BIOCHEMICAL AND BIOLOGICAL STUDIES ON SOME MICROBIAL INSECTICIDES

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5-SUMMARY

The growing concern over the hazards of synthetic chemical insecticides has stimulated development and employment of alternative means of insect control. One approach gaining in prominence is the utilization of microbial products such as *Bacillus thuringiensis* (*B.t.*).

The present study is concerned with the isolation and characterization of *B.t.* isolates from Egyptian soil and their active purified proteins using modern molecular and biochemical methods together with phenotypic methods. The existence of insecticidal protein genes will also be identified.

During the course of this study, the following results were obtained:

1) Isolation of *Bacillus thuringiensis* with insecticidal activity from local soils:

Soil samples were collected from different fields in Kfr El-Shiekh and from Fayoum governorates that had not previously been treated with Bt. and 19 isolates were found to be *Bacillus thuringiensis*. These 19 isolates were bioassayed for their insecticidal activity against *S.ltttoralis* larvae. All the tested isolates showed a virulent effect to *S.littoralis* 3rd instar larvae. However the mortality rate differed according to the bacterium isolate and range from 8% to 83% , the highest mortality percentage were obtained with *B.t.* isolates Fay1 (83%), Fay2 (83%), Kfr1 (81%), Fay4 (79%).

2) Distinguishing among isolates

a) Morphological and Physiological Identification of the isolated strains

The Fay1 Fay2 ,Kfr1 , Fay4 isolates were gram-positive, rod-shaped cells containing only one endospore. All the isolates were characterized by the presence of the crystals with different

sizes (big, medium, and small), and different shapes (irregular, spherical, flat square, and bipyramidal shape). Isolate Fay4 has only one shape of crystal (bipyramidal whereas isolates Fay1, Fay2, Kfr1 having mixed type of crystals with different shapes. The four isolates were able to tolerate high salt concentration (7%), produced acids from only glucose and galactose, hydrolyze starch and casein.

b) Molecular characterization of *Bacillus thuringiensis* isolates:

The present study tried to employ all the possible molecular biology techniques for answering the question about the genetic diversity among the *B.t.* isolates Fay1, Fay2, Fay4, Kfr1. These methods included the fractionation of the cellular protein contents of the vegetative and sporulated cells grown under the exact conditions, plasmid DNA profiles, RAPD-PCR product profile analysis, and serological relatedness of the crystal toxin proteins.

The results obtained could be summarized as follows:

1) The protein contents of the vegetative and sporulated cells were compared. The total proteins were separated on SDS- polyacrylamide gel electrophoresis. The results of such procedures were able to distinguish between the four *B.t.* isolates which can be classified into three types Fay1, (Fay2 =Kfr1), Fay4. The patterns of sporulated cells indicated that isolates Fay1, Fay2, Kfr1, and Fay4 produced proteins that have a molecular weight of 130 kDa and 65 kDa, which are responsible for their insecticidal activity.

2) Study of the differences in the plasmid contents of the four *B.t.* isolates: All tested vegetative cells of four isolates contained plasmids and the number of plasmids found were varied from 3-7 per

cell, with estimated molecular weight of higher than 10000 mega dalton.

3) RAPD-PCR

In the present study five primers OPO11, OPO13, OPO15, OPO19, and OPD18 were used. Primers OPO11, OPO15, OPD18 showed that isolates Fay2, Kfr1 produced exact pattern, and the rest of the isolates Fay1, Fay4 were different.

4) The double immunodiffusion assays with antibodies to lepidopteran toxin proteins showed difference in the precipitation patterns among the four isolates and hence genetic diversity.

3) <u>separation and purification of 65 kDa insecticidal protein:</u>

In this study, a method for separation and purification of 65 kDa insecticidal protein was developed. This methods allows recovery of this protein from crude mixture of spore-crystal by solubilizing of the crystalline proteins by treating with strong alkali and then lowering the pH to the isoelectric point of the 65 kDa protein by hydrochloric acid. As a result highly purified protein was obtained.

5) <u>Characterization of genes coding for insecticidal proteins by PCR</u>

PCR technology and primers specific for *B.t.* endotoxin genes were used to study the presence of genes encoding protein responsible for insecticidal activity. A set of primers specific for *Cry1, Cry111,* and *Cry1V* genes that insecticidal for lepidopeterans, coleopterans, and dipteranas were selected. The *Cry1A(b)* was found in all of the four tested *B.t.* isolates. The coleopteran toxin gene(s) product(s) were detected in isolates Fay1, Fay4 , of the four isolates, Whereas the *Cry1V* gene products were found in 3 out of four tested isolates (kfr1, Fay2, Fay1).