BIOLOGICAL CONTROL

Bacillus thuringiensis Survey in Brazil: Geographical Distribution and Insecticidal Activity Against *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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Levantamento de *Bacillus thuringiensis* no Brasil: Distribuição Geográfica e Atividade Inseticida Contra *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

RESUMO - O total de 3408 cepas de *Bacillus thuringiensis* foi isolado de 1448 amostras de solo provenientes de 10 estados, em quatro regiões brasileiras, abrangendo 96 municípios. As cepas foram avaliadas contra lagartas de *Spodoptera frugiperda* (J. E. Smith) sendo que apenas 62% mataram entre 81% e 100%, e 1758 não causaram mortalidade. O Sul destacou-se com 16,6% de cepas eficientes (mortalidade>75%) do total obtido por região, seguido das regiões Centro Oeste (3,1%), Sudeste (1,1%), e Nordeste (0,4%).

PALAVRAS-CHAVE: Insecta, bactéria, isolado nativo, controle biológico, patologia

ABSTRACT - A total of 3408 strains of *Bacillus thuringiensis* were collected from 1448 soil samples in 10 Brazilian states, four different geographical regions, covering 96 counties. These strains were evaluated against *Spodoptera frugiperda* (J.E. Smith) larvae. Only 62% killed between 81% and 100% and 1758 caused no mortality. Highest proportion of efficient strains (larval mortality above 75%) was found from the total isolated per region in the South Region (16.6%), followed by Western Central (3.1%), Southeast (1.1%) and Northeast Region (0.4%).

KEY WORDS: Insect, bacteria, native isolate, biological control, pathology

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is one of the most important insect pests of maize in Brazil, and the control is frequently achieved using chemical insecticides. In recent years, microbial insecticides have become a viable alternative to control fall armyworm and are considered a safe tool in Integrated Pest Management (Moscardi 1999, Valicente & Costa 1995). One of the most important insect pathogens in the world today is the bacteria *Bacillus thuringiensis*, accounting for 1-2% of the global insecticide market (Lambert & Peferoen 1992) and for over 90% of all commercial biopesticides sales with more than 100 products reported in the literature (Glare & O'Callaghan 2000).

B. thuringiensis is a rod-shaped, Gram-positive bacteria that occurs naturally in soil, dead insects, water and grain dust (Lambert & Peferoen 1992). During the sporulation process this bacteria produces a large crystal protein (δ endotoxins) that is toxic against many insect pests. These proteins may constitute 1/3 of the total proteins in the cell (Herrnstadt *et al*. 1986). When orally ingested by insects this crystal protein is solubilized in the midgut, forming proteins called delta endotoxins. The toxicity of these crystals to the insects is determined by the presence of the specific receptors in the midgut epitelium (Lambert & Peferoen 1992). Delta endotoxins produced as a parasporal body or crystaline inclusions are very specific in relation to the host, and many *B. thuringiensis* genes from different strains have been cloned in plants with the objective to transform these plants and make them more resistant to the insect pests (Adang *et al*. 1987, Vaeck *et al*. 1987, Koziel *et al*. 1993).

The number and variety of *B. thuringiensis* strains and insecticidal toxins increase everyday at an amazing rate. Boucias & Pedland (1998) estimated that 60,000 isolates are held in collections throughout the world. However, *B. thuringiensis* sometimes shows toxicity toward only one order of insect species and even within an order (e.g. Lepidoptera); dramatic differences in sensitivity are found among species, i.e. the beet and fall armyworm (*Spodoptera* spp.) are difficult to control with *Bt*-based bioinsecticides based on strains

HD1, but the tobacco budworm (*Heliothis virescens* Fabr.) and the diamond black moth (*Plutella xylostella* L.) are not (Baum *et al*. 1998). *B. thuringiensis* is not much efficient against *S. frugiperda* (Beegle & Yamamoto 1992), but it is efficient against *Trichoplusia ni* (Hueb.) and *H. virescens*. Aronson *et al.* (1991) reported that the solubility of the crystal is a main factor in the insecticidal efficiency of *B. thuringiensis* and suggest that this factor may explain the low susceptibility of *S. frugiperda* to *B. thuringiensis*.

The main objectives of this study were to survey *B. thuringiensis* in soils from different regions of Brazil, and evaluate the insecticidal activity of the collected strains against *S. frugiperda* larvae.

Materials and Methods

Soil Sampling. Most samples were collected from soil, but in some regions, grain dust of rice and corn plants were also sampled. Soil samples were obtained from fields with corn and beans mixed together, soybean, "milheto", sesame, pasture, rice, cotton, sorghum and areas growed with wild plants close to the main crops. Some samples were collected in the Northeast region, next to the sea (wet climate), agreste (mid-dry) and sertão (dry climate). Samples were also collected in Southern Brazil, from soils of Cerrado with high amount of aluminum, as well as in the South and Western Central regions, from fertile and dark soils. Sampling was also done in some farms located between the sampled places and towns or in any type of different soil seen during the trip.

Isolation of *B. thuringiensis* from Soil. Soil samples were collected from the ground surface. The soil samples were put inside plastic bags, identified and taken to the Laboratório de Controle Biológico at Embrapa Milho e Sorgo, located in Sete Lagoas, MG, Brazil. One gram of each soil sample was diluted in 5 ml of saline solution (0.8 g of NaCl and 100 ml of distilled water). After 15h of constant shaking, 1.0 ml of this suspension was transferred to an eppendorf tube and labeled. The Eppendorf tube was left 30 min. in waterbath at 65ºC and, immediately after, it was cooled on ice for 5 min. Samples were taken again to the vortex to be homogeinezed for 30 seconds and 50 ml of this suspension was poured on a plate containing medium [1.0 g of glicose, 8.0 g of nutrient broth, 0.02 g $FeSO_4$, 0.02 g $ZnSO_4$, 2.0 g yeast, 0.02 g $MnSO_4$, 0.3 g $MgSO_4$, 12.0 g agar and penicillin (40 mg/L)], with pH adjusted to 7.5. Plates were incubated at 30ºC for 24h to 48h. A sample of each colony was taken and observed with a phase contrast microscope.

Samples showing crystals were subcultured individually and incubated at 30ºC for 48h. These strains were tested later against fall armyorm larvae. All strains were grown in liquid media and conserved as a pellet and frozen at -80ºC, following the laboratory protocol.

Bioassays with *B. thuringiensis*. Two-day old healthy *S. frugiperda* larvae were used to determine the efficiency for each strain. The insects were reared on artificial diet containing the followed components and amounts/L of diet: cooked-bean grains (123.6 g), wheat germ (59.3 g), live beer-brewing yeast (38.0 g), ascorbic acid (3.82 g), nipagin (2.36 g), sorbic acid (1.23 g), agar (15.35 g), formaldehyde (3.1 g), phosphoric acid (0.131 ml) and propionic acid (1.3 ml).

The larvae were transferred individually to 50 ml disposable cups containing, each one, 5 g of artificial diet previously immersed in *B. thuringiensis* suspension containing spores and crystals. This suspension was obtained with *B. thuringiensis* colonies scrapped from the plates, so the concentration of spores and crystals were very high. Larvae were kept at 25ºC, 70% humidity and 14 h/10h photophase. Mortality was evaluated daily. Twenty-five larvae per bioassay were used, performing a total of 90.000 larvae. Efficient strains were those that killed more than 75% of the larvae at the 8th day of evaluation. Strains affording more than 25% mortality were regarded as active (Bernhard *et al*. 1997). According to Hossain *et al*. (1997), *B. thuringiensis* isolates that promoted 50% or more larval mortality were considered active strains.

Results and Discussion

Soil Sampling. A total of 3408 strains of *B. thuringiensis* were collected from 1448 soil samples in 10 Brazilian States, four different geographical regions, covering 96 visited counties (Table 1). The soil samples were distributed as follows: 611 samples from the Northeast, 513 from the Western Central, 223 from the Southeast and 101 from the South. However, strains of *B. thuringiensis* were found only in 395 (74.91%) samples from the Northeast, 386 (75.24%) samples from the Western Central, 165 (73.99%) samples from the Southeast and 65 (64.35%) samples from the South (Fig. 1).

Survey of *B. thuringiensis*. Strains of *B. thuringiensis* were found in all sampled Brazilian regions: 455 (15.25%) strains in the Northeast , 1317 (39.78%) strains in the South, 24 (0.81%) strains in the Southeast and 1186 (44.16%) strains in the Western Central region (Fig. 2).

South region showed the highest percent of efficient strains (16,6%), followed by Western Central (3,1%), Southeast (1,1%), and Northeast (0,4%) (Fig. 2). The searching sites are indicated in the Fig. 3, with some emphasis to the region of Jataí, Rio Verde and Santa Helena, all belonging to the Western Central. Additional studies are needed in order to determine the toxicity against insects of other species and/or orders.

A possible explanation for the gradual increase of percentage of efficient strains, from South to Northeast, is a greater number of *B. thuringiensis* strains found in the majority of the areas sampled in the maize production regions from south and southeast of Brazil. In the Northeast the majority was found in areas where maize is not cultivated. Martin & Travers (1989) analysed 785 *B. thuringiensis* strains originating from very diverse habitats and found no strong correlation between insect environments and densities of *B. thuringiensis*. The authors concluded that the presence of insects did not predict the presence of *B. thuringiensis* in a particular soil sample and that *B. thuringiensis* is distributed ubiquitously in the agricultural soil of Bangladesh. Simple correlation and regression analyses showed that the sand percentage and the available copper levels in the soil had significant negative and positive contributions, respectively,

Table 1. Brazilian counties where soil samples were collected.

Region	State	County
Northeast	Alagoas	Arapiraca, Maceió, Palmeira dos Índios, São Miguel dos Campos e União dos Palmares
	Bahia	Barreiras, Barrinha, Carrapichel, Juazeiro, Jurenal, Maçaroca, Mimoso do Oeste, Pindobaçu e Senhor do Bonfim
	Ceará	Araripe, Brejo Santo, Crato, Jati, Juazeiro do Norte, Mauriti, Missão Velha, Palestrina, Pena Forte e Quixabinha
	Pernambuco	Arcoverde, Araripe, Airi, Belém de São Francisco, Caruaru, Cruz das Maltas, Custódia, Exu, Floresta, Ibimirim, Lagoa Grande, Orocó, Ouricuri, Pesqueira, Petrolina, Salgueiro, Santa Maria da Boa Viagem, São João do Belmonte e Serra Talhada
	Sergipe	Itabaiana, Lagarto, Poço Verde e Simão Dias
Southeast	Minas Gerais	Boa Esperança, Carmo do Parnaíba, Conquista, Coqueiral, Curvelo, Diamantina, Guapé, Inhauma, Itumbiara, Janaúba, Lagoa Formosa, Lassance, Paraopeba, Patrocínio, Patos de Minas, Prudente de Morais, Santana do Pirapama, Sete Lagoas, Serro, Teixeiras, Uberaba, Uberlândia e Viçosa
	São Paulo	Assis, Floreal, Florestal, Gurolândia, Magda, Maracaí e Pereira Barreto
South	Paraná	Cascavel, Colônia Penha, Foz do Iguaçu, Marechal Cândido Rondon, Melissa e São Miguel do Iguaçu
Western Central	Goiás	Goiânia, Inhaumas, Jataí, Nerópolis, Rio Verde e Santa Helena
	Mato Grosso do Sul	Água Clara, Anhanduí, Bataguaçu, Campo Grande, Casa Verde, Nova Alvorada e Rio Pardo

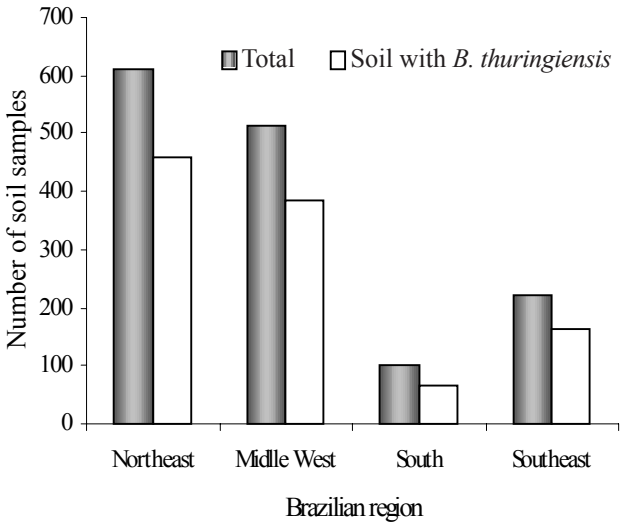

Figure 1. Total number of soil samples and number of soil samples with *B. thuringiensis* strains collected in different Brazilian regions.

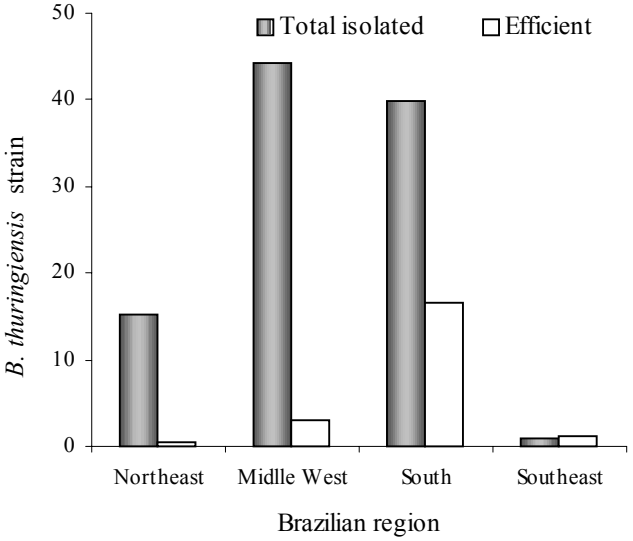

Figure 2. Percent of *B. thuringiensis* strains isolated from different Brazilian region and their efficiency (mortality > 75%) against *S. frugiperda*.

to the abundance and distribution of *B. thuringiensis* on the agricultural land (Hossain *et al*. 1997). According to Bernhard *et al*. (1997), activity did not appear to be correlated with origin, indicating a relatively ubiquitous distribution of the selected activities and of *B. thuringiensis* in general. Our results supported observations that nontoxic *B. thuringiensis* is common in natural soils (Bel *et al*. 1997, Ohba & Aizawa 1986b, Ohba *et al*. 1988, Hastowo *et al*. 1992). Surveys in different geographical regions have shown that *B. thuringiensis* is ubiquitous in natural soils, as well as in insect-breeding environments (DeLucca *et al*. 1981, Hastowo *et al*. 1992, Ohba & Aizawa, 1986b, Pádua *et al*. 1982).

Bioassays with *B. thuringiensis* Strains. Strains isolated

Figure 3. Brazilian states (gray areas) where samples were collected from.

from soil were 3408, with 1758 showing no pathogenic effect against *S. frugiperda*, 1041 causing less than 20% mortality, and 62 causing 81% to 100% mortality (Table 2). The most frequent isolates were those that appeared to be nontoxic at relatively low concentrations.

Using single-dose assays Bernhard *et al.* (1997) observed that 44% of the isolates killed less than 25% of four lepidoptera larvae tested (*H. virescens, Pieris brassicae* L., *Spodoptera littoralis* Boisduval *and Agrotis ypsilon* Rottenburg). According to Martin & Travers (1989) more than 60% of 1753 strains evaluted showed toxicity to some lepidopteran and dipteran species. Iriarte *et al.* (1998) reported that most of the *B. thuringiensis* isolates showed insecticidal activity (above 25% mortality) against some lepidopteran species. Isolates assayed showed significant insecticidal activity against the lepidopterans *Heliothis armigera* (76.1%), *Spodoptera exigua* (50.5%) and *P. xylostella* (19.7%). Among 97 isolates tested against the two lepidopterans (*P. xylostella* and *S. exigua*), 19 were found to be highly toxic for at least one of the two species (Ferrandis *et al*. 1999). Chilcott & Wigley (1993) showed that the percentage of isolates obtained from soil, with toxicity against the lepidopteran larvae, ranged from 37% to 88%. Bravo *et al*. (1998) founded *B. thuringiensis* in 456 of the 503 soil sample analyzed. The soil sample analyzed contained a high background level of other spore-forming bacteria.

Table 2. *B. thuringiensis* strains from different Brazilian regions and their pathogenicity to *S. frugiperda* larvae.

Number of strains	Mortality (%)
1758	0
1041	1 a 20
335	21 a 40
124	41 a 60
88	61 a 80
62	81 a 100

The high proportion of inactive strain observed in our study agree with results observed by other authors. Ohba & Aizawa (1986a) observed that nontoxic *B. thuringiensis* strains are widely distributed in natural environments and predominate among other strains. Martin & Travers (1989) reported that 60% of the strains they isolated from world wide soils were inactive to lepidopteran species. Abdel-Hameed & Landen (1994) observed that 26% of isolates from Swedish soils were inactive to some lepidopteran, dipteran and coleopteran species.

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