

**GENETIC IMPROVEMENT AND MOLECULAR
EVALUATION OF *BACILLUS THURINGIENSIS*
TO CONTROL SOME MAJOR INSECT
PESTS IN EGYPT**

By

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ABSTRACT

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The discovery of highly toxic isolates reveals the usefulness of screening studies for novel *Bt* strains is considered very important for genetic improvement. Protoplast fusion of the two strains *Bt* El-Sharkia (*Bt* Sh) and Damietta (*Bt* D) was applied to obtain new fusion *Bt* strain with high ability to control potato tuber moth and cotton leaf worm insects as a genetic improvement. The two strains of *Bacillus thuringiensis* used in this study were obtained from previous studies during M.Sc. They were registered at the NCBI database under accessions number: LC070660 for El-Sharkia and LC070661 for Damietta strains. They were tested chemically according to API 20E test and to colony morphology which confirmed that the two strains were round, about 3-5 mm in diameter, raised or flat slightly shiny, grayish white opaque with entire edge and having a granular surface. The microscopic examination also showed that the *Bacillus* strains were gram-positive, rod-shaped bacteria, all the previous techniques results confirmed that the two strains are *Bacillus*. SDS-PAGE analysis detected 3 protein bands with MWs at 140, 130, and 125 KDa in the two *Bacillus* strains and their fusion which related to Cry proteins. The two bacterial strains were grown before protoplast fusion and their antimicrobial susceptibility to 28 antibiotics (100 µg/ml) was tested, separately, to identify their resistance and susceptibility to these antibiotics. The two strains showed resistance to ampicillin, while *Bt* Sh strain was resistant to nitrofurantoin and susceptible to cefpodoxime. On the contrary, the *Bt* D strain was resistant to cefpodoxime, and susceptible to nitrofurantoin, the two strains were susceptible to the rest of the 25 other antibiotics. According to heat tolerance, Sh strain was able to tolerate up to 50°C, however, D strain was

unable to tolerate 50°C. Both isolates represented high growth rates at 35°C. For salt stress tolerance, the two strains were grown on different concentrations of NaCl (0.5-8%) and could grow at the lowest concentration of NaCl (0.5%). Sh strain grew on NaCl up to 6.5%, however, D strain was unable to grow on 6.5% NaCl. Successfully, the two strains Damietta and El-Sharkia were integrated according to their differences in the antibiotic resistance to obtain protoplast fusion. At the same time, the two strains showed different pattern according to their tolerance to heat (50°C) and salt (6.5%); *Bt* Sh was tolerant to heat and salt and contains *cry*

genes type II, while *Bt* D was sensitive to heat and salt and contains *cry* genes types I and II. Selection of the desirable fusant was applied by adding the previous three antibiotics to the selective medium and salinity and heat. Only the fusant strain combined the antibiotic resistance, salinity and heat tolerance patterns and type (I & II) of *cry* genes as their corresponding two parental strains was chosen.

The protoplast fusion (fusant) between the two strains revealed high toxicity to the new nates of potato tubers moth; *Phthorimea operculella* with 80% mortality compared with the parent strains 20-23%. The mortality% for the cotton leaf worm in the parent strains was 90%, while the fusant exhibited 80% mortality.

Keywords: *Bacillus thuringiensis*, protoplast fusion, *cry* genes, antibiotics, cotton leaf worm and potato tuber moth

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