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SCREENING OF *BACILLUS THURINGIENSIS* ISOLATES PATHOGENIC TO *SPODOPTERA FRUGIPERDA* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

To verify the susceptibility of *Spodoptera fugiperda*, the most important pest of maize, to *Bacillus thuringiensis* (*Bt*), this study was carried out with 58 isolates belonging to W. H. O. Collaborating Centres for Entomopathogenic *Bacillus* of Institut Pasteur, Paris. For each isolate, 20 larvae of second instar were used, being individualized in well-contained artificial diet and where 3 x 10^7 cells of this entomopathogenic bacterium were applied in each well. The selected isolate was evaluated concerning its LC_{50}, with 6 concentrations and control, in three replications, totaling 1,050 insects. The mortality, in both cases, was followed during seven days after treatment. *Bt morrisoni* isolate was the most pathogenic with 80% of mortality. Other 7 isolates caused mortality between 40 and 15%, 3 isolates below 15%, and all remainders were not active to this pest. In the virulence assay *Bt morrisoni* showed an LC_{50} of 8.6 x 10^6 cells / mL.

KEY WORDS: Biological control, entomopathogen, fall armyworm, integrated pest management.

RESUMO

SELEÇÃO DE ISOLADOS DE *BACILLUS THURINGIENSIS* PATOGÊNICOS PARA *SPODOPTERA FRUGIPERDA* (J.E. SMITH) (LEPIDOPTERA: NOCTUIDAE). Para verificar a suscetibilidade de *Spodoptera frugiperda*, a mais importante praga do milho, a *Bacillus thuringiensis* (*Bt*), foi conduzido este estudo com 58 isolados pertencentes ao Centro de Pesquisas sobre *Bacillus* Entomopatogênicos do Instituto Pasteur, França. Para cada isolado, foram testadas 20 lagartas de segundo ínstar, individualizadas em placas contendo dieta artificial e 3 x 10^7 celulas, deste entomopatógeno. Para o isolado mais patogênico foi determinada a CL_{50}. Para tanto, foram utilizadas 6 concentrações e a testemunha, em três repetições, totalizando 1.050 insetos. A mortalidade, em ambos os casos, foi avaliada até 7 dias após a inoculação dos isolados. *Bt morrisoni* foi o mais patogênico com 80% de mortalidade, sendo que sete isolados causaram mortalidades entre 40 e 15% e três isolados abaixo de 15%. Os demais isolados não se mostraram ativos para este inseto praga. Nos ensaios de virulência, o isolado *Bt morrisoni* mostrou uma CL_{50} de 8,6 x 10^6 celulas / mL.

PALAVRAS-CHAVE: Controle biológico, entomopatógeno, lagarta-do-cartucho, manejo integrado de pragas.

INTRODUCTION

Bacillus thuringiensis (*Bt*) is a bacterium that produces insecticidal crystal proteins during sporulation, highly toxic to various pests (ARANDA et al., 1996). It is the most successful commercial microbial insecticide, which is also starting to replace conventional insecticides. During the 1980s, new techniques, especially those afforded by recombinant DNA technology, and changes of public and political attitudes towards pesticide usage increased the *Bt* research by industries, government and academia, as well (VAN FRANKENHUYZEN, 1993). The development of transgenic plants expressing the *Bt* toxins and their significant use in integrated pest management increased the research for new isolates of this microorganism against lepidopteran pests (MOAR & TRUMBLE, 1990). An increment to the selling of 80% at

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the beginning of the 1990's is due to new formulations and new production techniques (MORAES et al., 1998).

This microorganism acts in the insect gut due to crystals composed of protoxins, which are discharged by an alkaline pH that causes their solubilization. These protoxins, exposed to digestive enzymes, are changed into in toxic polypeptides (delta-endotoxins). The activated toxins cross the peritrofic membrane, join the specific receptors in the apical membrane of the columnar cells of the midgut and insert themselves into the membrane. Following the binding, the toxic fragment or part of it inserts into the membrane making pores. The formation of pores in the plasmatic membrane of the cells causes an ionic unbalance between the cytoplasm and the outside environment of the cell. The first effects are the stoppage of feeding and the paralysis of the gut, which brings on the insect's death (HÖFTE & WHITELEY, 1989; KNOWLES, 1994).

We report the selection of *Bt* isolates with potential to control the fall armyworm (*Spodoptera frugiperda* J.E. Smith). This insect is one of the most important pests of maize, being firstly registered in the Southern United States in 1797 (WISEMAN et al., 1966). In Brazil, it causes a 20% loss in the plant production and, in cases of severe attacks, the total destruction of the plants (CRUZ, 1988). The use of chemical pesticides as a prophylactic method causes some problems such as ecological instability, pollution, risk during application, high costs and death of natural enemies. In fact, all these issues increase the interest in alternative strategies for the management of this pest.

MATERIAL AND METHODS

Bacterial isolates. The isolates used in our research were obtained from the International Entomopathogenic *Bacillus* Centre (Institute Pasteur - Paris) called "type I" (the first identification of each subspecies). The *Bacillus thuringiensis* (*Bt*) subspecies were 58 as follow: *Bt thuringiensis, Bt nigeriensis, Bt tochigiensis, Bt toguchini, Bt finitimus, Bt tolworthi, Bt yunnanensis, Bt leesis, Bt alesti, Bt darmstadiensis, Bt pondicheriensis, Bt konkukian, Bt kurstaki, Bt londrina, Bt colmeri, Bt seoulensis, Bt semiyoshiensis, Bt toumanoffi, Bt shandongiensis, Bt malaysiensis, Bt fukuokaensis, Bt kyushuensis, Bt japonensis, Bt andalousiensis, Bt sotto, Bt thompsoni, Bt neoleonensis, Bt oswaldocruzi, Bt kenyae, Bt pakistani, Bt novosibirsk, Bt brasiliensis, Bt galleriae, Bt israelensis, Bt coreanensis, Bt huazhongensis, Bt canadensis, Bt dakota, Bt silo, Bt sooncheon, Bt entomocidus, Bt Indiana, Bt mexicanensis, Bt jinghongiensis, Bt aizawai, Bt tohokuensis, Bt monterrey, Bt guiyagiensis, Bt morrisoni, Bt kumamotoensis, Bt jegathesan, Bt higo, Bt ostriniae, Bt yosoo, Bt amagiensis, Bt roskildiensis, Bt champaises, Bt dendrolimus.* The replication of the isolates was done in Erlenmeyer (1,000 mL) using Usual Medium Glucose - 1% (DE BARJAC & LECADET, 1976) kept on a shaker with 180 rpm, under 28°C, for 48 hours. After this period, the suspension was centrifuged at 5,000 rpm and washed with sterilized water. The concentration of bacteria cells was determinate in Neubaeur chamber and optical microscopy.

Insects. Larvae of *S. frugiperda*, second instar, were obtained from maize fields in "Viamão" (Southern Brazil) and maintained at 30° C and 12 hours photoperiod, reared according to BOWLING (1967). The insects were used after 20th generation.

Pre-selective assays. A volume of 100 µL of the $3x10^8$ cells / mL suspension was dropped onto the surface of the artificial diet inside a well of a multi-well tissue culture plate. A *S. frugiperda* larva of 2nd instar was placed in each plate, totaling 20 insects for each strain. The assays were carried out inside a climate-controlled chamber, under 25 ± 2° C, 65 ± 5% RH and 12 hours photoperiod. Mortality was evaluated during 7 days. The data were corrected by ABBOT (1925) and analyzed by Duncan's *t* test (P = 0.05).

LC_{50} assays. The most effective isolate were evaluated concerning the LC_{50}. In the beginning, pre-selective assays were carried out to determine the limits of the concentrations to be used are in these experiments. After that, the chosen concentrations were 8.6×10^5, 3×10^6, 8.6×10^6, 3×10^7, 8.6×10^7 and 3×10^8 Cells / mL and control, with three replications, totaling 1,050 insects per strain. The amount of solution and the method of cultivation used are the same as the ones in the pre-selective assays. The data obtained were analyzed by Polo-PC (LeOra Software 1987).

Subletal effects on development of *S. frugiperda* larva. To verify the effect of *Bt* isolates on the development of survival larvae from each treatment, these larvae were compared to those of the control group in order to identify the differences of development.

RESULTS AND DISCUSSION

The results showed that *Bt morrisoni* and *Bt galleriae* were the most pathogenic isolates, with 80.0% and 40.0% mortality, respectively. Nine isolates caused mortality between 30% and 5%, and the other 47 isolates were inactive for *S. frugiperda*. The Duncan test (5% probability) showed that *Bt morrisoni* was statistically different from the others. The isolates *Bt galleriae* and *Bt entomocidus* isolates were different from isolates *Bt monterrey* and *Bt mexicanensis* (Fig. 1).

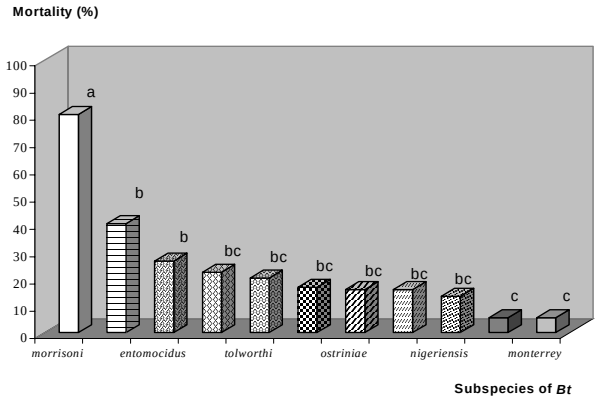

Fig. 1 - Mortality of *Spodoptera frugiperda* inoculated with different subspecies of *Bacillus thuringiensis*.

The results with *Bt morrisoni* and *Bt alesti type I* are similar to those obtained by HERNANDEZ (1988) using *S. frugiperda* larva, with mortality of 80% and 25% for *Bt morrisoni type* 5 and *Bt alesti type I*, respectively. However, the same author verified different results for *Bt galleriae type* 112 and *Bt entomocidus type* 5 using 3 x 10^7 cells / mL, with 97% and 80% mortality, respectively. For *Bt tolworthi type* 10, HERNANDEZ (1988) reported a 100% mortality, using 3 x 10^7 cells / mL and 8 x 10^6 cells / mL. Such data are different from those obtained in this work.

The isolate *Bt morrisoni* showed an LC_{50} of 8.6 x 10^5 cells / mL, with a confidence interval of 6.0 x 10^5 to 1.2 x 10^6 cells / mL. No data are available in the literature concerning the LC_{50} of this strain. This parameter is important because it shows the potency of the microorganism, which is likely to be used as a microbial insecticide in the pest control (HADDAD, 1998).

Similar to our research, HERNANDEZ (1988) tested 52 isolates, and only two showed high mortality (100%). BOHOROVA et al. (1996) tested 352 native isolates of *Bt* against *S. frugiperda* and only one caused mortality between 70 and 80%; other 149 isolates killed between 0-10% of the insects. VALICENTE et al. (1997, 1998) tested 728 isolates and only 15 were efficient (mortality between 90 and 100%), with 429 being innocuous and 405 killing less than 10% of the larvae.

All these results show a great difference among the isolates of the same subspecies of *B. thuringiensis* concerning their toxicity against *S. frugiperda*. This may be related to different protein genes of each strain in the same subspecies, which results in various degrees of toxicity. VAN FRANKENHUYZEN (1993) pointed out that, besides the affinity of binding to the brush border membrane vesicles of the midgut of susceptible insect species, due to by diversity in toxicity spectra, other factors such as protoxin stability, differential solubilization of crystals and subsequent proteolytic processing are important, and emphasized that toxicity appears to be a function of the capacity of the toxin to form a pore in the membrane after binding to the receptor.

DAVIDSON (1992) mentioned some reasons that make insects resistant to this pathogen such as pH, enzymes, petritrofic membrane, enzymatic detoxification and antimicrobian properties of gastric juice. GARCIA et al. (1992) pointed out that the gastric juice of *S. frugiperda* contains an inhibit factor, which decreases the pathogenicity of *Bt*.

It must be pointed out that the surviving larvae in each treatment with *Bt morrisoni* did not reach the fourth instar. According to VAN FRANKENHUYZEN (1993), intoxication is associated with immediate feeding inhibition. This can be associated to the delaying of the larvae development and the reduction in the consumption, as observed by LOPES et al. (1995) for *S. frugiperda*, by LAMBERT et al. (1996) for *S. littoralis* and by REGEV et al. (1996) for neonate *S. exigua* larvae.

From the practical point of view, the control was complete, because the surviving larvae had their damage potential affected, not being able to cause injury to the crop. In the field, these weakened larvae could be easily killed by natural enemies. This situation shows a great advantage of the biological control compared to the chemical control, allowing for the contribution of the natural enemies to obtain a satisfactory control level of insect pests.

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REFERENCES

ABBOT, W.S. A method of computing the effectiveness insecticide. *J. Econ. Entomol.*, v.18, p.265-267, 1925.

ARANDA, E.; SANCHEZ J.; PEFEROEN M.; GÜERECA L.; BRAVO, A. Interactions of *Bacillus thuringiensis* crystal proteins with the midgut epithelial cells of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.*, v.68, p.203-212, 1996.

BOHOROVA, N.; MACIEL, A.M.; BRITO, R.M.; AGUILART, L.; IBARRA, J.E.; HOISINGTON, D. Selection and characterization of mexican isolates of *Bacillus thuringiensis* active against four major lepidopteran maize pests. *Entomophaga*, v.4, p.153-165, 1996.

BOWLING, C.C. Rearing of two lepidopterous pests of rice on a common artificial diet. *Ann. Entomol. Soc. Am.*, v.60, p.1215-1216, 1967.

CRUZ, I. Manejo de pragas de milho no Brasil. In: CURSO SOBRE MANEJO Y CONTROL DE PLAGAS EN MAIZ Y SORGO, 1988. Sete Lagoas, MG. *Diálogo XXV*. p.17-31.

DAVIDSON, E. W. Development of insect resistance to biopesticides. *Pesqui. Agropecu. Bras.*, v.27, p.47-57, 1992.

DE BARJAC, H.; LECADET, M. M. Dosage biochimique d'exotoxine thermostable de. *Bacillus thuringiensis* d'après l'inhibition d'ARN-polymerases bacteriennes. *C. R. Acad. Sci.*, v.282, p.2119-2122, 1976.

GARCIA, M.A.; SIMÕES M.; HABIB, M.E.M. Possible reasons of resistance in larvae of *Spodoptera frugiperda* (Abbot & Smith, 1797) infected by *Bacillus thuringiensis* var. *kurstaki*. *Rev. Agric.*, Piracicaba, v.57, p.215-222, 1982.

HADDAD, M.L. Utilização do Polo-PC para análise de Probit. In: ALVES, S.B. (Ed.) *Controle Microbiano de Insetos*. Piracicaba: FEALQ, 1998. p.999-1012.

HERNANDEZ, J.L.L. Évaluation de la toxicité de *Bacillus thuringiensis* sur *Spodoptera frugiperda*. *Entomophaga*, v.32, p.163-171, 1988.

HÖFTE, H. & WHITELEY. H.R. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiol. Rev.*, Birmingham, v.53, p.242-255, 1989.

KNOWLES, B.H. Mechanism of action of *Bacillus thuringiensis* insecticidal d-endotoxins. *Adv. Insect Physiol.*, v.24, p.275-308, 1994.

LAMBERT, B.; BUYSSE, L.; DECOCK, C.; JANSENS, S.; PIENS, C.; SAEY, B.; SEURINCK, J.; VAN AUDENHOVE, K.; VAN RIE J.; VAN VLIET, A.; PEFEROEN, M.A *Bacillus thuringiensis* insecticidal crystal protein with high activity against members of the family Noctuidae. *Appl. Environ. Microbiol.*, v.62, p.80-86, 1996.

LOPES, L.C.C.; BOUCIAS, D.G.; SOARES JR., G.G. *Bacillus thuringiensis* endotoxin effects on *Spodoptera exigua* and *Spodoptera frugiperda* larvae infected with baculovirus. *Environ. Entomol.*, v.24, p.239-242, 1995.

MOAR, W.J. & TRUMBLE, J.T. Comparative toxicity of five *Bacillus thuringiensis* isolates and formulations against *Spodoptera exigua* (Lepidoptera: Noctuidae). *Fla. Entomol.*, v.73, p.195-197, 1990.

MORAES, I.O.; CAPALBO; D.M.F.; ARRUDA, R.O.M. *Produção de bactérias entomopatogênicas*. In: ALVES, S.B. (Ed.) *Controle Microbiano de Insetos*. Piracicaba: FEALQ, 1998. p.815-843.

REGEV, A.; KELLER, M. STRIZHOV, N.; SHEN, B.; PRUDOVSKY, E.; CHET, I., GINZBERG, I.; KONCZ-KALMAN Z.; KONCZ, C.; SCHELL, J.; ZILBERSTEIN, A. Sinergistic activity of a *Bacillus thuringiensis* d-endotoxin and a bacterial endochitinase against *Spodoptera littoralis* larvae. *Appl. Environ. Microbiol.*, v.62, p.3581-3386, 1996.

VALICENTE, F.H.; VASCONCELOS, M.J.V. DE; PAIVA, E. Efeito de diferentes concentrações e tempos de exposição de *Bacillus thuringiensis tolworthi* em larvas de *Spodoptera frugiperda*. In: CONGRESSO BRASILEIRO DE ENTOMOLOGIA. 16., 1997, Salvador, BA. *Resumos*. Salvador: UFBA / EMBRAPA, 1997. p.129.

VALICENTE, F.H.; VASCONCELOS, M.J.V. DE; PAIVA, E.; FARIA, A.L.; SOUZA, E.D.C.; BARRETO, M.R. Caracterização através da PCR de cepas de *Bacillus thuringiensis* de diferentes regiões do Brasil. In: SIMPÓSIO DE CONTROLE BIOLÓGICO, 6., 1998, Rio de Janeiro, RJ. *Anais*. Rio de Janeiro: EMBRAPA / FIOCRUZ, 1998. p.164.

VAN FRANKENHUYZEN, K. The challenge of *Bacillus thuringiensis*. In: ENTWISTLE, P.F.; CORY, J.S.; BAILEY, M.J.; HIGGS, S. (Eds.) *Bacillus thuringiensis, An Environmental Biopesticide: Theory and Practice*. Chichester: John Wiley, 1993. p.1-23.

WISEMAN, B.R.; PAINTER, R.H.; WASSOM, C.E. Detecting corn seedling differences on the greenhouse by visual classification of damage by the fall armyworm. *J. Econ. Entomol.*, v.59, p.1211-1214, 1996.

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