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بالرسالة صفحات

لم ترد بالأصل

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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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APPROVAL SHEET FOR SUBMISSION

Title of [Ph.D.] Thesis: Production of a genetically modified strain of bacteria *Bacillus thuringiensis*

Name of the candidate: Nahed Abdel Ghaffar Abdel Aziz Ibrahim

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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*

Degree: (Ph.D.) Thesis. Faculty of Science, Cairo University 2000/2001.

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 *cry*1C (which encodes an insecticidal protein highly specific to *S. littoralis*) and *cry*1Ag (1Ac like) were used. *Hind*III digested *cry*1C was ligated into the *Hind*III site of the shuttle vector pHT7593. *Hin*cII digested *cry*1Ag was ligated into *Sma*I site of pHT7593 and into *Sma*I 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 *cry*1C-harboring was toxic to larvae of *S. littoralis*.

The pHT7593-bearing *cry*1C, pHT-bearing *cry*1Ag and pHTNC3-harboring-*cry*1Ag were transferred into the Cry⁻ Bt4 *Bt* strain. PHT-bearing *cry*1C was also transferred into the Cry⁻ *kurstaki*-HD73 (which contained only *cry*1Ac) native recipient, using electroporation. The introduction of the *cry*1Ag gene into Cry Bt4 resulted in the formation of bipyramidal crystals. The introduction of both *cry* genes 1C and 1Ag resulted in the multiplication of bipyramidal crystals. Further, the introduction of *cry*1C into Cry⁻ *kur*-HD73 shows multiplication in bipyramidal crystals.

Binding of Cry1Ac, Cry1Ag, and Cry1C to midgut epithelial cell membrane protein of *S. littoralis* on ligand blots were investigated. Cry1C-Cry1Ag hybrid toxins were also used for this purpose. Results show ICP of Cry1C interact with BBMV of *S. littoralis* specifically with ~ 110 kd, and Cry1Ag / or Cry1Ac binds two proteins of Molecular masses ~ 110 kd and ~ 20 kd. A combination of Cry1C and Cry1Ag / or Cry1Ac bind the same ~ 110 kd and ~ 20 kd proteins of BBMV.

In bioassays, *cry*1Ac-expressing *kur*-HD73 and cry1Ag-expressing Bt4 *Bt* strains caused mortality of *S littoralis* larvae only slightly. When Cry1C and Cry1Ag were applied together, an additive effect was obtained. In the presence of only Cry1C, the LC_{50} was 63 ppm. In the presence of Cry1Ac, the LC50 was 197 ppm, and it was 103 ppm in presence of Cry1Ag. In presence of Cry1C co-expressed with Cry 1Ag or with Cry1Ac, the LC_{50} decreased to 2.2 ppm and 6.6 ppm respectively.

Thus a combination of the Cry proteins of 1C and 1Ag /or 1Ac 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*, acrystalifours *Bt*, transformed *Bt*, brush border membrane vesicles (BBMV).

Supervisors: Prof.Dr. Sanaa Osman Abd-Allah,
Prof.Dr. Mohamed Sayed Salama

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Chairman Of Chemistry Department
Faculty of science – Cairo University.

DEDICATION

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

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