be used in the development of effective and sustainable strategies to control the lepidopterans *H. armigera*, *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includes*. We identified eight *Bt* strains highly toxic to *H. armigera*. These strains were further tested against *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includes* and molecularly characterized.

Material and methods

Insects

The *H. armigera*, *A. gemmatalis*, *C. includens*, *D. saccharalis*, and *S. cosmioides* larvae used in this study were obtained from established colonies maintained in the Laboratory of Biological Control, Embrapa Maize and Sorghum Research Center. The insect colonies had been reared for several generations at standard laboratory conditions of 25±2°C, 70±10% relative humidity, and a photoperiod of 12 h:12 h (light:dark).

Bt strains

A total of 150 *Bt* strains from Embrapa Maize and Sorghum *Bt* Collection were analyzed in this study. The strains were previously isolated from soil samples collected in different regions of Brazil and stored as pellets in a freezer at − 20°C (Valicente and Barreto 2003). The strain subsp. *kurstaki* HD-1 was provided by the United States Department of Agriculture (Columbus, Ohio, USA). The strain HD-1 was selected to be used as a reference strain in the bioassays because it has been employed in several commercial biopesticides directed against lepidopteran insects.

Selective bioassays

To select *Bt* strains with high insecticidal activity against *H. armigera* larvae, we performed surface contamination bioassays with the bacterial culture containing spore/crystal mixture. Each *Bt* strain was cultured in 40 mL of Luria–Bertani (LB) medium enriched with salts and glucose (Valicente and da Silva 2014) at 200 rpm, 28±2°C for 72 h. A sample of each bacterial culture was observed by a phase-contrast microscope to confirm the *Bt* sporulation and the presence of crystals. Bioassays were carried out by spreading 150 μL of the bacterial culture on the top and lateral surface of the artificial diet with 1 cm^3 placed into a plastic container. The treated diet was allowed to air dry at room temperature, and first-instar larvae were individually placed into each container. The *Bt* strain HD-1 and autoclaved deionized water were used as positive and negative controls, respectively. The plastic containers were kept in a climate-controlled room at 25±2°C, 70±10% relative humidity, photoperiod of 12 h:12 h (light:dark), and larval mortality was recorded after 7 days. Each bioassay was performed in triplicate with 24 larvae per replicate. The *Bt* strains that caused mortality above 75% against *H. armigera* larvae were evaluated against *A. gemmatalis*, *C. includens*, *D. saccharalis*, and *S. cosmioides* larvae as described above. The artificial diets used in the bioassays were prepared as reported in Table S1.

Dose-mortality bioassays

The insecticidal efficacy of the *Bt* strains against *H. armigera* larvae was evaluated using dose-mortality bioassays. The *Bt* strains were streaked on sporulation medium (Valicente and da Silva 2014) and incubated at 28±2°C for 96 h. Posteriorly, the bacterial cells were harvested, frozen overnight, lyophilized for 18 h, and used to prepare ten dilutions containing spore/crystal mixture (from 20 to 2000 ng/cm^2). A total volume of 35 μL of each dilution was applied uniformly over the diet previously poured into the wells of the plastic bioassay trays (Bio-BA 128©; BioServ) and air-dried at room temperature. One first instar larva was placed into each well, and the

trays were sealed with self-adhesive plastic sheets (BIO-CV-16; CD International Inc., Pitman, NJ, USA). The bioassay trays were placed in a climate-controlled room at 25 ±2°C, 70±10% relative humidity, and a photoperiod of 12 h:12 h (light:dark). The *Bt* strain HD-1 was used as a positive control and autoclaved deionized water as a negative control. Larval mortality was recorded after 7 days. Three replicates with 24 larvae were used for each of the ten dilutions. The lethal concentration values (LC_{50} and LC_{90}) were calculated by probit analysis (Finney 1971) using PoloPlus software. LC_{50} and LC_{90} values were considered significantly different when fiducial limits did not overlap.

Characterization of crystal protein (SDS–PAGE)

The *Bt* strains were grown in 5 mL of modified LB medium at 200 rpm, 28±2°C for 96 h, and then the proteins were extracted from 1.5 mL of grown culture as described previously (Lecadet et al. 1992). A volume of 15 μL of the protein samples was mixed with 5 μL of Bolt™ LDS Sample Buffer 4× (Life Technologies), boiled for 5 min, and separated in a Bolt® 4–12% Bis-Tris Plus gel using Bolt™ MES SDS Running Buffer (Life Technologies), following manufacturer's instructions. The molecular mass of the proteins was determined using the protein ladder SeeBlue® Plus2 Pre-Stained Standard (Life Technologies).

Screening of *cry* and *vip* genes

The DNA extraction was performed as described by Shuhaimi et al. (2001) with some adaptations. PCR reactions consisted of 3 μL of genomic DNA (90 ng), 0.25 μL of each primer (10 μM), 0.5 μL of dNTPs (10 mM), 0.4 μL of $MgCl_2$ (25 mM), 1 μL of 10× reaction buffer, 0.2 μL of Taq DNA polymerase (Kapa Biosystems) and autoclaved deionized water to a final volume of 10 μL. PCR amplifications were carried out in an Eppendorf Mastercycler thermal cycler as follows: an initial denaturation at 95°C for 2 min, followed by 30 cycles of amplification with 1 min of denaturation at 95°C, 1 min of annealing at 45–57°C, 1 min of extension at 72°C, and a final extension at 72°C for 10 min. PCR amplifications using the *vip* and *cry2* primers were carried out following conditions described previously (Ben-dov et al. 1997; Hernández-Rodríguez et al. 2009). The PCR products were visualized on 1–2% agarose gel using the L-PIX Molecular Image transilluminator (Loccus Biotecnologia). The primers used in this study are listed in Table 1.

Statistical analysis

After arcsine square root transformation, the mortality data were subjected to one-way ANOVA followed by Tukey's test to identify differences among the treatments, and values of $P < 0.05$ were considered statistically significant. Statistical analyses were conducted using custom scripts in R version 3.6.3 (R Core Team 2019). Data were expressed as mean percentage mortality ± standard error of the mean (SE).

Results

Insect bioassays

Eight out of 150 *Bt* strains examined against *H. armigera* through the selective bioassays showed larval mortality greater than 75% (426, 520B, 1636, 1641, 1644, 1648, 1657, and 1658), corresponding to 5.33% of the strains (Table 2). Dose-response bioassays were performed to evaluate the LC_{50} value of the selected *Bt* strains. Comparatively, the strain 1641 was approximately 10-fold more toxic to *H. armigera* larvae than the strain 426, with LC_{50} values of 159.44 ng/cm^2 and 1697.81 ng/cm^2, respectively (Table 3). The strains 1641, 1636, and 1658 were the most effective against *H. armigera*, with LC_{50} values of 159.44, 185.02 and 192.98 ng/cm^2, respectively. The LC_{50} values of these strains were statistically similar to the strain HD-1 that showed LC_{50} of 192.3 ng/cm^2. The LC_{50} of the strain 1644 was not determined since the highest concentration tested (2000 ng/cm^2) was not enough to kill 50% of the insects.

The insecticidal activity of the *Bt* strains that caused mortality above 75% in *H. armigera* was assayed against *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includens*. Our results showed that most of the strains were also active against the other lepidopteran species tested, with mortality superior to 75% and statistically similar to the strain HD-1. However, the strain 1648 did not show insecticidal activity against *A. gemmatalis*, *S. cosmioides* and *C. includes*, and the strain 520B was not toxic to *S. cosmioides* and *D. saccharalis*. In addition, the strains 1648 and 426 displayed relatively low toxicity against *D. saccharalis*, while the strain 426 showed low activity against *S. cosmioides* (Table 2).

Molecular characterization and SDS-PAGE protein profile

The PCR amplification using specific primers revealed that the *cry* and *vip* gene contents of the *Bt* strains were variable (Table 4). The *vip2*, *cry1Fa*/*1Fb*, and *cry2Ac* genes were the less frequent occurring in three strains, while *cry1B*, *cry2Ab2*, and *vip3* genes showed the highest frequency as they were found in eight strains, followed by *cry2Aa1* and *vip1* that were present in seven strains. The strains 520B and 1657 harbored *vip1*, *vip2* and *vip3* genes, whereas the other strains harbored at least one *vip* gene.

Table 1 Characteristics of the primers used to detect the *cry* and *vip* genes in the *Bt* strains.

Genes recognized	Sequence (5′-3′)	Tm (°C)	Size (bp)	Reference
cry1Ac	F-GTTAGATTAAATAGTAGTGG	53	180	Cerón et al. 1994
	R-TGTAGCTGGTACTGTATTG			
cry1B	F-CTTCATCACGATGGAGTAA	55	367	Cerón et al. 1994
	R-CATAATTTGGTCGTTCTGTT			
cry1C	F-AAAGATCTGGAACACCTTT	55	130	Cerón et al. 1994
	R-CAAACTCTAAATCCTTTCAC			
cry1D	F-CTGCAGCAAGCTATCCAA	55	290	Cerón et al. 1994
	R-ATTTGAATTGTCAAGGCCTG			
cry1Ea/cry1Eb	F-GGAACCAAGACGAACTATTGC	57	147	Cerón et al. 1995
	R-GGTTGAATGAACCCTACTCCC			
cry1Fa/cry1Fb	F-TGAGGATTCTCCAGTTTCTGC	57	177	Cerón et al. 1995
	R-CGGTTACCAGCCGTATTTCG			
cry1G	F-ATATGGAGTGAATAGGGCG	50	235	Cerón et al. 1995
	R-TGAACGGCGATTACATGC			
cry2Aa1	F-GTTATTCTTAATGCAGATGAATGGG	45	498	Ben-Dov et al. 1997
	R-GAGATTAGTCGCCCCTATGAG			
cry2Ab2	F-GTTATTCTTAATGCAGATGAATGGG	45	546	Ben-Dov et al. 1997
	R-TGGCGTTAACAATGGGGGGAGAAAT			
cry2Ac	F-GTTATTCTTAATGCAGATGAATGGG	45	725	Ben-Dov et al. 1997
	R-GCGTTGCTAATAGTCCCAACAACA			
vip1	F-TTATTAGATAAACAACAACAAGAATA TCAATCTATTMGNTGGATHGG	50	585	Hernández-Rodríguez et al. 2009
	R-GATCTATATCTCTAGCTGCTTTTTCAT AATCTSARTANGGRTC			
vip2	F-GATAAAGAAAAAGCAAAAGA ATGGGRNAARRA	50	845	Hernández-Rodríguez et al. 2009
	R-CCACACCATCTATATACAGTAATATTT TCTGGDATNGG			
vip3	F-TGCCACTGGTATCAARGA	47	1621	Hernández-Rodríguez et al. 2009
	R-TCCTCCTGTATGATCTACATATGCAT TYTTRTTRTT			

(F) Forward; (R) Reverse

Table 2 Toxicity of the *Bt* strains against different lepidopteran species.

Strain	*Helicoverpa armigera*	*Anticarsia gemmatalis*	*Spodoptera cosmioides*	*Chrysodeixis includens*	*Diatraea saccharalis*
HD-1	100±0.00 c	100±0.00 b	100±0.00 d	100±0.00 c	96.79±1.62 c
426	100±0.00 c	100±0.00 b	27.29±8.22 b	100±0.00 c	43.91±14.50 b
520B	98.33±1.67 c	100±0.00 b	13.13±5.37 ab	85.55±1.12 b	12.50±6.49 ab
1636	98.55±1.45 c	98.55±1.45 b	100±0.00 d	100±0.00 c	100±0.00 c
1641	98.55±1.45 c	98.24±1.75 b	76.99±8.18 c	86.75±4.55 bc	95.65±4.35 c
1644	100±0.00 c	100±0.00 b	95.65±4.35 cd	96.96±3.03 bc	100±0.00 c
1648	98.06±1.94 c	23.33±13.02 a	8.33±4.81 ab	7.19±3.73 a	40.68±6.34 b
1657	78.46±3.92 b	100±0.00 b	91.30±2.51 cd	97.10±2.90 bc	100±0.00 c
1658	98.55±1.45 c	97.91±2.08 b	100±0.00 d	100±0.00 c	97.09±1.46 c
Control	5.55±3.67 a	7.19±3.73 a	2.77±2.77 a	4.34±4.34 a	0.00±0.00 a

Percentage of mortality values (means ± SE) followed by the same letter in the column do not differ statistically (one-way ANOVA followed by Tukey's test at $P < 0.05$)

Table 3 LC_{50} and LC_{90} of the *Bt* strains against first instar larvae of *Helicoverpa armigera* after 7 days of bioassay.

Strain	LC_{50} (95% FL) (ng/cm^2)	LC_{90} (95% FL) (ng/cm^2)	Slope ±SE	x^2	df	*P*-value
HD1	192.30 (143.91–259.12) a	1500.16 (956.19–2837.46) a	1.43±0.09	11.42	7	0.12
426	1697.81 (957.75–4122.17) b	113070.33 (28991.06–1114099.39) b	0.70±0.09	2.69	6	0.84
520B	1038.46 (703.54–1763.35) b	32318.73 (12864.33–134318.93) b	0.85±0.09	2.16	7	0.95
1636	185.02 (139.19–250.20) a	1198.40 (774.97–2211.50) a	1.57±0.10	9.10	6	0.16
1641	159.44 (127.09–206.36) a	1727.80 (1050.88–3533.70) a	1.23±0.12	3.95	5	0.56
1644	N.D.					
1648	898.22 (568.48–1862.44) b	14616.01 (5283.82–95079.79) b	1.05±0.11	9.92	7	0.19
1657	1500.08 (808.41–4339.18) b	54054.12 (13059.16–1024236.37) b	0.82±0.09	10.21	6	0.11
1658	192.98 (150.34–245.35) a	1314.54 (917.58–2146.60) a	1.53±0.11	6.72	6	0.34

LC_{50} and LC_{90} values designated by different letters are significantly different due to non-overlap of 95% CIs

N.D. Not determined. Less than 50% mortality was obtained even at concentrations as high as 2000 ng/cm^2

Protein analyses by SDS-PAGE revealed that the strains 426, 520B, 1636, 1641, 1644, 1648, 1657, and 1658 have a protein profile similar to the strain HD-1 with the presence of two principal proteins of about 140 and 55 kDa (Fig. 1). These molecular masses are related to the Cry1, Cry9 (130–140 kDa), and Cry2 (50–75 kDa) protein classes and could reflect the insecticidal activity of the strains since these classes of Cry proteins usually show toxicity toward lepidopteran species.

Discussion

Currently, several control methods, including synthetic insecticides, *Bt* crops, biological control and cultural practices have been adopted in the integrated pest management directed against the insect pests *H. armigera*, *A. gemmatalis*, *S. cosmioides*, *C. includes*, and *D. saccharalis* to achieve more effective crop protection (Bortolotto et al. 2015; Blanco et al. 2016; Mascarin et al. 2018; Vieira et al. 2019). The economic, environmental and social benefits offered by the insect-resistant *Bt* crops and *Bt* bioinsecticides have driven increasing adoption of these strategies for controlling a wide variety of insect pests (Lacey et al. 2015; Dively et al. 2018; Romeis et al. 2019). However, insect populations can evolve resistance to *Bt* pesticidal proteins under field conditions, compromising the effectiveness of the transgenic plants and bioinsecticides (Jurat-Fuentes et al. 2021). This scenario boosts the identification of novel *Bt* strains as well as genes that can be applied in such insect management strategies.

In this study, the insecticidal activity of *Bt* strains was investigated to find promising strains for the development of biopesticides against lepidopteran insects. We demonstrated through the selective bioassays that only 5.3% of the strains evaluated were highly toxic to *H. armigera* larvae. In addition, among the selected strains, 1641, 1636, and 1658 displayed the highest toxicity against *H. armigera* as they had the lowest LC_{50} values. Some studies have shown that the proportion of strains toxic to different lepidopterans is usually low as reported in our study (Valicente and Barreto 2003; Apaydin et al. 2008; Santos et al. 2009; Silva et al. 2012; Azzouz et al. 2015; Lone et al. 2017).

Table 4 *cry* and *vip* genes present in the *Bt* strains.

Strains	Gene profile
HD-1	*cry1Ac*, *cry2Aa1*, *cry2Ab2*, *cry2Ac*, *vip1*, *vip3*
426	*cry1Ac*, *cry1B*, *cry1C*, *cry1D*, *cry1EA/1Eb*, *cry1Fa/1Fb*, *cry2Aa1*, *vip1*
520B	*cry1B*, *cry1C*, *cry1D*, *cry1Ea/1Eb*, *cry2Aa1*, *cry2Ab2*, *vip1*, *vip2*, *vip3*
1636	*cry1Ac*, *cry1B*, *cry1G*, *cry2Aa1*, *cry2Ab2*, *vip2*, *vip3*
1641	*cry1Ac*, *cry1B*, *cry1D*, *cry1Ea/1Eb*, *cry1G*, *cry2Aa1*, *cry2Ab2*, *vip1*, *vip3*
1644	*cry1B*, *cry1C*, *cry1D*, *cry2Ab2*, *cry2Ac*, *vip1*, *vip3*
1648	*cry1B*, *cry1C*, *cry1D*, *cry2Aa1*, *cry2Ab2*, *vip3*
1657	*cry1B*, *cry1D*, *cry1Ea/1Eb*, *cry1Fa/1Fb*, *cry1G*, *cry2Ab2*, *cry2Ac*, *vip1*, *vip2*, *vip3*
1658	*cry1Ac*, *cry1B*, *cry1Fa/1Fb*, *cry1G*, *cry2Aa1*, *cry2Ab2*, *vip1*, *vip3*

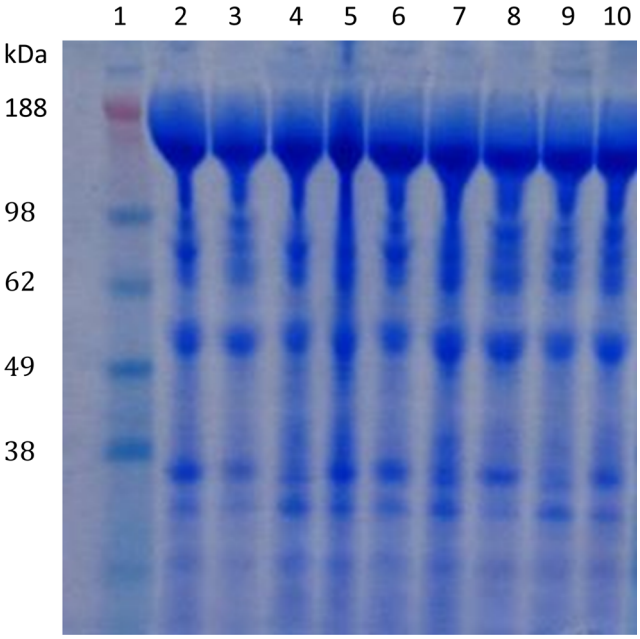

Fig. 1 Protein profile of the *Bt* strains toxic against *Helicoverpa armigera* analyzed by SDS-PAGE. 1: SeeBlue Plus2 Pre-stained Protein Standard (Life Technologies), 2: 426, 3: 528B, 4: 1636, 5: 1641, 6:1644, 7: 1648, 8: 1657, 9: 1658, 10: HD-1

We found that the strains with toxicity against *H. armigera* varied considerably their biological activity against *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includens*. The strain 1648 had low or no toxicity to these other lepidopteran species, whereas the strains 1636, 1641, 1644, 1657, and 1658 caused high levels of mortality in all these species. Furthermore, the strains 426 and 520B were not effective against *D. saccharalis* and *S. cosmioides*. Our results demonstrated that while some *Bt* strains had a broad spectrum of activity against the lepidopteran pests, other strains were more specific. These results indicate that evaluation of the insecticidal activity of the *Bt* strains against the target insects through laboratory assays is a critical step to select the strains that cause high levels of mortality and to determine the feasibility of their use as biological control agents.

The differential susceptibility of the lepidopteran species to the *Bt* strains tested in our study may be due to the differences among the receptors present in their midgut since the existence of specific receptors for the Cry and Vip pesticidal proteins is essential for their activity (Pardo-López et al. 2013; Chakroun et al. 2016). The pH, protease composition and activity of the larval midgut affect the solubilization and proteolytic processing of the Cry and Vip proteins. Accordingly, differences in these features may also lead to variations in the susceptibility (Fortier et al. 2007; Talaei-Hassanloui et al. 2014; Jurat-Fuentes and Crickmore 2017; Kunthic et al. 2017). In addition, the synergistic or antagonistic interactions between the Cry and Vip proteins, as well as the expression level of these proteins are factors that can influence the toxicity of *Bt* strains (Chen et al. 2014; Lemes et al. 2014).

Bt strains active against more than one insect pest are especially attractive for the development of biopesticides because a broader range of insect species could be controlled with their use. Thus, this is an important aspect that should be considered when selecting the *Bt* strains. The Cry and Vip proteins can be order-specific and even species-specific (Chakroun et al. 2016); however, strains that present diversity of genes might have increased activity and target a wider spectrum of insect pests. Moreover, the presence of several *cry* and *vip* genes that express pesticidal proteins with different modes of action can prevent or at least delay the development of resistance in the target insect populations. In agreement with our results indicating that the strains harbor genes coding for Cry1, Cry2 and Vip3 proteins, some studies with *H. armigera*, *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includens* have evaluated the activity of different proteins belonging to these classes, and demonstrated high levels of toxicity (Ruiz de Escudero et al. 2014; Sebastião et al. 2015; Mushtaq et al. 2017; Falcon et al. 2019; Bel et al. 2019).

We found that the *Bt* strains 426, 520B, 1636, 1641, 1644, 1648, 1657, and 1658 showed a high diversity of *cry* and *vip* genes; however, we cannot rule out the possibility that other genes that are present in the strains but were not screened in our study may contribute to the overall toxicity of these strains. Further studies at the genomic, transcriptomic, and proteomic levels will be required to accurately identify all *cry* and *vip* genes that may be associated with the toxicity of these strains. Nonetheless, our results suggest that the selected strains might be a potential source of genes for the development of insect-resistant transgenic crops.

In this study we report the identification and characterization of *Bt* strains that have great potential to be employed in the formulation of bioinsecticides for the management of *H. armigera*, *A. gemmatalis*, *D. saccharalis*, *S. cosmioides*, and *C. includes.* Further studies in greenhouse and field conditions should be performed to evaluate the effectiveness of these strains in suppressing the target insect pest populations under agricultural environments.

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Declarations

Conflict of interest The authors declare no competing interests.

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