Bacillus thuringiensis in Brazil: Geographical Distribution and Fermentation Media for Production

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The fall armyworm, *Spodoptera frugiperda* (Fig. 1) is one of the most important insect pests in maize in Brazil. The damage it causes may reduce yields up to 34%. Chemical insecticides are currently used to control this pest in the field. *Bacillus thuringiensis (Bt),* however, may be a viable and economic alternative for the control of this important corn insect pest.

The objectives of this research were three-fold, 1) to survey the presence of *Bt* strains from different regions in Brazil, 2) to bioassay all collected *Bt* strains against *S. frugiperda* larvae, and 3) to develop fermentation media for *Bt* production using by-products.

Most samples were collected from soil, and others from grain dust, leaves and water. For *Bt* isolation, 1 g of soil was diluted in 5 mL of saline solution (0.8 g of NaCl and 100 mL of distilled water). Plates were incubated at 30°C for 48 h. A map of Brazil (Fig. 2) shows the locations of the samples collected and the maize production regions.

All *Bt* strains were tested against 2-day-old *S. frugiperda* larvae. Concentrations of 10^6, 10^7 and 10^8 spores/mL were used. In a first bioassay, sterilized rice was used to grow three strains, T09 (from Institut Pasteur) and 344 (from Embrapa) (both *Bt* serovar *tolworthi*) and 1644 (from Embrapa). A total of 50 and 100 g of sterilized rice were inoculated with 20 mL and 40 mL of *Bt* fermented in liquid media (LB supplemented with salts ($MgSO_4$, $FeSO_4$, $ZnSO_4$ and $MnSO_4$) pH of 7.5), respectively. After 5 days of incubation at 30°C, the rice was washed 5 times with water. Following determination of the spores count, the *Bt* strains were tested against *S. frugiperda* larvae. In a second bioassay, glucose and soybean flour were added to 50 and 100 g of rice before

FIG. 1. Fall armyworm and damage caused on a corn leaf.

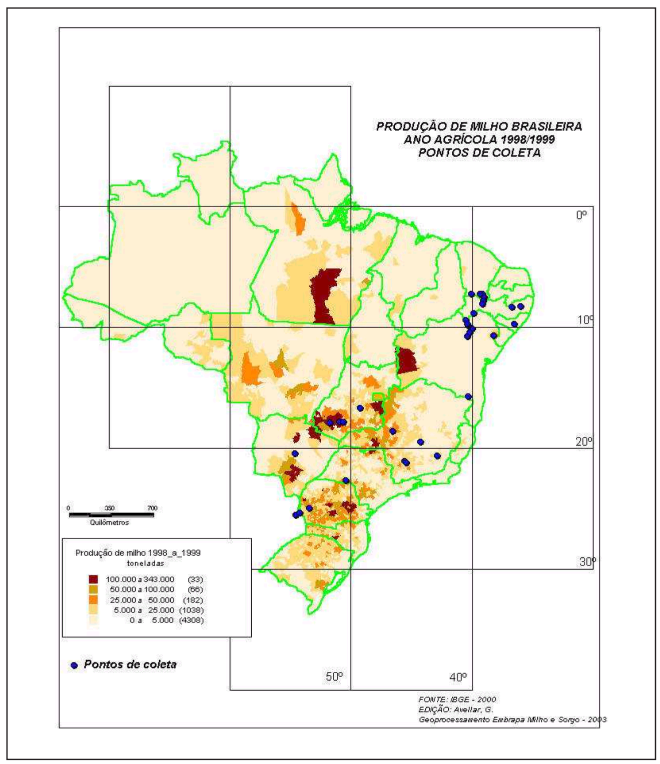

FIG. 2. Map of Brazil with locations of the samples collected and the maize production regions.in blue dots the locations of the samples collected. The brown and ochre color show the maize production regions.

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TABLE 1- Mortality of fall armyworm, *Spodoptera frugiperda*, following assay with *Bacillus thuringiensis* grown on rice and containing by-products, glucose and soybean flour.

Treatment	Bt(T09)	Concentration	Mortality (%)
100g rice	40ml	1.4×10^7	21
		1.4×10^8	55
		1.4×10^9	100
50g rice	20ml	1.2×10^7	26
		1.2×10^8	87
		$1,2 \times 10^9$	100

TABLE 2- Mortality of fall armyworm, *Spodoptera frugiperda*, using *Bt* grown on sterilized rice. 2004.

Strain	Amount of rice/ inoculum	Concentration	Mortality (%)
T09	50g/20mL	4.5×10^6	8.3
		4.5×10^7	91.6
		4.5×10^8	100
T09	100g/20mL	7.4×10^6	8.3
		7.4×10^7	86.4
		7.4×10^8	100
1644	50g/20mL	4.0×10^6	30.4
		4.0×10^7	35.0
		4.0×10^8	100
1644	100g/20ml	4.4×10^6	13
		4.4×10^7	21.7
		4.4×10^8	90.5
344	50g/20ml	4.8×10^6	21.7
		4.8×10^7	75.0
		4.8×10^8	100
344	100g/20ml	3.1×10^6	31.8
		3.1×10^7	90.5
		3.1×10^8	100

sterilization. After sterilization, 20mL of fermented liquid of the strain T09 were added to 50 g of rice, and 40 mL to 100 g of rice, and incubated at 30˚C for 5 days. In a third bioassay, 50 and 100 g of rice were inoculated with 20 mL of the strains T09, 344 and 1644, and were maintained for 5 days at 30˚C. Rice was afterward washed 5 times with water, spores were counted and *Bt* strains were tested against *S. frugiperda* larvae.

A total of 4460 *Bt* strains were isolated from 1760 soil samples from different regions of Brazil. All strains were tested against *S. frugiperda* larvae and 150 strains caused mortality above 75%. Highly insecticidal and non-highly insecticidal strains were found in all regions surveyed.

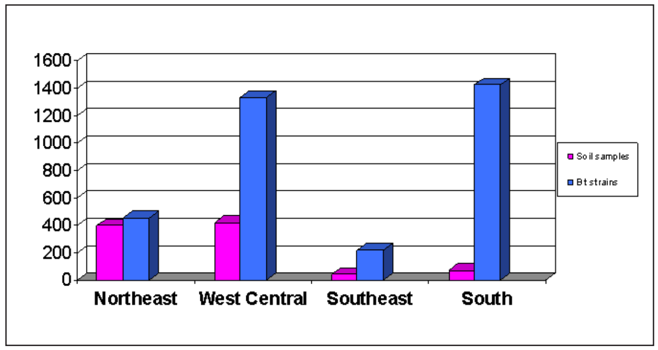

FIG. 3. Soil samples and *Bt* strains collected from different regions from Brazil.

Results of the first bioassay showed that the use of 100g of rice did not increase the final production of spores, as all treatments produced about 4×10^8 spores/ mL. Mortality was 100% when the spore/ crystal highest concentrations of 1.4×10^9 was used. Results of the second bioassay showed that the addition of by-products to rice increased the percent mortality (Table 1).

These results showed that it is possible, viable and feasible to use by-products to grow *B. thuringiensis*. The cost of production is low, around U$0.07 per hectare. Also, strain 344 has been tested in the field and has showed excellent results with no need for application of chemical insecticides to control fall armyworm.

References

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