ELECTROPHORETIC CHARACTERIZATION OF TOTAL PROTEIN FROM THE SUPERNATANT OF *Bacillus thuringiensis* STRAINS WITH DIFFERENT MORTALITY EFFECTS ON *Spodoptera frugiperda* LARVAE.

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Novel *Bacillus thuringiensis* (B.t.) insecticidal proteins, recently isolated from the culture supernatant of certain strains in the vegetative growth phase (vegetative insecticidal proteins, or Vips), have provided adequate levels of control against some economically important crop pests (Estruch *et al.*, 1996; Yu *et al.*, 1997). Thus, a characterization of some B.t. strains regarding the effect of their culture supernatant on the mortality of *Spodoptera frugiperda* (fall armyworm), the most important pest of the maize crop in Brazil, has initiated. In this work, the total protein profile of the supernatant of two foreign (T09, Pasteur Institute, France; and HD125, UNAM, Mexico) and two 'domestic' (344 and 520B, Valicente *et al.*, subm.) strains were analyzed. These strains have shown contrasting effects of their supernatant in the control of *S. frugiperda* larvae (Barreto *et al.*, 1998). The total protein of their supernatant was extracted and purified according to a established protocol (Estruch *et al.*, 1996), and their composition was preliminarily studied by polyacrylamide electrophoresis under both denaturing and non-denaturing conditions (SDS- and Native-PAGE). Seven μg of total protein per strain (evaluated by the method of Bradford, 1976) were resuspended in loading buffer with SDS + β-mercaptoethanol, boiled for 2 min, loaded into a 12.5% acrylamide-SDS mini-gel, and ran for 3.5 hours at 65 V. The gel was afterwards stained by a sensitive silver-based technique, as described in Blum *et al.* (1987). The electrophoretic banding patterns obtained for the four strains under denaturing conditions is shown in Figure 1. The results demonstrated that each strain displays a very different peptide composition, contrasting to a very similar banding pattern among these four strains when considering the δ-endotoxin fraction (Valicente *et al.*, subm.). The presence of specific bands of ~80 kDa (Figure 1, arrows) in the T09 and HD125 strains might be diagnostic of the Vip activity of the supernatant, based on other previous studies (Estruch *et al.*, 1996). Furthermore, preliminary feeding experiments with these purified supernatant proteins (data not shown) confirmed the mortality tendency exhibited by the whole unprocessed supernatant directly applied to the *Spodoptera* larvae (Barreto *et al.*, 1998).

The banding pattern of each strain supernatant was also assessed by native-PAGE, in order to differentiate heated (1-2 min at 95ºC) and non-heated protein samples (Figure 2), which helps classify the insecticidal portion of the secreted B.t. proteins (present in the supernatant) in a thermal-sensitive Vip fraction and a thermal-stable β-exotoxin fraction (Estruch *et al.*, 1996). Again, seven μg of total protein per strain, per heat treatment, were resuspended in the same loading buffer without SDS and β-mercaptoethanol, applied in a 12.5% non-denaturing polycrylamide gel, ran and stained in the same conditions described above. The pH of the separating gel was 8.3, which is appropriate for proteins with an acidic net charge. Despite a similar effect of the T09 and HD125 strains in the mortality of *S. frugiperda* larvae (Barreto *et al.*,

1998), the electrophoretic pattern in the non-heated samples of these and the other strains appeared to be very different. In the heated samples, several bands disappeared relatively to the non-heated counterpart, suggesting that they belong to the thermal-sensitive fraction of the total supernatant protein. The presence of some thermal-sensitive bands common to T09 and HD125 may correlate with the supernatant insecticidal Vip activity against fall armyworm, since the migration of this protein through the gel is insured by its acidic isoeletric point (pI). However, the analysis of more strains is certainly required to confirm this hypothesis, since the non-lethal supernatant of the 344 and 520B strains also showed thermal-sensitive bands (Figure 2), although not necessarily with the same electrophoretic pattern of migration. Bands that decreased their intensity relatively to the non-heated counterpart also correspond to thermal-sensitive peptides, for which a longer heating is needed for complete disappearance (data not shown). Bands with a similar intensity that were present in both heated and non-heated samples of a single strain probably belong to the thermal-stable fraction.

A series of experiments regarding (i) the isolation of individual proteins based on the net charge composition, (ii) application of those individual fractions on larval-feeding assays, (iii) purification of those individual protein fractions for amino acid sequencing, and (iv) synthesis of oligonucleotides to serve as gene-specific probes for B.t. DNA library screening, are currently underway with a two-fold purpose: to better dissect the protein composition in the supernatant of B.t. strains effective in the control of *S. frugiperda* larvae, as well as to help isolate the *Vip* gene(s) possibly related to those larval-controlling effects.

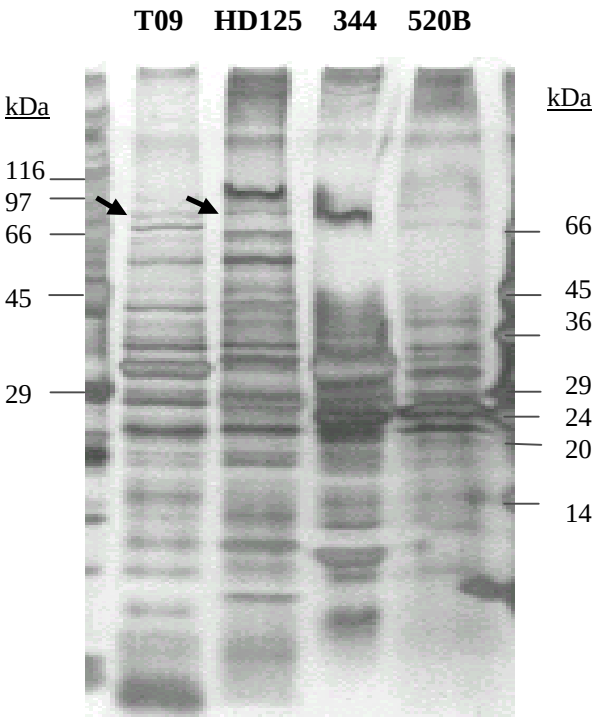

Figure 1: SDS-PAGE (12.5% gel) of total protein extracted from the supernatant of four B.t. strains.

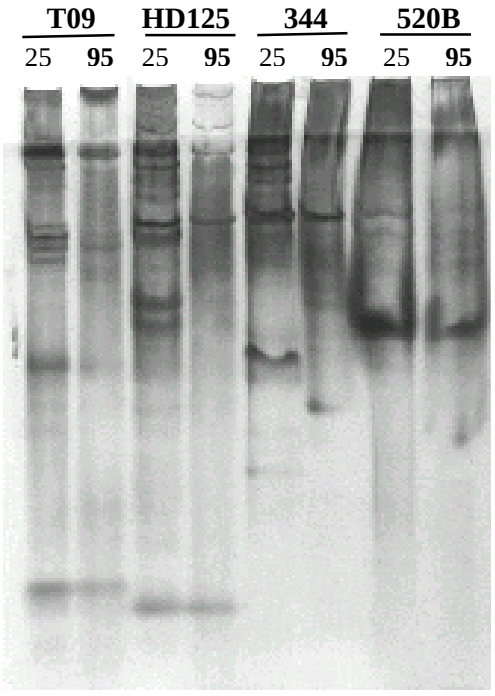

Figure 2: Non-denaturing PAGE (12.5% gel) of heated and non-heated samples of total protein extracted from the supernatant of the same four B.t. strains.

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