Screening of functional insecticidal molecules using shuffled libraries.

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The cotton boll weevil, *Anthonomus grandis,* and the Mexican bean weevil, *Zabrotes subfasciatus*, are economically important pests causing severe losses to agriculture due to their damages to cotton floral buds and common bean seeds. Biotechnological approaches employing proteins involved in plant defense, such as δ-endotoxins (Cry proteins) and a-amylase inhibitors (aAIs), are powerful tools for the control of insect-pests. Because of their specificity, these proteinaceus factors are only toxic to certain insects. However, several combinatorial methods, associated with phage display technique, are now available to generate novel molecules by genetic manipulation of genes that can be searched for insecticidal proteins with higher potency and different specificities. Aiming to develop active defense factors for important insect-pests, such as the cotton boll weevil and Mexican bean weevil, two libraries, with 10^7 and 10^6 mutant recombined genes were constructed using DNA shuffling and phage display strategies, based on the recombination of two *cry* genes and two *αAIs* genes, respectively. The search for high-affinity ligands among phage-displayed random Cry toxins and a-amylase inhibitor mutants yielded enrichment after five round cycles of phage display selection. A large number of mutations in the amino acid sequences were found for the different mutants screened, which were evaluated for the toxicity and α-amylases specificities against these insects. Our data indicate that the new screened toxins and αAIs exhibit a high potential for use on the cotton boll weevil and Mexican bean weevil control and highlight that DNA shuffling and phage-display combined techniques are, together, an efficient strategy allowing both, to generate large number of mutants *in vitro* and to select new molecules with improved activities towards insect-pests.

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