MORTALITY EFFECT OF THE CULTURE SUPERNATANT OF FIVE STRAINS OF *Bacillus thuringiensis* ON THE DIET OF *Spodoptera frugiperda* LARVAE.

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Insecticidal δ-endotoxin proteins from *Bacillus thuringiensis* (B.t.), which form crystal inclusions during the sporulation phase of the bacteria, has been used widely as commercial bioinsecticides in Integrated Pest Management (IPM) programs (Höfte and Whiteley, 1989). Recently, it has been demonstrated that other vegetative insecticidal proteins (Vips), identified in the culture supernatant of certain B.t. strains, were capable to cause death to some economically important insect pests (Estruch *et al.*, 1996; Yu *et al.*, 1997). As a preliminary evaluation of the potential applicability of these proteins against the most important insect pest for the tropical maize genotypes cultivated in Brazil, the culture supernatant of five B.t. strains were tested in their mortality effect on fall armyworm (*Spodoptera frugiperda*) larvae. Forty-ml bacterial cultures were grown for 16 hr at 30ºC, and centrifuged for 15 min at 30,500 x g to pellet the cells as much as possible; the supernatant was then collected with care and filtered through 3MM paper to minimize even further the presence of bacterial cells. Considering that the Vips are a thermal-sensitive fraction in the pool of proteins secreted by B.t. cells, contrasting to the thermal-stable fraction of β-exotoxins (Estruch *et al*., 1996), 5 ml of each of these five supernatants were heated for 20 min at 95ºC to denature and inactivate the Vip fraction irreversibly. In these conditions, only the insecticidal activity of the β-exotoxins was expected to remain (Estruch *et al*., 1996). The difference in larval mortality between a non-heated and heated supernatant were used to estimate its Vip content with biological activity (Figure 1). Artificial diets were briefly soaked in heated and non-heated supernatants from each strain before applying to 2-day-old larvae on an individual basis, i.e., one piece of soaked diet per larva, per container (50 ml-plastic cups with lids). Mortality ratios in a total of 24 individuals per strain, per heat-treatment of the supernatant, were assessed during the whole larval period. The supernatants tested here came from two foreign B.t. strains (T09 - Pasteur Institute, France; HD125 - UNAM, Mexico) and three tropical strains, which were obtained from field conditions in Brazil and previously evaluated regarding their δ-endotoxin contents and efficiency in controlling *Spodoptera frugiperda* (344 and 520B, Valicente *et al*., subm.; 606B, data not shown). The results suggested that (i) the Vip contents of the two foreign strains are higher and/or more active than the three brazilian strains tested against tropical fall armyworm larvae, (ii) the amount of Vips relative to β-exotoxins appeared to be higher for HD125 than for T09, and (iii) there is no apparent correlation between the δ-endotoxin and the Vip contents in a single strain. The latter conclusion is supported by combining these with other results (Valicente *et al*., subm; data not shown) that have revealed B.t. strains with high (T09, HD125, 344 and 520B) and low (606B) efficiencies in controlling fall armyworm, as a consequence of their crystal (δ-endotoxins) content and composition. Taken together, at least

three combinations were obtained, i.e., high endotoxin and Vip activity (T09 and HD125), high endotoxin and low Vip activity (344 and 520B), and low endotoxin and Vip activity (606B).

The results above described were obtained using 'time' (i.e., cultures of the same age - 16 hr-old) as the standardizing parameter for comparisons between supernatants of different strains. To verify whether the number of cells (optical density) at the exponential phase of growth (healthier cells) could be used as a better normalizing parameter for comparisons among strains, the growth curve of those five B.t. strains was evaluated by checking both their culture optical density at 600 nm and the total level of proteins in the corresponding supernatant. Values of O.D. (at 600 nm) up to 1.5 units were annotated and plotted as a function of time (Figure 2). The results suggested clearly that these strains grow at different rates, so that culture age might not be the best normalizing parameter. However, by comparing the levels of total protein from each supernatant, estimated according to the method of Bradford (1976), in which dye-protein complexes absorb light at a wavelength of 595 nm, it was noticed the tendency for a trade-off between cell division (growth) and protein secretion: faster-growing strains tended to show less protein secreted to the medium, and vice-versa, based on a similar optical density (Table 1). To confirm these trends statistically, similar experiments with more strains are currently underway. Taken together with other data regarding the minimal culture time required to achieve a concentration of proteins in the supernatant with measurable mortality effects during the larval period (data not shown), the results here obtained indicated that culture age is good enough as the normalizing parameter to compare the supernatant from different B.t. strains in their effect on *S. frugiperda*. Other preliminary SDS- and native-PAGE experiments showed that the protein banding pattern displayed by each supernatant is different, with the possibility of some bands being closely associated with high *S. frugiperda* mortality (Loguercio *et al*., 1998).

The results, here presented, stimulate further evaluation of supernatants from other B.t. strains against fall armyworm, in the search for more efficient Vip activities as alternative bioinsecticides to be used in IPM programs. A collection of ~800 other strains, also isolated from brazilian field conditions (Valicente *et al*., subm.), are currently under assessment of their Vip contents and activity against *S. frugiperda*. In addition, the composition of secreted proteins in the supernatant of these and other B.t. strains is being characterized further, aiming to pinpoint the specific Vip(s) responsible for most, if not all, the biological activity of B.t. culture supernatants against tropical races of *S. frugiperda*.

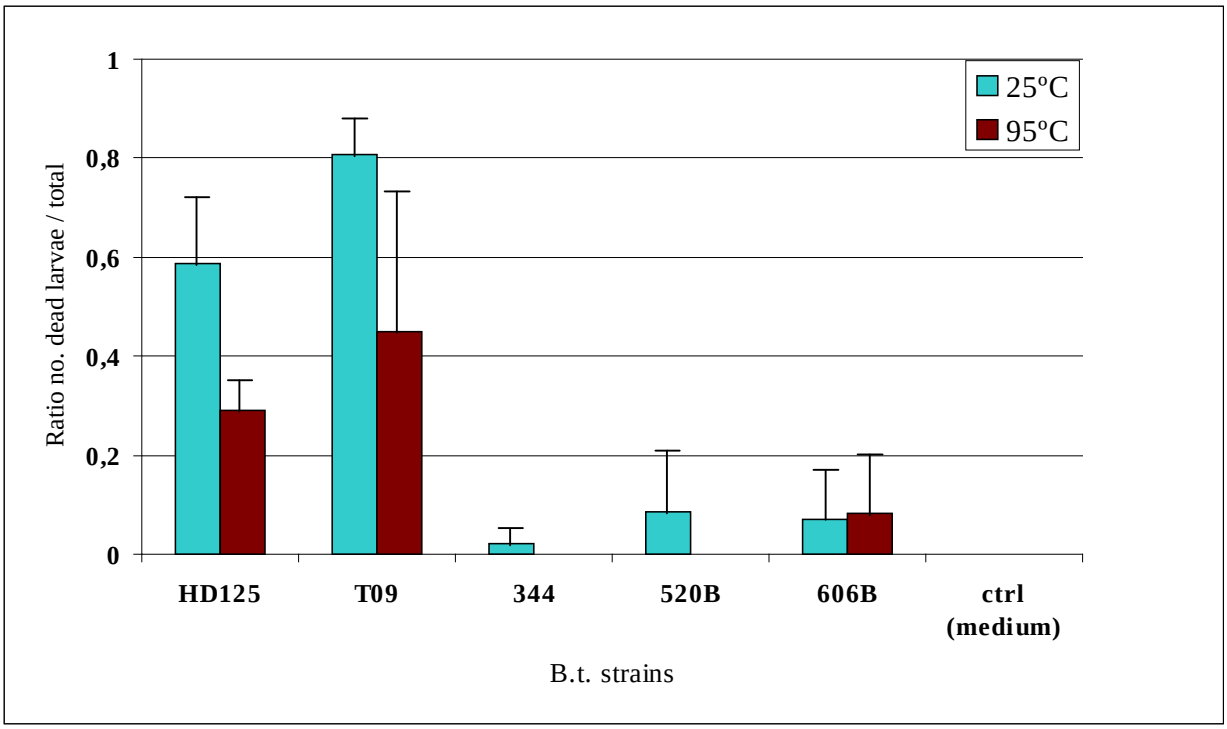

Figure 1: Mortality of *Spodoptera frugiperda* during the larval period, after feeding with diet soaked in the supernatant of five B.t. strains (obs: the error-bars in the graph correspond to two replicas of the experiment, except for HD125 and T09 at 25ºC, in which the bars refer to four replicas).

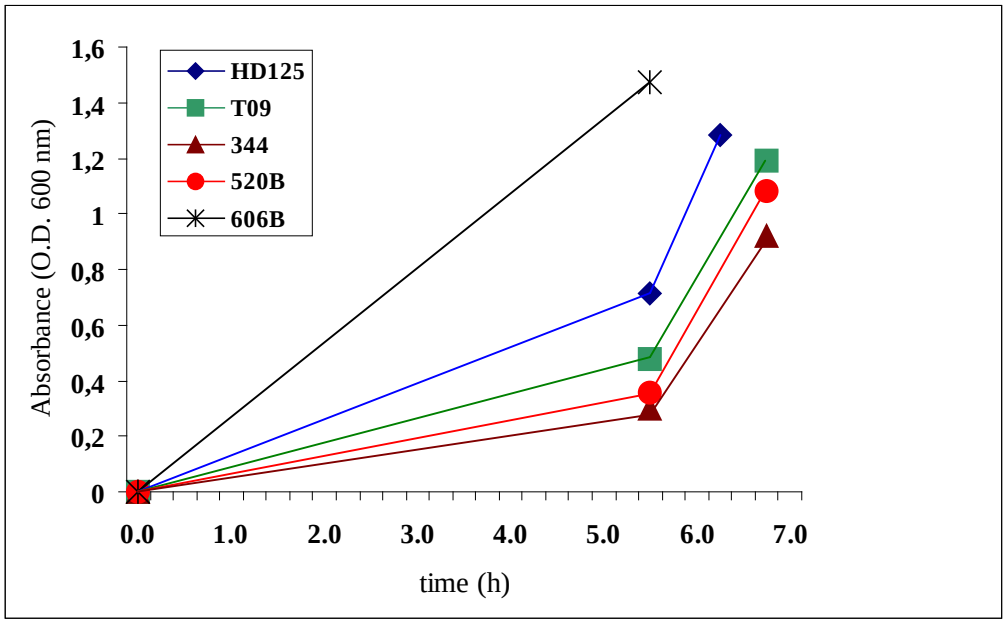

Figure 2: Non-normalized growth curve of the five B.t. strains

Table 1: Values of absorbance at 595 nm corresponding to total protein (Bradford, 1987) in the supernatants of the five B.t. strains, incubated up to an optical density between 0.9 and 1.5 units at 600 nm (see Figure 2):

B.t. strains	Total protein (O.D.)(1)	Culturing time (h:min)(2)
606B	0.332	5:30
HD125	0.546	6:15
T09	0.557	6:45
520B	0.619	6:45
344	0.611	6:45

(1) Values of absorbance at 595 nm after application of the Bradford technique for estimation of total protein amount.

(2) Time of incubation in which the optical density of the culture at 600 nm reached values between 0.9 and 1.5; at those times the supernatant was collected for total protein measurement (see above)

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