MOLECULAR CHARACTERIZATION OF THE *Bacillus thuringiensis* (BERLINER) STRAINS 344 AND 1644, EFFICIENT AGAINST FALL ARMYWORM *Spodoptera frugiperda* (J. E. SMITH)

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ABSTRACT - *Bacillus thuringiensis* (Berliner) strains 344 and 1644 belonging to the CNPMS/Embrapa (Maize and Sorghum National Research Center) Microorganism Bank, located in Sete Lagoas, MG, showed to be highly efficient in controlling fall armyworm, *Spodotpera frugiperda* (J.E. Smith). Strains 344 and 1644 were isolated from soil samples originated from Iguassu Falls and Rolândia, respectively, Paraná State, Brazil. While strain 344 harbors *cry1Ab, cry1B, cry1E* and *cry1Fb* genes, strain 1644 showed cry1B, cry1C, and cry1D and cry1Fb genes. The strain 344 crystal has bipyramidal shape and showed LC_{50} equal to 8.21 x 10^6 spores/mL. Strain 1644 has cuboid crystal and showed LC_{50} equal to 2.07 x 10^6 spores/mL. Comparative analysis of protein profile, plasmid and amplification with primers ERIC allowed the differentiation of the evaluated strains.
Key words: insect pathology, *Bacillus thuringiensis*, c*ry* genes.

CARACTERIZAÇÃO MOLECULAR DAS CEPAS 344 E 1644 DE *Bacillus thuringiensis* (BERLINER), EFICIENTES NO CONTROLE DA LAGARTA-DO-CARTUCHO, *Spodoptera frugiperda* (J. E. SMITH)

RESUMO - As cepas 344 e 1644 de *Bacillus thuringiensis* (Berliner), do Banco de Microrganismos da Embrapa, situada em Sete Lagoas, MG, mostram-se muito eficientes no controle da lagarta-do-cartucho, *Spodoptera frugiperda* (J.E. Smith). Essas cepas foram isoladas de amostras de solo das cidades de Foz do Iguaçu e Rolândia, respectivamente, no estado do Paraná, Brasil. Enquanto na cepa 344 estão presentes os genes *cry1Ab, cry1B, cry1E* e *cry1Fb*, a cepa 1644 apresentou os genes *cry1B, cry1C, cry1D e cry1Fb*. O cristal da cepa 344 possui formato bipiramidal e apresentou CL_{50} igual a 8,21 x 10^6 esporos/mL. Já a cepa 1644 possui cristal cubóide e CL_{50} igual a 2,07 x 10^6 esporos/mL.

Análises comparativas dos perfis protéico, plasmidial e por amplificação com primers ERIC permitiram a diferenciação das cepas avaliadas.

Palavras-chave: patologia de insetos, *Bacillus thuringiensis*, genes *cry*.

Fall armyworm, *Spodoptera frugiperda*, is considered one of the most important insect pests in maize crop in Brazil and may reduce yield production up to 34% (Carvalho, 1970). The life cycle of this insect pest is completed within 30 days under laboratory conditions, and the number of eggs may vary from 100 to 200 eggs per clutch/female and a total of 1500 to 2000 eggs/female. Therefore, the potential damage that this insect may cause in the field is high. Chemical control is widely used to control this insect, however efficient *Bacillus thuringiensis* (*Bt*) strains may become feasible to control *S. frugiperda* in the field.

Bacillus thuringiensis is a Gram-positive bacterium that occurs naturally in soil, water, dead insects and grain dust (Lambert & Peferoen, 1992). During the stationary and/or sporulation phase, this bacterium produces a sporangium that contains an endospore and one or more crystalline proteinaceous inclusions called δ-endotoxins. These δ-endotoxins are toxic to a great number of insects and turns *Bt* into a valuable tool to be used in the Insect Pest Management (IPM). More than 200 different genes coding for δ-endotoxins were already identified, and the vast majority are active against a few order of insects. The activity of the δ-endotoxins is restricted to the midgut of the insect. When larvae were fed with a great amount of toxin, they suffer paralysis and die (Glare & O'Callaghan, 2000). The proteins that form these crystals comprises 20 to 30% of the total protein of the bacteria during the sporulation phase (Boucias & Pendland, 1998). Molecular weight of these crystals varies depending on the method of the dissolution, and upon solubilization in basic pH, become protoxins that are composed of Cry proteins or insecticidal crystal proteins (ICPs) with molecular masses ranging from 25 to 140 kDa (Céron et al., 1995; Berhnard et al., 1997). Usually, crystals that are generally bipyramidal in shape are active against lepidopterans. Crystal shapes also include triangles, cuboids, ovoid, flat, and amorphous in many subspecies. Kronstad et al. (1983) report that *cry* genes occur in plasmids and in some subspecies in bacterial chromosomes. Interest in *Bt* plasmids started at the end of the 1970s when a correlation was established between the formation of crystals and the presence of certain plasmids. Later on, attention was focused on the location of *cry* genes in plasmids and on the transfer of plasmids between different strains of *Bt* (González et

al., 1982). However, little attention was paid to the importance of plasmid patterns as a tool for strain characterization.

Boucias & Pendland (1998) estimate 60,000 *Bt* isolates in collections all over the world. Embrapa Maize and Sorghum Research Center, located in Sete Lagoas, Brazil, has a *Bt* Bank with more than 4500 isolates originated from soil samples, dead insect and grain dust.

In terms of specificity, *Bt* shows differences in toxicity regarding insect species, but it is not constant within the same order (e.g. Lepidoptera). Dramatic differences in sensitivity were found among species, i.e. *Spodoptera* spp. are difficult to control with *Bt* based bioinsecticides (strain HD1), however *Heliothis virescens* and *Plutella xylostella* are not (Baum et al., 1999). Beegle & Yamamoto (1992) also confirmed that *Bt* is not much efficient in controlling *S. frugiperda.* Aronson et al. (1991) reported that crystal solubility is the main factor of the *Bt* insecticidal efficiency and suggested that this factor may explain the low susceptibility of *S. frugiperda* to *Bt*. So far, *Bt kurstaki* has been the most used strain ever in commercial products, and some of them are the strain HD-1 belonging to the Dulmage collection (Nakamura & Dulmage, 1988).

Serotyping is the most widely accepted subspecific classification technique for varieties of *Bt*, even if strains from the same serovar do not necessarily share the biochemical, genetic, or toxicological attributes. Although serotyping is a reliable and straightforward technique, it is performed only in a few laboratories around the world, in particular the Pasteur Institute in France, where a *Bt* collection is held. Therefore, alternative techniques, especially molecular techniques, are being developed to try to overcome those constrains (Phucharoen et al., 1999).

The enterobacterial repetitive intergenic consensus (ERIC) sequences, also known as intergenic repeat units (IRUs) are present in many copies in the genomes of *Escherichia coli*, *Salmonella typhimurium*, and other enterobacteria (Hulton et al., 1991). In ERIC-PCR a band pattern is obtained by amplification of genomic DNA located between ERIC elements or between ERIC elements and other repetitive DNA sequences and generate distinctive electrophoretic patterns among different strains. ERIC-PCR involves the use of primers of 22 nucleotides with high homology to repetitive intergenic sequences that are dispersed throughout the procaryotic kingdom (Versalovic et al., 1991).

Two *Bt* strains isolated in Paraná State, Brazil, showed to be very efficient in controlling fall armyworm in laboratory bioassays. The objectives of this work were to characterize these strains using different molecular techniques as a valuable routine characterization and check their potential to be used against fall armyworm in the field.

Material and Methods

B. thuringiensis strains: Strains were isolated, used and kindly provided by Valicente & Barreto (2003). These authors showed that strains 344 and 1644 were the most promising in preliminary tests against fall armyworm larvae.

Bioassay with B. thuringiensis and LC$_{50}$: Two-day old healthy *S. frugiperda* larvae were used to determine the efficiency of each strain and the laboratory conditions were 25°C, 70% humidity and 14 h photophase. The insects were reared in artificial diet and transferred and maintained individually in 50 mL disposable plastic cups, each one containing 5.0 g of artificial diet previously immersed in *Bt* suspension containing spores and crystals. This suspension was obtained by scrapping *Bt* colonies from the plates and the concentration varied from 10^3 to 10^8 spores/mL. Mortality was daily evaluated. Four replicates with twenty-five larvae per bioassay were used. LC$_{50}$ was obtained using two-day old larvae. MSTAT software was used to calculate the LC$_{50}$.

PCR - cry1 genes: PCR was performed using fresh DNA that was isolated using *Bt* colony grown overnight in solid medium. For DNA isolation a loopful of strains 344 and 1644, were mixed with 100 µL distilled autoclaved water, frozen at -80°C for 15 minutes and immediately after immersed into boiling water for five minutes. Primers were selected from the highly conserved regions of the *cry1* genes. Also, it was used *cry1* gene primers designed by Cerón et al. (1994) and Cerón et al. (1995). Table 1 shows the sequence of each primer, the *cry1* gene subgroup identified and the predicted size of the PCR product. Identification of known *cryI* genes was done using 5 µL of DNA sample with 1.5 U *Taq* DNA polymerase (Invitrogen), 250 nM each deoxynucleoside triphosphate, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 400 nM each primer and 3 mM MgCl$_2$, in a final volume of 25 µL. PCR cycles were as follows: a single denaturation step for 3 min at 95°C, followed by 29 cycles, each consisting of a denaturation step at 95°C for 1 min, annealing temperature depending on the primer set used (usually between 50 and 57°C) for 1 min, and extension for 1 min at 72°C and a final extension step at 72°C for 5 minutes. To avoid false negatives, each PCR reaction was performed at least three times. PCR products were analyzed using a 3% agarose gel electrophoresis in TAE buffer 1X (40 mM Tris-acetate, 1 mM EDTA pH 8.0) at 110 V for 1h and stained with ethidium bromide solution (1 µg/mL) for 30 min. The results were visualized under a UV light using Gel Logic 200 (Kodak, Rochester, USA).

Genomic DNA Extraction: Fresh 30 mL LB broth cultures of strains 344 and 1644 were centrifuged at 3000 x *g* for 5 min at 4°C, and the pellets were washed in 10 mL of J buffer (1 M Tris-HCl, 0.1 M EDTA, 0.15 M

TABLE 1. Primers used for c*ry1* genes characterization of *Bacillus thuringiensis*.

Name	Sequence (5´- 3´)	Gene	Fragment (bp)	Reference
CJ01	TTATACTTGGTTCAGGCCC	*cryIA(a)*	246	Céron et al., 1994
CJ02	TTGGAGCTCTCAAGGTGTAA	*cryIA(d)*		
CJ03	CAGCCGATTTACCTTCTA	*cryIA(d)*	171	Céron et al., 1994
CJ02	TTGGAGCTCTCAAGGTGTAA	*cryIA(d)*		
CJ04	AACAACTATCTGTTCTTGAC	*cryIA(b)*	216	Céron et al., 1994
CJ05	CTCTTATTATACTTACACTAC	*cryIA(c)*		
CJ06	GTTAGATTAAATAGTAGTGG	*cryIA(c)*	180	Céron et al., 1994
CJ07	TGTAGCTGGTACTGTATTG	*cryIA(c)*		
CJ08	CTTCATCACGATGGAGTAA	*cryIB*	367	Céron et al., 1994
CJ09	CATAATTTGGTCGTTCTGTT	*cryIB*		
CJ10	AAAGATCTGGAACACCTTT	*cryIC*	130	Céron et al., 1994
CJ11	CAAACTCTAAATCCTTTCAC	*cryIC*		
CJ12	CTGCAGCAAGCTATCCAA	*cryID*	290	Céron et al., 1994
CJ13	ATTTGAATTGTCAAGGCCTG	*cryID*		
CJ14	GGAACCAAGACGAACTATTGC	*cryIEa*	147	Céron et al.,1995
CJ15	GGTTGAATGAACCCTACTCCC	*cryIEb*	147	
CJ16	TGAGGATTCTCCAGTTTCTGC	*cryIFa*	177	Céron et al.,1995
CJ17	CGGTTACCAGCCGTATTTCG	*cryIFb*	177	
Cry1Ab51320F	AATTTGCCATCCGCTGTA	*cry1Ab*	418	Valicente (data
Cry1Ab51740R	TTGTGGTAGAAGCGTAGCGA	*cry1Ab*	418	not published)

NaCl [pH 8.0]). Pellets were resuspended in 4 mL of J buffer, and lysozyme was added to a final concentration of 4 mg/mL, followed by incubation at 37°C for 30 min. Then, 10 μL of RNase (10 mg/mL) were added, and suspensions were incubated for 15 min at 37°C. Next, 200 μL of 20% sodium dodecyl sulfate were added and incubated for 20 min at 65°C, followed by the addition of 30 μL of proteinase K (10 mg/mL) and incubation overnight at 65°C. A total of 1.15 mL of 5 M NaCl was then added, gently mixed in ice for 15 min, and centrifuged at 3900 x *g* for 20 min at 4°C. The supernatant was mixed with an equal volume of isopropanol and centrifuged at 17000 x *g* for 20 min at 4°C. The pellet was washed with 70% ethanol, air dried, and dissolved in 100 μL TE buffer. DNA was

quantified by spectrophotometry (NanoDrop, Wilmington, USA) and samples were stored at -20°C until further use.

Eric-PCR amplification and electrophoretic analyses: ERIC primers were used as follows: ERIC 1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC 2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Versalovic et al., 1991). PCR assays were performed in a 20 μl reaction mixture containing 30 ng of template DNA, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 0.5 μM of each primer, 2.5 mM $MgCl_2$, 150 μM deoxynucleoside triphosphate mixture and 1 U of *Taq* DNA polymerase (Invitrogen). PCR amplifications were performed under the following conditions: 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, 41 cycles with a final temperature of 20°C. Eric-PCR patterns were visualized by agarose gel electrophoresis. Amplification products were loaded onto 1.2% agarose gel and run in TAE buffer 1X at 50 V during 1 h. Gel was stained with 1 μg of ethidium bromide/mL and documented with a Gel Logic 200 (Kodak, Rochester, USA).

Plasmid DNA Extraction: *B. thuringiensis* strains 344 and 1644 were cultivated in 200 mL of Luria-Bertani (LB) medium, at 200 rpm and 28°C until O.D.$_{600 nm}$ was between 1.5 and 2.0. The culture cells were centrifuged at 8000 x g for 10 minutes at 4°C and the pellet resuspended in 10 mL of solution A (20 mM Tris-HCl pH 8.0; 5 mM EDTA pH 8.0; 20% sucrose), containing 15 mg/mL of lysozyme. Samples were incubated at 37°C for 90 minutes and 20 mL of solution B were added (0.2 M NaOH e 0.1% SDS). Suspensions were mixed up and maintained at room temperature for 5 minutes. Ten mL of solution C (3 M potassium acetate pH 5.5) were added to the samples and homogenized, maintained in ice for 15 minutes and centrifuged at 8000 x g for 10 minutes at 4°C. Supernatant was removed, filtered through two layers of sterile gaze and 25 mL of isopropanol added to the supernatant. This solution was incubated at -20°C for one hour. Samples were centrifuged again at 8000 x g for 20 minutes and the supernatant discarded. Pellet was dried at room temperature and resuspended in 10 mL of TE (10 mM Tris; 1 mM EDTA; pH 8.0).

Plasmid DNA Purification with cesium chloride ultracentrifugation: During plasmid DNA purification, 11 g of cesium chloride were added in each sample, which was homogenized until complete dissolution. Then, 800 μL of ethidium bromide (10 mg/mL) were added and the samples transferred to new tubes and centrifuged at 150000 x g for 24 hours at 20°C. Under a UV light, the band containing the plasmid DNA was removed and transferred to a 1.5 mL microtube. Ethidium bromide removal was done by adding one volume of 1-butanol saturated with water. Samples were agitated for 5 minutes and centrifuged at 10000 x g for 3 minutes at room

temperature. The organic phase was discarded and to the inferior phase an equal volume of 1-butanol saturated with water was added. This procedure was repeated four times until the organic phase was completely clear. This phase was transferred to a new microtube and three volumes of water were added. Plasmids were precipitated with two volumes of absolute ethanol and incubated during 30 minutes at 4°C. After centrifugation at 16000 x g for 20 minutes, the supernatant was discarded and the pellet washed with 200 µL of ethanol 70%. The samples were centrifuged at 16000 x g for 10 minutes and the supernatant discarded. Pellet was dried at room temperature, resuspended in 50 µL of TE (10 mM Tris; 1 mM EDTA; pH 8.0) and quantified in spectrophotometer (NanoDrop, Wilmington, USA).

DNA electrophoretic analysis: Plasmid DNA samples were analyzed in 0.5% agarose gel with TAE buffer 1X, and 1.5 µg of plasmid DNA was used to load a gel that ran at 10 V for 16 hours. The agarose gel was dyed for one hour with ethidium bromide at 1 µg/mL and the excess washed with deionized water for one hour. Plasmid DNA was observed under the UV light and registered in the Gel Logic 200 (Kodak, Rochester, USA).

Protein electrophoresis: Protein analysis were performed by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% SDS-PAGE). Bacterial strains 344 and 1644 were cultivated in 50 mL of Luria-Bertani (LB) for four days at 30°C under continuous shaking. The culture cells were centrifuged at 16000 x g for 10 minutes and the pellet resuspended in 10 mL of 0.01% triton. This procedure was repeated three times. In the last wash, 5 mL of 0.01% triton was added with 50 mM Tris-HCl pH 8.0 and 10 mM NaCl. The mixture was centrifuged at 16000 x g for 5 minutes and pellet was resuspended in 5.0 mL of 50 mM buffer bicarbonate pH 10.5 with 10 mM β-mercaptoetanol and incubated for 3 hours at 37°C under constant shaking. Samples were centrifuged at 16000 x g for 10 minutes. The supernatant was incubated with trypsin (200 µg/mL) at 37°C for two hours. Reaction was inactivated with 1mM PMSF. Polyacrylamide gel was run at 10 mA for one hour.

Results and Discussion

B. thuringiensis strains and subspecies identification: *B. thuringiensis* strain 344 was identified as *Bacillus thruringiensis sv tolworthi* serovar H9 by Institute Pasteur, IEBC number T09 046. Strain 1644 was identified only as *Bacillus thuringiensis*. Strain 344 has bipyramydal crystals and the strain 1644 cuboid crystal, both confirmed using Scanning Electron Microscope analysis (Figure 1) (Valicente & Souza 2004).

Bioassay with B. thuringiensis and LC$_{50}$: Lethal concentration 50 was determined using doses ranging from 10^3 to 10^9 spores/mL against two-day-old healthy fall armyworm
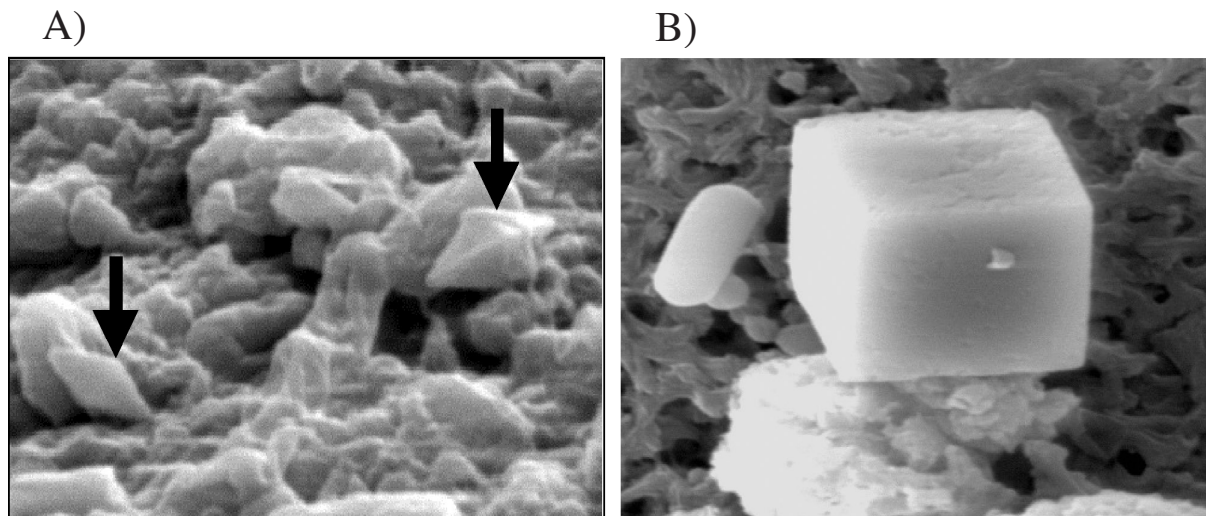
A)

B)

FIGURE 1. Crystal protein of *Bacillus thuringiensis*. **A)** Strain 344 with a bipyramidal shape - Scanning Electron Microscope analysis with magnification of 20,000X. **B)** Strain 1644 with a cuboid shape - Magnification of 10,000X (Valicente & Souza, 2004).

larvae. LC_{50} was 8.21 x 10^6 spores/mL for strain 344 while strain 1644 showed LC_{50} equal 2.07 x 10^6 spores/mL.

Identification of *cry1* genes: PCR was used to detect *cry1* genes from strains 344 and 1644 that showed to be efficient in controlling fall armyworm. The expected sizes of the PCR products of the *cry1* genes ranged from 130 bp to 418 bp. Strain 344 harbors genes *cry1Ab, cry1B, cry1E* and *cry1Fb*. A product size for *cry1B* was 367 bp, *cry1E* 147 bp and *cry1Fb* was 177 bp (Céron et al., 1994; Céron et al., 1995). Another set of primers was used, cry1Ab51320F and cry1Ab51740R, to verify *cry1Ab* gene that generated a PCR product of 418 bp (Valicente, data not published). Strain 1644 harbors *cry1B, cry1C*, with 130 bp fragment, *cry1D* (290 bp*)* and *cry1Fb* genes. Some of these results are shown on Figure 2.

The strain 344, although also very efficient in killing fall armyworm, does not harbor *cry1C* gene. Our data are in agreement with those reported by Loguercio et al. (2001) who showed that the presence of the *cry1C* genes does not necessarily correlate with high toxicity to *S. frugiperda* larvae and suggest that other proteins present in the *Bt* strains analyzed might be more important for toxicity. These findings are corroborated by Valicente & Fonseca (2004). Additionally, other factors, such as differences in toxin expression or synergistic effects between some cry toxins, could account in toxicity of *Bt* strains (Monnerat et al., 2006).

ERIC-PCR: The amplification with ERIC primers of 344 and 1644 *Bt* strains revealed multiple bands, ranging between 200 and 3000 bp (Figure 3). The 344 strain presented a
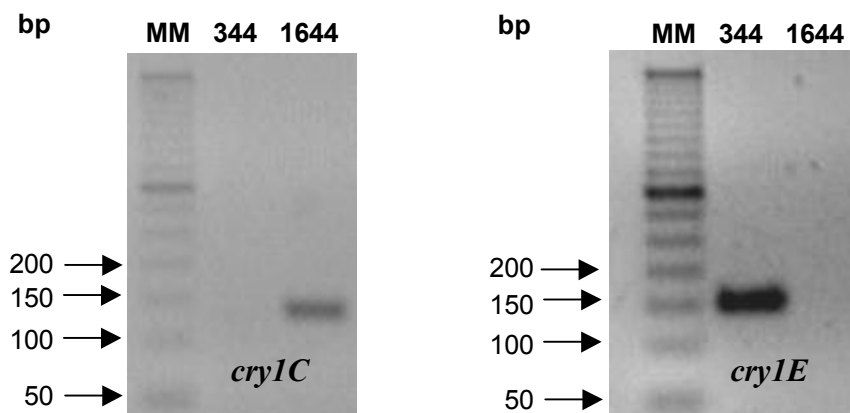
FIGURE 2. PCR analysis of the *cryIC* and *cryIE* genes of the strains 344 and 1644. MM - Molecular weight 50 pb Ladder (Invitrogen).
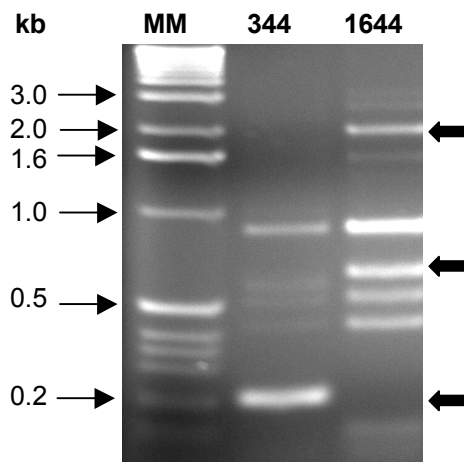

FIGURE 3. Comparative analysis of the strains 344 and 1644 based on PCR-amplified fragment bands using ERIC1-R and ERIC2 primers. MM - Molecular weight 1 Kb Ladder (Invitrogen).

fragment of approximately 200 bp allowing its differentiation from the 1644 strain. Diversely from 344 strain, 1644 strain showed at least two unique bands with sizes around 750 and 2000 bp. With ERIC-PCR, strain 344 was discriminated from the 1644, indicating that these strains are genetically different. This technique has been used for strain identification and for target prevision, without the need to carry out all the tedious and time-consuming bioassays (Carozzi et al., 1991). The use of repetitive DNA sequences such as ERIC for bacterial classification is becoming frequent, and has allowed comparisons of possible genetic similarities among different bacterial genomes (Versalovic et al., 1991; Louws et al., 1994; Selenska-Pobell et al., 1995). Our study supports the data presented by Shangkuan et al. (2001) and Lima et al. (2002) that revealed that ERIC elements are present in the genomes of *Bt*. Our results also

support the conclusion of De Bruijn (1992) that the ERIC PCR could become a powerful tool to differentiate strains of microorganisms. ERIC-PCR fingerprint analyses of *Bt* strains showed to be simple, fast, and reproducible, it may become a standardized characterization procedure.

Plasmid pattern of *B. thuringiensis* strains: In this work we compared the plasmid patterns of two *Bt* strains. While strain 344 presented about 11 plasmids, strain 1664 showed at least eight. The relevance of plasmids in *Bt* strains is assumed by the regular presence of a set of plasmids, which can vary in number from 1 to 17 (Aptosoglou et al., 1997; Gonzáles & Carlton, 1980). The profile of strains indicated plasmids of different sizes, ranging from 3 kb to higher values than 12 kb (Figure 4). These variations in the number and molecular weight of the DNA plasmid represent the
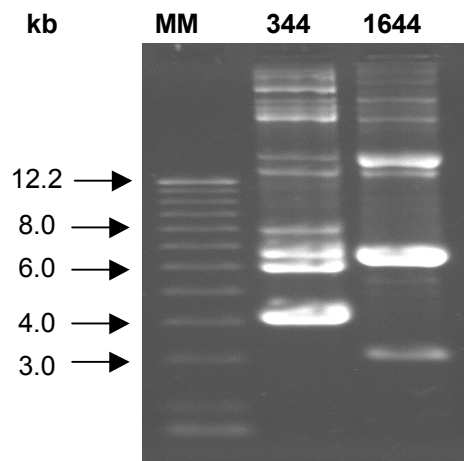

FIGURE 4. Plasmid pattern of Bacillus thuringiensis strains 344 and 1644. MM - Molecular weight 1 Kb Ladder (Invitrogen).

genetic divergence between the strains of that species. Although *Bt* plasmids have been studied either to locate *cry* genes or to transfer them to different strains and species, plasmid patterns have frequently been used to characterize strains (Ibarra & Federici, 1987; Ibarra et al., 2003). Reyes-Ramírez & Ibarra (2008) evaluated the plasmid patterns of several strains of Bt and observed that, with one exception, all strains showed a unique plasmid pattern. In this report, we were able to compare the plasmid patterns of the strains 344 and 1644 of Bt. These strains also showed a unique plasmid pattern and the results obtained are supported by Reyes-Ramírez & Ibarra (2008). The information obtained from

this comparison showed the importance of this tool as a strain characterization procedure and indicates the complexity and uniqueness of this feature.

Protein electrophoresis: SDS-PAGE profile shows that the strains 344 and 1644 harbour multiple and different protein profiles, with molecular masses ranging from 30 to 205 kDa. Strain 344 showed an active toxin fragment of 60 kDa after trypsin digestion, while strain 1644 showed a toxic fragment of 55 KDa (Figure 5).

Morphological characterization of crystal inclusions of the selected *Bt* strains showed typical bipyramidal and cuboidal crystals, common to most lepidopteran-active strains. These data are in agreement with the
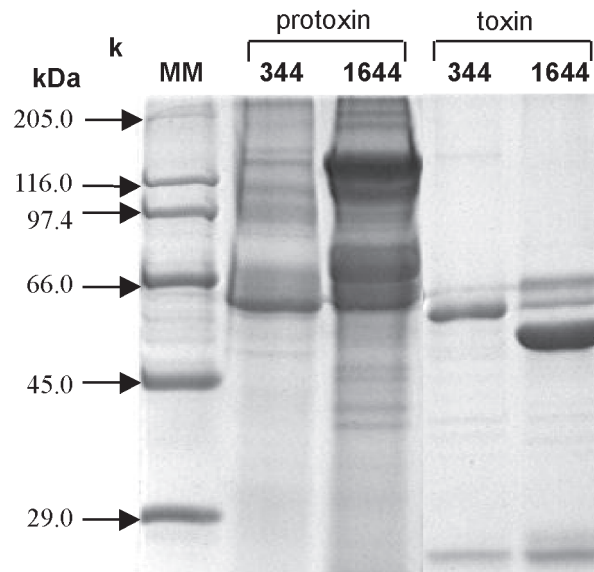

FIGURE 5. Protein analysis of strains 344 and 1644. MM - Molecular Marker SDS 6H (Sigma).

protein composition observed in the SDS-PAGE analysis. However, a high diversity of plasmid profiles was observed, suggesting important variability among these *Bt* strains, which agrees with the different *cry* gene contents found in these strains.

Conclusions

Comparative analyses of *cry* genes, amplification with ERIC primers, plasmid pattern and protein profile allowed the clear differentiation of the evaluated strains. It may also help in the establishment of a new subspecies-level classification of *Bt*. Due to this discrimination, these analysis can be an useful tool in the characterization of *Bt* strains, something highly valuable in intellectual property claims.

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