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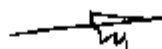
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**Production of a genetically modified strain of
bacteria *Bacillus thuringiensis***

Dissertation submitted to the Faculty of Science, Cairo
University for the Degree of Doctor of Philosophy

In
Biochemistry (Molecular Biology)

By
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ABSTRACT

Name: Nahed Abdel Ghaffar Abdel Aziz Ibrahim

Title of thesis: Production of a genetically modified strain of bacteria *Bacillus thuringiensis*

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This work has been carried out in order to construct a potent *Bt* strain active against the Egyptian cotton leaf worm *S. littoralis*. Toward this target, the δ -endotoxin crystal protein genes *cryIC* (which encodes an insecticidal protein highly specific to *S. littoralis*) and *cryIAg* (I Ac like) were used. *HindIII* digested *cryIC* was ligated into the *HindIII* site of the shuttle vector pHT7593. *HincII* digested *cryIAg* was ligated into *SmaI* site of pHT7593 and into *SmaI* site of pHTNC3. The three plasmid constructs were used to transform *E. coli* strain JM109. Colonies likely to contain recombinant plasmids were screened for the production of toxin proteins. Positively identified transformants produced the expected size protein, detected by partial purification of protein, and is truncated upon trypsin digestion. Protein extracts of clone 3 *cryIC*-harboring was toxic to larvae of *S. littoralis*.

The pHT7593-bearing *cryIC*, PHT-bearing *cryIAg* and pHTNC3-harboring-*cryIAg* were transferred into the Cry⁺ Bt4 *Bt* strain. PHT-bearing *cryIC* was also transferred into the Cry⁺ *kurstaki*-HD73 (which contained only *cryIAc*) native recipient, using electroporation. The introduction of the *cryIAg* gene into Cry⁺ Bt4 resulted in the formation of bipyramidal crystals. The introduction of both *cry* genes IC and IAg resulted in the multiplication of bipyramidal crystals. Further, the introduction of *cryIC* into Cry⁺ *kur*-HD73 shows multiplication in bipyramidal crystals.

Binding of CryIAc, CryIAg, and CryIC to midgut epithelial cell membrane protein of *S. littoralis* on ligand blots were investigated. CryIC-CryIAg hybrid toxins were also used for this purpose. Results show ICP of CryIC interact with BBMV of *S. littoralis* specifically with ~ 110 kd, and CryIAg / or CryIAc binds two proteins of Molecular masses ~ 110 kd and ~ 20 kd. A combination of CryIC and CryIAg / or CryIAc bind the same ~ 110 kd and ~ 20 kd proteins of BBMV.

In bioassays, *cryIAc*-expressing *kur*-HD73 and *cryIAg*-expressing Bt4 *Bt* strains caused mortality of *S. littoralis* larvae only slightly. When CryIC and CryIAg were applied together, an additive effect was obtained. In the presence of only CryIC, the LC₅₀ was 63 ppm. In the presence of CryIAc, the LC50 was 197 ppm, and it was 103 ppm in presence of CryIAg. In presence of CryIC co-expressed with Cry IAg or with CryIAc, the LC₅₀ decreased to 2.2 ppm and 6.6 ppm respectively.

Thus a combination of the Cry proteins of IC and IAg /or IAc could result in effective insect control. With this approach, a combination of Cry proteins can be designed rather than discovered. Selecting host backgrounds suitable for large-scale fermentation can augment crystal protein production.

Key words: Toxin gene, *cry* gene, *Bacillus thuringiensis*, shuttle vector, *Spodoptera littoralis*, acrysalifours *Bt*, transformed *Bt*, brush border membrane vesicles (BBMV).

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DEDICATION

*To the soul of my mother
To my husband Nabil Omar
To my lovely Kids; Pasent, Omar, and Ahmed*

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