

Insecticidal effects of Cry8H mutants generated by DNA Shuffling and Phage Display techniques against fall armyworm (*Spodoptera frugiperda*).

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Bacillus thuringiensis is a gram-positive bacterium that produces δ -endotoxins active against diverse insect pest species and other invertebrates, which cause extensive agricultural damages in the world. The *cry* genes isolated from *B. thuringiensis* have been widely used to develop transgenic plants with enhanced resistance to specific insect pests. Despite of the toxins (Cry proteins) *B. thuringiensis* of the diversity, the rapid molecular evolution (DNA shuffling) has been contributed to generate more potent and specific Cry proteins against diverse agricultural pests. In this context, the present work aimed to develop new mutant for Bt toxins with enhanced activity towards the main insect pest of maize and cotton, the fall armyworm (*Spodoptera frugiperda*). A library was constructed using the recombination of a new *cry8* gene combining DNA shuffling and Phage Display techniques. The gene denominated, *cry8H*, was initially amplified by PCR using specific primers containing the *Sfi* I restriction site and submitted to DNase I fragmentation. The products of this reaction (30-50 bp fragments) were submitted to a new PCR reaction without primers in order to obtain new recombinant genes. The recombinant genes were digested by *Sfi* I, subcloned in the pCOMB3X phagemid and expressed in *Escherichia coli* (XL1 Blue) generating a combinatory library of mutant genes *cry* with 10⁵ transformants. The clones obtained were screened for Phage Display using *S. frugiperda* gut ligands and the select molecules exhibited a higher insecticidal activity ranging from 2 – 6 times in relation to the wild type. This result demonstrated that the combination of DNA shuffling and Phage Display techniques is efficient a tool to generate new *cry* genes encoding insecticidal proteins with potential use in the *S. frugiperda* control. Supported by: EMBRAPA, FACUAL, FIALGO, CNPq and CAPES.