

ESTADO DA ARTE DE *Bacillus thuringiensis*/*Spodoptera frugiperda*. STATE OF THE ART OF *Bacillus thuringiensis*/*Spodoptera frugiperda* CONTROL.

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Fall armyworm, *Spodoptera frugiperda* is one of the most important corn insect pests in Brazil and its damage may reduce yield up to 34%. Control of this insect in the field is done with chemical insecticides. However, in recent years microbial insecticides became a viable alternative to control insect pests. One of the most important insect pathogens in the world today is the bacteria *Bacillus thuringiensis* (*Bt*), accounting for 1-2% of the global insecticide market and for over 90% of all commercial biopesticides sales. *Bacillus thuringiensis* is a gram positive rod-shaped, spore forming bacteria and produce a large crystal protein during sporulation. Occurs naturally in soil, dead insects, water and grain dust. Today, it is estimated that 60,000 *Bt* strains have been collected in the world. However, *Bt* is reported not efficient in controlling *S. frugiperda*. A survey of *B.t.* strains was done mainly in corn producing regions. Most samples were from taken soil, but in some regions from grain dust, rice, mixed cropping of corn and beans, soybeans, sesame, pasture, rice, cotton, sorghum and in some natural wild plants close to the main crops. These samples were collected in the Northeast region that is humid climate and next to the sea, agreste (mid-dry) and sertão (dry climate), in the Southeast of Brazil 2 areas were chosen: one is the Cerrado area that is characterized by the high amount of aluminum in soil and, also in some fertile dark soils, in the South region (fertile soils) and in the Center East (cerrado). Sampling was also done in some properties that connected the sampled cities or in any type of different soil seen during the trip.

So, the objectives were survey of *B.t* strains from different regions in Brazil, test strains against fall armyworm in laboratory and molecular characterization of the most efficient strains.

Soil sample were taken from the surface and heat shock treatment was applied as follows: water bath at 65°C / 30 minutes and ice immediately after. Material was plated, incubated and colonies observed in phase contrast microscope. Colonies showing crystals were re-isolated. These were the strains used in the bioassays. 2-day old healthy *S. frugiperda* larvae were tested. Mortality was evaluated daily up to the 8th day. Total of 90.000 larvae were used. Strains were considered efficient if mortality was superior to 75%

Results: total of 1448 soil samples were collected and analyzed from 10 different States and including 4 different geographical regions from Brazil. So far, a total of 3408 strains were isolated. These strains are preserved at -20°C and -80°C (pellet:

spores and crystals). Molecular characterization was done using PCR to characterize the most efficient strains. It was used general and specific primers for *cryI* genes.

Total protein(crystals) was analyzed in Poliacrylamide gel and showed a molecular weight of 117KDa. After trypsin treatment- 66 KDa. Lower molecular weight bands are possibly Cry2 proteins. Also Vip (Vegetative Insecticidal Protein) and proteins were analyzed. PCR analysis showed that most of strains showed *cryIAb* and *cryIE* genes, some for *cryIB* and *cryID* and none for *cryIF* and *cryIC*. Polypeptides corresponding to Vip3A activities have a molecular weight in the range of 80-90KDa and showed promising mortality against fall armyworm. PCR was also used to characterize Vip-like gene, and only one strain showed positive result. However, partial sequencing from the amplified and purified PCR fragments revealed high nucleotide identity (>90%) with Vip3A genes.

3408 strains were isolated from the soil samples, however 1758 showed no effect on *S. frugiperda* mortality (0%), 1041 strains killed from 1 to 20%, and 62 strains showed a higher activity between 81 and 100% of mortality. Regarding the number of efficient strains per State/Region, they were as follow: 2, 4, 14 e 37 strains to the regions Northeast (Alagoas State = 1 and Ceará State= 1), South (Paraná State = 4), Southeast (Minas Gerais State= 12 and São Paulo = 1) and West Central (Goiás State = 32 and Mato Grosso do Sul State = 2), respectively. The State of Goiás (including the cities of Jataí, Rio Verde and Santa Helena) showed the best sites to collect soil samples from where the best *Bt* strains were isolated. However, South Region was the place that proportionally contributed with the highest amount of efficient strains (16,6%) and the other regions showed 3,1%, 1,1%, e 0,4% of efficient strains to the West Central, Southeast e Northeast, respectively, in relation to number of total strains obtained per region.

Research at CNPMS/EMBRAPA, continues to isolate, characterize and test the most efficient strains against fall armyworm.