THURSDAY

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The amount of carbon and nitrogen used to produce *Bacillus thuringiensis* biopesticide may influence the quality of the final product. This research used different levels of carbon and nitrogen in 3 bioassays with 5 treatments each, and LB medium was the check treatment. The first bioassay used 5g/L of maize glucose for all treatments with yeast ranging from 5g/L to 60g/L. The second bioassay used 30g/L of yeast for all treatments with maize glucose ranging from 5g/L to 60g/L. The third bioassay used increasing amounts of nutrient ranging from 1g/L of maize glucose and 3g/L of yeast up to 20g/L of maize glucose and 60g/L of yeast. All media were enriched with salts (FeSO4, ZnSO4, MnSO4, MgSO4). The seed culture was produced using LB medium plus salts, at a stirrer speed of 200rpm, for 18 hours at 30°C. All media were sterilized and inoculated with Bt strain 344 (*B. thuringiensis tolworthi*) and maintained at 30°C for 72 hours at a stirrer speed of 250rpm. The H was measured at regular intervals, heat resistant spores were expressed as c.f.u/mL, cell mass produced in g/L-lyophilized, and spore counting per mL of medium. Results showed that pH followed the same pattern for all media tested, decreasing in the first 12-14 hours and increasing up to 8.7 (no pH control was made). The number of spores reached 4.9 x 109 spores/mL, and the lowest amount of 1.09 x 109 spores/mL. In the second bioassay the maximum number of spores was reached within 48h. Cumulative cell mass produced more than 30.0g/L in many treatments were the amount of nitrogen was higher. Mortality of 2-day-old *Spodoptera frugiperda* larvae was a 100% when treated with spores withdrawn at 24 hours from bioassay 3, and a 100% after 48 hours with spores withdrawn from bioassay 2 and 3.

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Expression of aminopeptidases in *Ostrinia nubilalis* (Hübner).

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The aminopeptidases N (APNs) are a large family of enzymes with probable role in food digestion that have been detected in the midgut of several lepidopteran species. Aside of their insect physiological role, they have become relevant because of their function as membrane binding proteins involved in the mode of action of *Bacillus thuringiensis* (Bt) crystal protein biopesticidal toxins. In the present study, the expressions of 5 *apn* genes and *one puromicyne-sensitive aminopeptidase* (*psa*) gene have been characterized in *Ostrinia nubilalis* (Hübner), a key pest of Bt-corn The analysis by RT-PCR showed that all aminopeptidases were expressed along the whole larval development. The relative tissue expression analyses in 5th instar larvae by qRT-PCR showed that all aminopeptidase genes were transcribed in the midgut. Moreover, 2 *apns* (*Onapn4* and *Onapn8*) were also expressed in Malpighian tubules, and the *Onpsa* transcripts were found at similar levels in those tissues as well as in the fat body and carcass. The *Onapn8* was expressed in the Malpighian tubules and in the midgut tissue without statistically significant differences, whereas the *Onapn4* had a very low level of expression in the Malpighian tubules. The *in silico* structural putative aminoacidic sequence differences between APNs and PSA seems to be correlated with their expression patterns. The structural similarity and expression of the analyzed APNs suggest that more than a single class may be involved in the Bt toxin binding in the migut.

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Characterization, distribution and cloning *cry1* genes efficient against fall armyworm, *Spodoptera frugiperda*, in Brazil.

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Brazil is located in South America and contains a rich and different biodiversity. A total of 4,459 Bt strains were isolated and evaluated regarding to *Spodoptera frugiperda* larval mortality, and 165 showed larval mortality above 75%. Molecular characterization was based on PCR electrophoresis profile using specific cry1 primers. Among these strains, 33 (20%) did not amplify the expected fragments; 103 (62,42%) amplified fragments corresponding to the presence of only one gene, while 25 (15,15%), 3 (1,8%) and 1 (0,6%) showed a profile of two, three and four different cry1 genes, respectively. SDS-page protein analyses were positive for the presence of cry1 genes. The most frequent (57.5%) was cry1D gene, whereas cry1Aa/cry1Ad and cry1C genes were the less frequent (1.2%). However, more than 60% of the evaluated strains presented cry1B and cry1E genes. Analysis of strains carrying cry1C, cry1B, cry1E, cry1F, cry1A, cry1G and cry1D genes showed that they were toxic to *S. frugiperda,* ranging from the most to the least toxic. The available sequences at http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/ were used for designing primers to clone cry1C and cry1F genes. The amplified fragments with the expected size, approximately 2,046 bp, were purified, cloned and transformed into competent cells. The sequencing of 5' and 3' ends allowed the confirmation of the identity of the genes. Some strains that presented unspecific fragments, were also cloned, amplified, sequenced and showed sequences corresponding to cry1-type genes. Colonies holding clones of cry1F, cry1Ca and cry1Cb genes, were obtained only for two of the evaluated strains.

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Plasmid capture system and its applications.

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