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EFFECTIVENESS OF *BACILLUS THURINGIENSIS* STRAINS AGAINST *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE)

Ricardo Antonio Polanczyk[1]; Rogério Fernando Pires da Silva[1]; Lidia Mariana Fiuza[2]*

[1]Health Plant Department, School of Agronomy, Universidade Federal do Rio Grande do Sul, RS, Brasil; [2]Centro de Biotecnologia do Estado do Rio Grande do Sul, Universidade do Rio Grande do Sul e Departamento de Microbiologia, UNISINOS, RS, Brasil

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ABSTRACT

The fall armyworm (*Spodoptera frugiperda*) is one of the most important pests of maize, causing up to 20% production losses when defoliation occurs near to flowering, or even complete destruction of plants. Among the alternatives to control this pest, the use of *Bacillus thuringiensis* (*Bt*) has gained attention due to its efficiency and low impact on natural enemies. Strains *Bt dendrolimus* HD 37, *Bt aizawai* HD 68, *Bt kurstaki* HD 73, *Bt darmstadiensis* HD 146, and *Bt thuringiensis* 4412 were tested against second instar larvae in *in vivo* assays. Suspensions of *Bt aizawai* HD 68 and *Bt thuringiensis* 4412, containing 3 x 10^8 cells/ml, induced mortality of 100% and 80.4%, respectively. To test virulence, cell concentrations of 8 x 10^5 to 3 x 10^8 cells/ml of strains *Bt aizawai* HD 68 and *Bt thuringiensis* 4412 were applied on the second instar larvae: LC_{50} were 6.7 x 10^6 and 8.6 x 10^6 cells/ml, respectively.

Key words: *Bacillus thuringiensis*, *Spodoptera frugiperda*, biological control, bioassays, fall armyworm

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda* Smith & Abbot), that attacks various cultures is one of the most important pests of maize in the Americas (17), causing about 20% production losses in Brazil (6). Although the use of chemicals is the prevailing method to control this pest, problems such as ecological disequilibrium, pollution, risks during application and high costs are present (16). Furthermore, the insecticides kill the fall armyworm natural enemies, favoring rapid reinfestation with serious damage to the culture (6). In fact, all these questions increased the interest in alternative strategies to manage this pest.

Nowadays, some methods, solely or together, get the satisfactory control of *S. frugiperda*. Among the entomopathogenic agents used in biological control of lepidopterous pests the *Bacillus thuringiensis* Berliner bacterium (*Bt*) has gained special attention as an alternative method (2, 11).

This microorganism acts in the insect gut due to cristals, composed by protoxins, discharged in the gut due to the alkaline pH that causes solubilization. These protoxins, in presence of digestive enzymes, are converted in toxic polypetides (delta-endotoxins). The activated toxins cross the peritrofic membrane, join to specific receptors in apical membrane of columnar cells of midgut, and insert themselves into the membrane (9, 11). The formation of pores disrupts the ionic gradients and osmotic balance in the apical membrane, resulting in cell swelling and lysis. This phenomenon leds to massive destruction of epithelium, causing death of larva (12).

Some time ago, the efficacy of this microorganism against *S. frugiperda* was considered questionable, but more recently the increment in researches on the use of *Bt* against this lepidopterous brought some interesting results (4, 10). The objective of this work was to report the selection of *Bt* strains with potential to control the fall armyworm. They could be used in the formulation of new biopesticides or in genetic transformation of host plants.

* Corresponding author. Mailing address: Laboratório de Microbiologia, Centro 2, UNISINOS, Caixa Postal 275, CEP: 93022-000, São Leopoldo, RS, Brasil. E-mail: fiuza@cirrus.unisinos.br

MATERIALS AND METHODS

Insects: S. frugiperda larvae were obtained from maize fields in Viamão (South Brazil) and reared in Bowling diet (5). The insects used in these assays belonged to the 20th generation and were mantained at 25 ± 2°C with 65 ± 5% relative humidity and 12h photoperiod.

Selective assays: These assays were carried out in a Biological Oxigen Demand chamber, in the same conditions described above. *Bt dendrolimus* HD 37 was provided by the Institut Pasteur, Paris and *Bt aizawai* HD 68, *Bt kurstaki* HD 73, *Bt darmstadiensis* HD 146 and *Bt thuringiensis* 4412 strains were provided by Plant Genetic Systems, Gent. They were grown in Usual Glicosed Medium (8) at 28°C and 180 rpm for 48h. The suspension was centrifuged at 5,000 rpm and washed 3 times with sterilized water. The concentration of bacterial spores was determined in a Neubaeur chamber using phase contrast microscopy at 400x. 100 ml of the both containing of 3 x 10^8 cells/ml were added to the surface of artificial diet previously put in mini-plates (30 mm of diameter), where larvae of 2nd instar of *S. frugiperda* were individualized (20 insects per strain). In controls, the broth was replaced by 100 µl of sterile water. The mortality was evaluated up to seven days after treatment. The data were corrected according to Abbot (1) and submitted to Duncan's *t* test (P = 0.05).

LC_{50} assays: The virulence of *Bt aizawai* HD 68 and *Bt thuringiensis* 4412 was evaluated by LC_{50} assays, using 8 x 10^5, 3 x 10^6, 8 x 10^6, 3 x 10^7, 8 x 10^7 and 3 x 10^8 cells/ml and control. Three replications were carried out, totalizing 1,050 insects per strain. To achieve the initial concentration of 3 x 10^8 cells/ml, the above mentioned method for cell counting was used. All other cell concentrations was obtained through dilutions of this suspension. The exact number of spores was determined in a Neubaeur chamber. The amount of suspension and the method for growth of microorganism were the same as in selective assays. The data were analyzed by Polo-PC (LeOra Software 1987).

Subletal effects: The possible effects of the strains on insects were verified through visual comparison between growth of surviving larvae and control insects.

RESULTS AND DISCUSSION

The *in vivo* activities of *Bt* strains tested on second instar *S. frugiperda* larvae are shown in Fig. 1. *Bt thuringiensis* 4412 and Bt *aizawai* HD 68 strains were the most pathogenic causing 80.40% and 100% of mortality, respectively. These strains were statistically different to others but not between them (Duncan, 5%). Similar results were obtained by Hernandez (10) for subspecies *Bt aizawai*, *Bt thuringiensis* and *Bt kurstaki*, observing mortality of 80%, 100% and 70%, respectively, to 3 x 10^7 cells/ml.

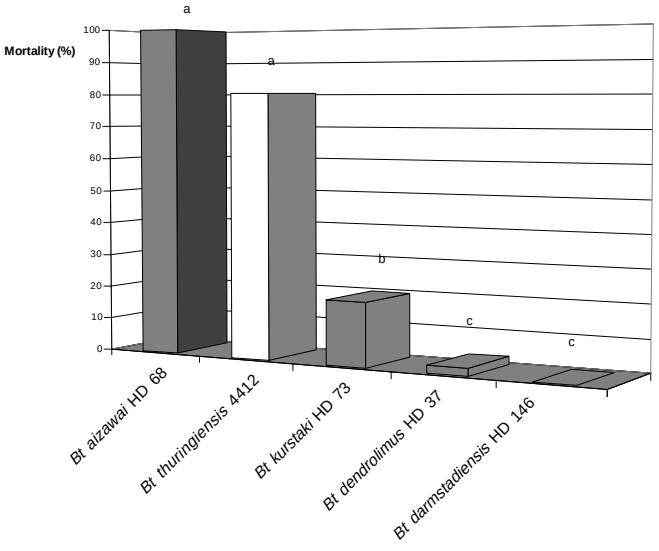

Figure 1. Mortality in selective assays of *Spodoptera frugiperda* second instar larvae and *Bacillus thuringiensis* strains at 3 x 10^8 cells/ml concentration.

The virulence assays showed that *Bt aizawai* HD 68 was the most active strain, with an LC_{50} of 6.7 x 10^6 cells/ml. *Bt thuringiensis* 4412 presented an LC_{50} of 8.6 x 10^6 cells/ml (Table 1). These LC_{50} values showed that a concentration 42.0% higher of *Bt thuringiensis* 4412 than the other strain was necessary to kill 50.0% of a *S. frugiperda* population in a certain period. Fig. 2 compares the virulence of the strains and shows that *Bt aizawai* HD 68 requires a lower concentration to be letal to 100% of larvae population.

The differences in toxicity of these strains to *S. frugiperda* may be related to the composition of cristals and their toxic potential. *Bt aizawai* HD 68 has two genes (*cryIA*(*a*), *cryID*) related to toxicity, while *Bt thuringiensis* 4412 has only one

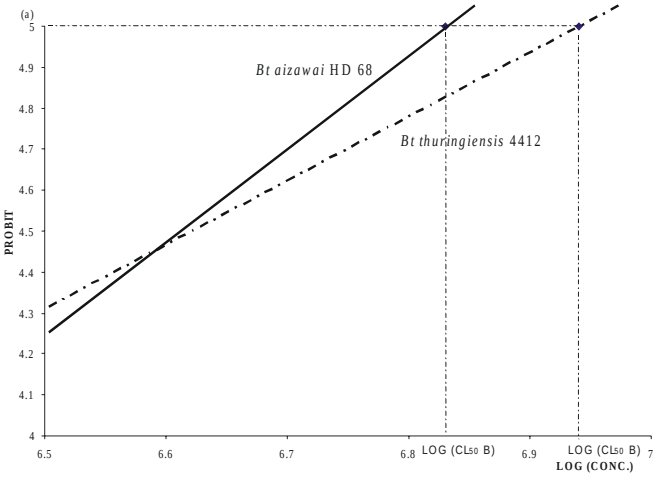

Figure 2. Comparative virulence of *Bt aizawai* HD 68 and *Bt thuringiensis* 4412 to *Spodoptera frugiperda* second instar larvae.

Table 1. LC_{50} of *Bacillus thuringiensis* strains to *Spodoptera frugiperda* second instar larvae.

Strains	LC_{50}*	(*l*L - *s*L)	Equation
Bt aizawai HD 68	6.7×10^6	4.7×10^6 - 9.2×10^6	y = - 0.412 + 0.792x
Bt thuringiensis 4412	8.6×10^6	6.0×10^6 - 12.0×10^6	y = 0.414 + 0.660x

* Results obtained using 1,050 larvae per strain.
(*l*L – *s*L) = Lower and superior limits (values) per LC_{50}.

(*cryIB*) (3). LC_{50} values of 77 ng/cm^2 for *cryID* and above 2,000 ng/cm^2 for *cryIA* (*a*,*b*,*c*) were observed (3). Chak *et al.* (7) described a new strain of *Bt* with *cryI* (*a*,*b*), *cryIC* and *cryID* genes, and emphasized that the high activity of *Bt aizawai* strains to *S. frugiperda* may be related to interactions between *cryIA* and *cryID* genes.

It must be pointed out that although the mortality to the selected strains in concentration of 3×10^8 cells/ml was incomplete, the surviving larvae had their development delayed, and didn't reach the fourth instar. From a practical point of view, the control can be considered effective, because the damage potential of surviving larvae was affected. This enhanced the possibility to use moderated toxic proteins as a helpful tool to control *S. frugiperda* in integrated pest management systems in maize. The subletal effects of *Bt* on *S. frugiperda* (13), *S. littoralis* (15) and *S. exigua* (14) were pointed out in other studies, where subletal doses caused reduction in consumption and delay in development. However, these effects were temporary, and the intensity decreased with the growth of larvae.

The high virulence presented by *Bt aizawai* HD 68 and *Bt thuringiensis* 4412 to *S. frugiperda* indicates their application in integrated pest management systems. The great majority of surviving larvae had their development delayed by the action of these pathogenic microorganisms. This aspect is very important, but rarely considered in the evaluation of effectiveness of biological agents.

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