Bacillus thuringiensis isolates entomopathogenic for *Culex quinquefasciatus* (Diptera: Culicidae) and *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)

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(With 1 figure)

Abstract

Samples of the *Bacillus thuringiensis* (Bt) were collected from soil and insects. Eight isolates were selected from rural soil, 15 from urban soil and 11 from insects. These were evaluated for entomopathogenicity against larvae of *Anticarsia gemmatalis* and *Culex quinquefasciatus*. The pathogenicity tests showed that a higher percentage of isolates were active against *A. gemmatalis* (60%) compared to *C. quinquefasciatus* (31%). Probit analysis (LC_{50}) indicated that against *A. gemmatalis* four of the isolates presented values similar to the reference strain against *A. gemmatalis*, while against *C. quinquefasciatus* one isolate showed an LC_{50} similar to the reference strain (IPS-82). SDS-PAGE characterisation of two isolates showed a 27 kDa protein fraction related to the Bt subspecies *israelensis* cytolytic toxin (cyt) gene. One 130 kDa protein, possibly related to the Bt crystal inclusions (cry1) gene, was identified in the other two isolates, which were more toxic for lepidoptera; another isolate presented a protein of 100 kDa. Some new local Bt isolates had similar LC50 probit values to the reference strains.

Keywords: Anticarsia gemmatalis, *Bacillus thuringiensis,* Cry proteins*, Culex quinquefasciatus*, isolation.

Isolados de *Bacillus thuringiensis* entomopatogênicos para *Culex quinquefasciatus* (Diptera: Culicidae) e *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)

Resumo

Amostras de *Bacillus thuringiensis* (Bt) foram coletadas do solo e de insetos. Oito isolados foram coletados de solo rural, 15 de solo urbano e 11 de insetos, os quais foram avaliados quanto a sua entomopatogenicidade contra larvas de *Anticarsia gemmatalis* e *Culex quinquefasciatus*. Os testes de patogenicidade mostraram uma alta porcentagem de isolados ativos contra *A. gemmatalis* (60%), comparado a *C. quinquefasciatus* (31%). Por análise de probit (CL_{50}), verificou-se que quatro isolados apresentaram valores similares aos da estirpe de referência contra *A. gemmatalis*, enquanto somente um isolado mostrou CL50 similar à estirpe de referência (IPS 82) contra *C.quinquefasciatus*. A caracterização por SDS-PAGE de dois isolados mostrou uma proteína de 27 kDa relativa à toxina citolítica (Cyt) de *B. thuringiensis* subespécie *israelensis*. Uma proteína de 130 kDa, possivelmente relacionada à família do gene *cry* 1, foi identificada em outros dois isolados, os quais foram mais tóxicos para lepidópteros, enquanto que os outros dois isolados apresentaram uma proteína de 100 kDa. Alguns novos isolados locais de *B. thuringiensis* apresentaram valores de CL50 similares às estirpes de referência.

Palavras-chave: Anticarsia gemmatalis, Bacillus thuringiensis, proteínas Cry, *Culex quinquefasciatus*, isolamento.

1. Introduction

The bacterium *Bacillus thuringiensis* has been used to control insects belonging to a variety of different orders. However, further studies are needed to obtain and characterise isolates effective against specific insects, especially for the control of Diptera (Saadoun et al., 2001; Praça et al., 2004; Cavados et al., 2005; Ohgushi et al., 2005; Jara et al., 2006; Armengol et al., 2007), Lepidoptera (Dias et al., 1999; Asano et al., 2000; Uribe et al., 2003; Martínez et al.,

2005; Armengol et al., 2007), Hymenoptera (Pinto et al., 2003) and Coleoptera (Martins et al., 2007).

In southern Brazil, some of the most important insect pests belong to the Culicidae (Diptera: Nematocera), with haematophagous habits having medical importance (e.g. Consoli and Oliveira, 1994) while representatives of the Noctuidae (Lepidoptera), such as the velvet bean caterpillar (*Anticarsia gemmatalis*), the corn earworm (*Helicoverpa zea*), the armyworm (*Spodoptera frugipeda*), the cutworms (*Agrotis ipslon*) and the semilooper (*Pseudoplusia includens*) cause damage and economic loss to important crops (Gallo et al., 2002).

The search for new *B. thuringiensis* isolates and their characterisation has been carried out by several research groups in different countries (Dias et al., 1999; Uribe et al., 2003; Pinto et al., 2003; Cavados et al., 2005; Jara et al., 2006), these isolates being important in the control of insects because they represent new alternatives in integrated pest management (IPM). The majority of natural *B. thuringiensis* isolates with insecticidal activity are effective against lepidoptera larva (Alves et al., 1998), with *B. thuringiensis* strains active against diptera or coleoptera being more difficult to isolate (Mohammedi et al., 2006; Armengol et al., 2007).

Bioassays have been used to examine new *B. thuringiensis* isolates from soil, leaves and insects that induce mortality in dipterous and lepidopterous insects and are equal, or superior, to *B. thuringiensis* reference strain *B.thuringiensis* IPS-82 and *kurstaki* strain HD-1 (Bobrowski et al., 2001; Jara et al., 2006).

Strains of *B. thuringiensis* produce several proteinaceous δ-endotoxins, known as crystal inclusion (Cry) proteins, classified according to the amino acid sequence determining their specificity (Crickmore et al., 1998). The Cry 2, Cry 4, Cry 10, Cry 11, Cry 16, Cry 17, Cry 19 toxins are generally described as active against dipterans while the Cry 1, Cry 9 and Cry 2 toxins are effective against lepidopterans. Moreover, *B. thuringiensis* subsp. *israelensis* also produces cytolytic toxins (Cyt) with recognised activity and specificity against dipterans (Crickmore et al., 1998; Armengol et al., 2007).

The purpose of the study reported in this paper was obtain and characterise novel *B. thuringiensis* isolates from soil and insect samples collected in the Serra Gaúcha, a hilly region in the southernmost Brazilian state of Rio Grande do Sul. Bioassays were conducted with isolates using *Culex quinquefasciatus* and *A. gemmatalis* Hübner and a protein profile study of the strains was carried out using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) characterisation.

2. Material and Methods

2.1. Isolation of B. thuringiensis

The *B. thuringiensis* strains were isolated from soil and insect samples from the Serra Gaúcha, Rio Grande do Sul, Brazil. The soil samples were collected from unused urban land and native vegetation in rural areas. Isolation of soil samples followed the methodology recommended by WHO (1985) and the collection and storage procedures were according to Lezama-Gutiérrez et al. (2001). Unless otherwise stated, all chemicals were purchased from Sigma (USA) and culture media components from Oxoid (UK) or Merck (Germany).

The urban *B. thuringiensis* isolates were obtained from naturally infected specimens of the common housefly, *Musca domestica* (Diptera: Muscidae), collected from vacant urban land, while the rural *B. thuringiensis* isolates were obtained from the moths *Anicla ignicans*, growing on wheat (*Triticum aestivum*) and *Agrotis malefida* (Lepidoptera: Noctuidae), growing on cabbage (*Brassica oleracea*). The isolation of *B. thuringiensis* from insects followed the methodology described by Alves et al. (1998). Briefly, the dead insects were dispatched and then macerated in saline solution (0.5% (w/v) NaCl) using a sterilised mortar and pestle, after which 1 ml aliquots were transferred to sterilised test-tubes, thermally shocked at 80 °C, appropriately diluted in saline and 100 µl portions inoculated into of Luria Bertani (LB) solid medium (containing (g l^{-1}): agar, 15; tryptone, 10; yeast extract, 5; NaCl, 5. pH 7.5) which was then incubated for 24 h at 28 °C and the *Bacillus* colonies selected using the same methodology as that used for the soil samples. The isolates were coded as follows: SR = rural soil; SU = urban soil; ID-MD = *M. domestica*; IL-ANI = *Anicla ignicans*; and IL-AGR = *Agrotis malefida* .

To select for endospore-forming bacteria in the soil-samples and the insect preparations described above, we suspended one gramme of soil in 10 ml of 0.87% (v/v) NaCl contained in sterile tubes and heated appropriate dilutions of the suspension at 80 ºC for 12 min in a water bath, after which 100 µl aliquots were plated onto nutrient agar (containing: g L^{-1}; peptone, 5; yeast extract, 2; NaCl, 5; agar, 15. pH 7.4) and incubated at 28 °C for 24 h. After incubation, we used the World Health Organization (WHO) colony morphology criteria (WHO, 1985) to select circular, scalloped-edged, non-pigmented presumptive *B. thuringiensis* colonies. The colonies were individually subcultured into LB broth supplemented with 10 g L^{-1} penicillin and incubated for 24 h at 28 °C to verify in phase contrast microscopy those exhibiting protein crystals considered to be *B. thuringiensis* (Silva-Werneck and Monnerat, 2001).

The *B. thuringiensis* isolates were conserved at -18°C in Eppendorf tubes containing NYSM medium (Difco nutrient agar supplemented with: 0.05% (w/v) yeast extract, 5×10^{-5} M $MnCl_2$, 7×10^{-4} M $CaCl_2$ and 10-3 M $MgC1_2$) and kept in the bacteria culture collection of the Pest Control Laboratory, Biotechnology Institute, Universidade de Caxias do Sul, Caxias do Sul, Brazil.

2.2. Bioassays

Bioassays were carried out on the isolates shown in Table 1 using the mosquitoes *C. quinquefasciatus* and the moth *A. gemmatalis*, which were reared in our laboratory under controlled conditions (25 ºC ± 1 ºC, 65% + 10%

relative humidity, 12 hours photophase) according to the procedures of Consoli and Oliveira (1994) for the former and Hoffmann-Campo et al. (1985) for the latter.

Mean percentage mortality was determined using a selective bioassay to identify isolates capable of killing at least 30% of the larvae tested at a specific concentration of the isolate. Virulence was assessed based on the dose-dependent toxicity of each isolate. Triplicate bioassays were used in all cases. For *C. quinquefasciatus* mosquitoes the entomopathogenicity of each isolate was assessed by adding 200 µl of spore suspension, containing 3×10^8 mL^{-1} spores, to 300 ml plastic cups containing 100 mL of de-chlorinated water supplemented with 0.001 g L^{-1} of brewers yeast and 25 third-instar *C. quinquefasciatus* larvae. Negative controls received 200 µL of de-chlorinated water instead of spore suspension and the positive controls received 200 µL of *B. thuringiensis* reference strain IPS-82, known to be highly entomopathogenic to dipterans (Fillinger et al., 2003), at the same spore concentration. Mortality was evaluated after 48 hours. For *A. gemmatalis* moths the entomopathogenicity of each isolate was assessed by applying 100 µl of spore suspension, containing 3×10^8 spores, to a disk (diameter = 25 mm) cut from fresh leaves of the common bean (*Phaseolus vulgaris* L.) contained in a Petri dish along with 10 third-instar *A. gemmatalis* larvae. The larvae remained in contact with the suspension for 24 hours, after which they were individually placed into 50 mL plastic cups containing a pathogen-free artificial diet (Parra, 2001). Negative controls received 200 µL of de-chlorinated water instead of spore suspension and the positive controls received 200 µL of *B. thuringiensis kurstaki* strain HD1, known to be highly entomopathogenic to lepidopterans (Lee et al., 2001), at the same spore concentration. The *A. gemmatalis* bioassays were evaluated on the 2nd and 7th day.

Virulence assessment was carried out on the isolates in Table 1, which presented the best growth and sporulation and were pathogenic for at least one species. Using the methods described above and appropriate controls we tested five isolates from rural soil, six from urban soil and five from insects (Table 2) at the following spore concentrations (spores mL^{-1}) were assessed: 1.5×10^3, 1.5×10^4, 1.5×10^5, 1.5×10^6 and 1.5×10^7. The 50% lethal concentration (LC_{50}) was calculated for each isolate using probit analysis (Finney, 1971). The mean fifty-percent lethal concentration (LC_{50}) values are given as probit values and their 95% confidence limits (CI) for a spore concentration of 1.5×10^7 mL^{-1} and n = 3. The toxicity of the isolates for each insect were compared using analysis of variance (ANOVA) and the SPSS Program (Kuo et al., 1992)

2.3. SDS-PAGE analysis

The protein profiles of the isolates that showing the best toxicity were analysed using SDS-PAGE in 12% (w/v) acrylamide gel. The proteins were extracted from the culture according to Lecadet et al. (1991) and 15 µL of each sample were loaded onto the gel. After electrophoresis, the gel was stained and fixed according to the method described by Laemmli (1970). The reference isolate was *B. thuringiensis* IPS 82.

3. Results

3.1. Isolation and evaluation of pathogenicity

A total of 721 bacterial colonies were isolated, 555 (77%) from urban and rural soil samples and 166 (23%) from insects. Of the colonies isolated, 172 (31% of 555) of the soil isolates and 59 (36% of 166) of the isolates from insects were identified as *B. thuringiensis*, corresponding to a total of 231 isolates. We selected 35 isolates in NYSM medium, which presented higher growth (colony diameter) and presence of spores four days after inoculation (8 from rural soil, 15 from urban soil and 4 each from each species of insect) and evaluated them for entomopathogenicity against *C. quinquefasciatus* and *A. gemmatalis* (Table 1).

The mean percentage mortality test (Table 1) showed that a higher percentage of isolates were active against *A. gemmatalis* (60%) than *C. quinquefasciatus* (31%). Four isolates (SR-CR2, SR-CP2, SR-CR3 and SR-AP13) from rural soil were significantly (p = 0.05) more toxic to *A. gemmatalis* than to *C. quinquefasciatus*. Isolate SR-AP5 produced 100% mortality against both *C. quinquefasciatus* and *A. gemmatalis*, while isolate SR-VV2 produced 100% mortality against *C. quinquefasciatus* only and isolate SR-CR2 showed the same mortality against *A. gemmatalis* (Table 1). The isolates from urban soil followed a similar pattern, except that none showed 100% mortality (Table 1).

Only four (21%) of the 12 isolates from insects produced more than 30% mortality against *C. quinquefasciatus*, with the muscid isolates ID-MD4 and ID-MD1 both showing significantly (p = 0.05) higher toxicity against mosquito larvae than moth larvae. Only 11 isolates obtained from insects were tested against *A. gemmatalis.* However, all of the 11 isolates tested showed mortality values higher than 30% when tested against *A. gemmatalis* larvae, with isolate IL-ANI4, obtained from a lepidopteran, causing 100% mortality. The isolates obtained from *M. domestica* produced in excess of 30% mortality both *C. quinquefasciatus* and *A. gemmatalis* larvae but were more effective against *C. quinquefasciatus*, with the isolates from the noctuids *A. ignicans* and *A. malefida* being more effective against *A. gemmatalis* (Table 1)

Isolate ID-MD4, obtained from the dipteran *M. domestica*, caused 100% mortality in *C. quinquefasciatus*, equal to that of reference strain IPS-82. However, the isolates obtained from lepidoptera were not highly pathogenic to diptera larvae but isolates IL-ANI4, IL-AGR3 and IL-AGR1 caused high mortality (77-100%) against *A. gemmatalis* larvae and were similar in potency to reference strain HD1 (Table 1).

The results of the virulence assessment are presented as probit values in Table 2 where for each source category the isolates are ordered by probit value in decreasing *A. gemmatalis* entomopathogenicity. Against *A. gemmatalis* some isolates (IL-ANI4, LC_{50} = 3.49; SR-AP5, LC_{50} = 3.78;

Table 1. Mean percentage mortality ± standard error (SE) for larvae of the moth *Anticarsia gemmatalis* and the mosquito *Culex quinquefasciatus* exposed to *Bacillus thuringiensis* isolates obtained from different sources. The positive controls were the reference strains *B. thuringiensis kurstaki* HD-1 and *B. thuringiensis israelensis* IPS-82, effective against lepidopterans and dipterans respectively, which both gave 100% mortality. For each source category, the isolates are ordered by decreasing *A. gemmatalis* toxicity.

Source categories and isolate code	Mean percentage mortality ± SE	
	A. gemmatalis	*C. quinquefasciatus*
Rural soil		
SR-AP5	100.00 ± 0.00	100.00 ± 0.00 ns
SR-CR2	100.00 ± 0.00	22.67 ± 9.33*
SR-VV2	66.33 ± 19.34	100.00 ± 0.00 ns
SR-CP2	60.00 ± 10.41	6.67 ± 1.76*
SR-CR3	60.00 ± 0.00	2.67 ± 1.33*
SR-VV1	55.33 ± 29.36	41.33 ± 4.81ns
SR-CB2	55 ±11.00	69 ± 31.00ns
SR-AP13	22.00 ± 4.16	4.00 ± 2.31*
Urban soil		
SU-SM1	88.67 ± 11.33	72.00 ±6.11ns
SU-GBA2	88.67 ± 11.33	44.00 ± 6.00*
SU-ALF6	88.67 ± 9.33	25.33 ± 9.84*
SU-SM2	77.33 ± 8.51	16.00 ± 6.11ns
SU-BF7	77,33 ±11.33	57.33 ± 3.53 ns
SU-GBA8	66.67 ± 3.33	21.33 ± 5.8*
SU-GBA1	56.00 ± 11.00	60.00 ± 10.07ns
SU-BF4	55.00 ±11.00	68.00 ± 4.00ns
SU-GBA4	53.33 ± 24.04	8.00 ± 4.62ns
SU-FAR3	44.00 ± 11.00	29.33 ± 4.81ns
SU-GAL1	40.00 ± 11.55	12.67 ± 8.67 ns
SU-GBA6	40.00 ± 20.00	5.33 ± 2.19 ns
SU-FAR4	20.00 ± 6.11	6.67 ± 6.67*
SU-GB1	26.67 ± 6.67	9.67 ± 3.18*
SU-SM12	26.67 ± 6.67	5.33 ± 2.03ns
Insects		
M. domestica		
ID-MD4	66.33 ± 5.17	100.00 ± 0.00*
ID-MD1	55.00 ± 3.00	98.67 ± 0.85*
ID-MD5	33.00 ± 1.80	40.00 ± 2.31ns
ID-MD3	NT	92.67 ± 2.37
A. ignicans		
IL-ANI4	100.00 ± 0.00	18.67 ± 2.73*
IL-ANI2	53.33 ± 3.88	5.33 ± 1.14*
IL-ANI3	40.00 ± 5.03	10.67 ± 2.20*
IL-ANIH	33.00 ± 6.26	6.67 ± 1.01ns
A. malefida		
IL-AGR3	88.67 ± 3.85	0.67 ± 0.09*
IL-AGR1	77.33 ± 4.37	0.67 ± 0.19*
IL-AGR2	55.00 ± 2.52	0.67 ± 0.04*
IL-AGR5	33.67 ± 7.97	5.33 ± 0.24ns

*Significant difference between the *A. gemmatalis* and *C. quinquefasciatus* columns by the Student's t-test assuming different variances (n = 3, p = 0.05). NT = Not tested, ns = non-significant.

SU-SM2, LC_{50} = 4.69; and SR-CR2, LC_{50} = 4.77) were as virulent as reference strain HD1 (LC_{50} = 3.47), while against *C. quinquefasciatus* isolate SU-BF4 (LC_{50} = 4.67) did not differ in virulence when compared with reference strain IPS-82 (LC_{50} = 5.13) (Table 2).

The protein profiles of isolates SR-AP5 and SR-CR2, obtained from rural soil, showed high molecular weight proteins of 130 kDa. Isolates ID-MD1 and ID-MD4, from *M. domestica*, also showed a 130 kDa protein band and this displaced similar electrophoretic patterns regarding the number and position of protein bands to those of the strain IPS-82 (Figure 1). Isolate SU-SM1, from urban soil, and isolates IL-ANI4 and IL-AGR3, from lepidoptera (Figure 1a) along with isolates SU-SM2 and IL-ANIH and (Figure 1b) showed higher virulence against *A. gemmatalis* and presented bands with a molecular weight of 25 kDa and 120 kDa. The rural soil isolate SR-VV2, the most virulent against mosquito larvae, presented a large protein band of approximately 100 kDa.

4. Discussion

Some of our new *B. thuringiensis* isolates from both soil and insects produced high mean percentage mortality in *C. quinquefasciatus*, *A. gemmatalis* or both, confirming the reports of various authors that both soil and insects are good sources for new entomopathogenic *B. thuringiensis* strains (Hansen et al., 1997; Schnepf et al., 1998; Kim, 2000; Cavados et al., 2001; Pinto et al., 2003; Quesada-Moraga et al., 2004; Hajaij et al., 2005; Hernandez et al., 2005).

The percentage of *B. thuringiensis* isolates obtained in our study (31% from soil and 36% from insects) was similar to that reported by Chilcot and Wigley (1993) but lower than the 82% obtained by Uribe et al. (2003) and the 90% reported by Bravo et al. (1998). The 36% of *B. thuringiensis* recovered from insects was similar to the 40% reported by Pinto et al. (2003), but less than the 60% obtained by Hernandez et al. (2005).

The pathogenicity tests (Table 1) showed a higher percentage of strains active against *A. gemmatalis* (60%) than *C. quinquefasciatus* (31%). In general, the isolates obtained from insects showed higher activity than those recovered from soil, and it was found that isolates obtained from dipterous and lepidopterous larvae were more effective against *C. quinquefasciatus* and *A. gemmatalis* larvae, respectively. Isolate ID-MD4, obtained from *M. domestica*, showed the same percentage mortality against *C. quinquefasciatus* as the reference strain IPS-82. Similar observations were reported by Cavados et al. (2005), who reported that bioassays of a *B. thuringiensis* isolate obtained from the black fly *Simulium pertinax,* which had been captured in a field in southeastern Brazil, was not only effective against *S. pertinax* but also had comparable toxicity against the important disease vector *Aedes aegypti* as reference strain IPS-82 which is only effective against mosquitoes.

The isolates obtained from lepidoptera showed higher activity for *A. gemmatalis*, with isolates IL-ANI3, IL-

Table 2. Mean virulence of *Bacillus thuringiensis* (*Bt*) isolates against the moth *Anticarsia gemmatalis* and the mosquito *Culex quinquefasciatus.* The mean fifty-percent lethal concentration (LC_{50}) probits and their 95% confidence limits (CI) are shown for a spore concentration of 1.5×10^7 mL^{-1} and n = 3. For each source category, the isolates are ordered by decreasing *A. gemmatalis* toxicity. The positive controls are the reference strains *B. thuringiensis* subsp. *kurstaki* strain HD1 and *B. thuringiensis* IPS-82, effective against lepidopterans and dipterans respectively.

Source categories and *Bt* isolate code	Probit values for larvae			
	A. gemmatalis		*C. quinquefasciatus*	
	Mean†	95% CI	Mean†	95% CI
Rural soil				
SR-AP5	3.78[b]	2.45 to 4.96	7.76[a]	6.86 to 12.64
SR-CR2	4.77[b]	1.14 to 5.76	7.22[b]	5.96 to 10.36
SR-VV2	7.66[a]	6.93 to 10.41	5.20[bc]	3.11 to 6.11
SR-CB2	7.98[a]	7.29 to 10.09	5.74[bc]	5.28 to 6.10
SR-VV1	8.09[a]	7.32 to 10.93	–	–
Urban soil				
SU-SM2	4.69[b]	5.08 to 5.74	–	–
SU-SM1	5.80[ab]	3.12 to 6.67	6.15[b]	5.67 to 6.56
SU-GBA1	6.39[ab]	5.57 to 7.15	6.40[b]	6.01 to 6.78
SU-ALF6	7.59[a]	6.59 to 18.51	6.00[bc]	5.25 to 6.53
SU-BF4	7.66[a]	6.90 to 10.25	4.67[c]	2.39 to 5.42
SU-FAR3	7.88[a]	6.94 to 14.55	–	–
Insects				
M. domestica				
ID-MD1	6.13[ab]	3.82 to 7.20	6.74[ab]	5.41 to 9.34
ID-MD4	6.24[ab]	3.72 to 7.55	7.82[ab]	6.64 to 12.41
A. ignicans				
IL-ANI4	3.49[b]	2.64 to 4.91	8.05[a]	7.32 to 10.23
IL-ANIH	7.37[a]	6.95 to 7.97	–	–
A. malefida				
IL-AGR3	7.34[a]	6.68 to 8.79	–	–
Positive controls				
Btk – HD1	3.47[b]	1.87 to 4.89	NT	NT
IPS-82	NT	NT	5.13[c]	4.81 to 5.36

† Within the same column, different superscript letters indicate a significant difference between means by the Duncan's test (n = 3, p = 0.05). A hyphen (–) indicates no activity and NT = not tested.

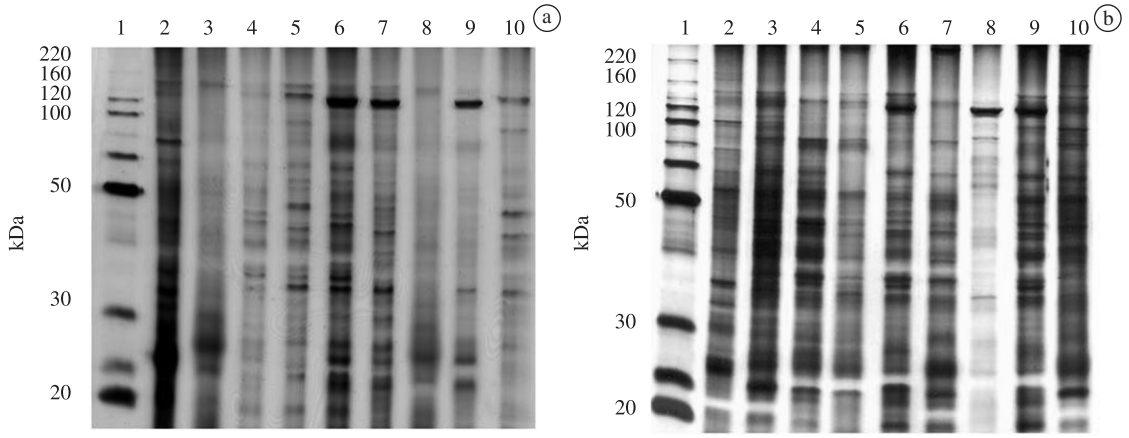

Figure 1. SDS-PAGE of the spore-crystal mixture (52-hour culture) of the isolates of *Bacillus thuringiensis*. a: 1 – molecular weight marker (Invitrogen); 2 – IPS-82; 3 – ID-MD1; 4 – SU-BF4; 5 – SR-AP5; 6 – IL-ANI4; 7 – SU-SM1; 8 – ID-MD4; 9 – IL-AGR3; 10 – SR-CR2; b: 1 – molecular weight marker (Invitrogen); 2 – IPS-82; 3 – SU-GBA1; 4 – SU-ALF6; 5 – SU-FAR3; 6 – IL-ANIH; 7 – SR-VV1; 8 – SR-VV2; 9 – SU-SM2; 10 – SR-CB2.

ANI4, IL-AGR1 IL-AGR2 and IL-AGR3 producing higher *A. gemmatalis* mortality and virulence compared to the isolates recovered from soil and *M. domestica*. Similar results were obtained with *B. thuringiensis* isolates obtained from insects by Aronson (1993). Regarding the isolates recovered from *A. ignicans*, it should be pointed out that Monnerat et al. (2002) and Porcar and Caballero (2000) obtained isolates from lepidoptera with high toxic activity against *S. frugiperda*, *H. armigera* and *S. littoralis.* Several authors have also reported that soil can be a source of entomopathogenic *B. thuringiensis* isolates with toxicity against lepidoptera (Silva et al., 2004; Praça et al., 2004; Bobrowski et al., 2001).

Our finding that only isolate SU-BF4 presented a CL_{50} similar to reference strain IPS-82 (Table 2) supports other studies which have found that most new *B. thuringiensis* isolates obtained from soil were less effective against dipteran insects (Praça et al., 2004; Martínez et al., 2005).

Martin and Travers (1989) also reported that *B. thuringiensis* isolates obtained from soil were more active against lepidoptera than mosquitoes, with more than double the number of isolates being entomopathogenic for lepidoptera than for diptera. Other authors have reported that of a large number of isolates, which were effective against lepidoptera, few or none were active against diptera (Dias et al., 1999; Kim, 2000; Martínez and Caballero, 2002). Our observations support the view that *B. thuringiensis* isolates from soil usually exhibit specificity to their hosts (Hansen et al., 1997; Mohammedi et al., 2006).

In our study, however, the isolates, which were virulent for *C. quinquefasciatus* were also virulent for *A. gemmatalis*, similar results having been reported by Silva et al. (2004) and Martínez et al. (2005). This suggests that soil samples containing *B. thuringiensis* isolates entomopathogenic for a specific insect species are rare compared to soil samples containing *B. thuringiensis* isolates with broad-spectrum activity.

The SDS-PAGE technique detected high molecular weight proteins in some isolates, with isolate SR-AP5 showing a 65 kDa band and SR-CR2 showing a 130 kDa band (Figure 1). The high lepidopteran toxicity of these strains may have been due to the presence of these proteins, which may have been related to the *cry* 1 gene described by several workers (Li et al., 2002; Monnerat et al., 2002; Uribe et al., 2003). The protein profile showing 65 kDa and 130 kDa bands is similar to pattern obtained from reference strain HD1 (Dias et al., 1999; Li et al., 2002; Medeiros et al., 2005). The relationship between the protein profile of isolates ID-MD1 and ID-MD4 and their toxicity to *C. quinquefasciatus* may have been due to the 27 kDa band in the protein profile of these isolates (Figure 1), since the Cyt protein, known to trigger cytolytic action against diptera, also resolves at 27 kDa (Crickmore et al., 1998; Tokcaer et al., 2006). Isolate SR-VV2 more virulent for *C. quinquefasciatus* than *A. gemmatalis*, displayed a band at approximately 100 kDa (Figure 1b), similar results have been reported for two isolates toxic against A. *aegypti* (Cavados et al., 2001). The isolates showing 25 kDa and 120 kDa bands (Figures I and II) were more virulent for *A. gemmatalis*, similar results having been reported for other soil isolates from Rio Grande do Sul (Pinto and Fiuza, 2003).

During the study reported in this paper, we obtained novel *B. thuringiensis* isolates, which showed similar virulence against *C. quinquefasciatus* and *A. gemmatalis* as the reference strains *B. thuringiensis* IPS-82 and HD1. A further interesting aspect of our research was the recovery of some *B. thuringiensis* isolates, such as SR-AP5 and SR-VV2, which were virulent to both of the test organisms, and which may have an important role in future biological control programs.

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