Characterization of Membrane-Associated Alkaline Phosphatase of Anticarsia gemmatalis Larvae Midguts: Possible Role as Bt Toxin Receptor

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Brazil is one of the largest worldwide soybean producers and losses related to spoilage caused by insect attacks are of great impact for soybean production in the country. The moth Anticarsia gemmatalis is responsible for the major defoliating pest afflicting soybean crops in Brazil. Recent strategies for pest management such as Bacillus thuringiensis endotoxins (Bt) expressed by transgenic plants against insect attacks are in development. These molecules bind to receptors on the midgut, including alkaline phosphatase, which is anchored by GPI to the cell membrane. In this work, we have identified and characterized a membrane-associated alkaline phosphatase (ALP) in the midguts of fifth instar larvae. After differential centrifugation, a membrane fraction was solubilized using CHAPS and solubilized proteins were separated by ultracentrifugation. The sample thus obtained was loaded onto a Q-Sepharose anionic column and the peaks with phosphatase activity were concentrated. This sample was selectively inhibited by levamisole. Zymography data confirmed the presence of a single isoform of alkaline phosphatase and it was also submitted to SDS-PAGE. Cytochemical localization of alkaline phosphatase in the midgut is also under investigation. In parallel, two strains of B. thuringiensis (var. morrisoni and kurstaki) were grown in nutrient broth and crystals were solubilized in alkaline conditions. The samples were submitted to SDS-PAGE where endotoxins were observed. To observe a possible role of ALP as Bt receptor, the endotoxins will be isolated and binding assays with alkaline phosphatase will be performed as a further step. These data serve as a basis for studying the possible interaction between these molecules, bringing about new prospects in controlling this pest.

Keywords: Alkaline Phosphatase; Anticarsia gemmatalis; Bt endotoxins.

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