

Research Thrusts

→ Structure/Function Analysis of Insecticidal Proteins Leads to Molecular Evolution for Improved Activity & Identification of Insect Targets

Soybean cysteine proteinase inhibitors (CPIs) and *Griffonia simplicifolia* leaf lectin have been expressed as bacterial recombinant proteins, and these exhibit insecticidal activity in western corn rootworm (WCR) and cowpea weevil feeding bioassays. The recombinant protein expression systems facilitated determination of functional residues involved in insecticidal activity using site-directed mutagenesis. A N-acetylglucosamine (GlcNAc) molecule in the midgut epithelium or peritrophic matrix of cowpea weevil is implicated as the primary target for the plant defensive lectin. Future research focus is identification of the insect target molecule that interacts with the plant protein to potentiate its insecticidal action.

Research established a phage display selection protocol that can differentiate insecticidal activity of plant-derived CPIs. The selection system is being used for in vitro directed molecular evolution of CPI insecticidal activity by targeted design mutagenesis or DNA shuffling. This is an alternative to screening plant germplasm for more efficacious forms of a specific class of insecticidal proteins. CPI affinity purification resulted in isolation of digestive cysteine proteinases from the larval midgut of WCR. A *Pichia pastoris* expression system was developed to express active recombinant WCR cysteine proteinases. These WCR cysteine proteinases are targets for directed molecular evolution of insecticidal CPIs.

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