ABSTRACT

Abeer Mahmoud Mohammad ' Microbiological studies using recombinant bacterial strain as bioinsecticide against cotton leafworm *Spodoptera littoralis*' Unpublished Ph.D. thesis, University of Ain Shams, Faculty of Agriculture, Department of Agric. Microbiology, 2007.

A total of seven *Bacillus thuringiensis* (*B.t.*) isolates were obtained from thirty-four bacterial collections from different Egyptian soils. Two of these seven isolates were found to be effective against the 2nd instars of *Spodoptera littoralis* larvae. These isolates were named as *B.t.* Mo-I and K-II.

Cry I gene of these strains, which is responsible for insecticidal protein, was identified by polymerase chain reaction (PCR) technique. The morphology of sporangia and parasporal inclusions was differentiated by phase contrast and electron microscope, respectively. It was found that *B.t.*Mo-I produces bi-pyramidal inclusions, whereas K-II produces cubical inclusions.

Recombinant *E. coli* strain, that contains the CryI gene, was tested for its efficacy against the 2nd instar larvae of *Spodoptera littoralis.* It was found to give 70 % mortality of the larvae.

Recombinant *E. coli* was propagated in MR medium, experimentally modified, to obtain the highest yield of cells and δ-endotoxin, using 100 ml medium / 250 ml conical flasks, at 200 rpm on rotary shaker. Maximum cell yield was obtained when sucrose in 20 g/L, and yeast extract were used respectively, as carbon and nitrogen sources, at pH 7.0 and 37°C incubation temperature, for attaining maximum cell yield and at pH 7.5 and 30°C incubation temperature, for producing maximum endotoxin concentration.

The effect of stress conditions on viability and activity of recombinant *E. coli* illustrated that, solar radiation showed a bad effect on both viability and efficacy after 3 days of exposure.

The drastic effect also happened when recombinant *E. coli* was exposed either to heat treatment (at 50^{o}C for more than 5 minutes) or to high concentrations of sucrose (> 20 % for more than 2 days) and for NaCl (> 5 % even after one day of exposure).

The best preservation method, for keeping viability and activity of recombinant *E. coli* against the larvae, was found to be freezing under glycerol, then granulation and finally lyophilization.

Keywords: *Bacillus thuringiensis,* CryI gene, Recombinant *E. coli*, Medium requirements, δ-endotoxin, Genetic stability.

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