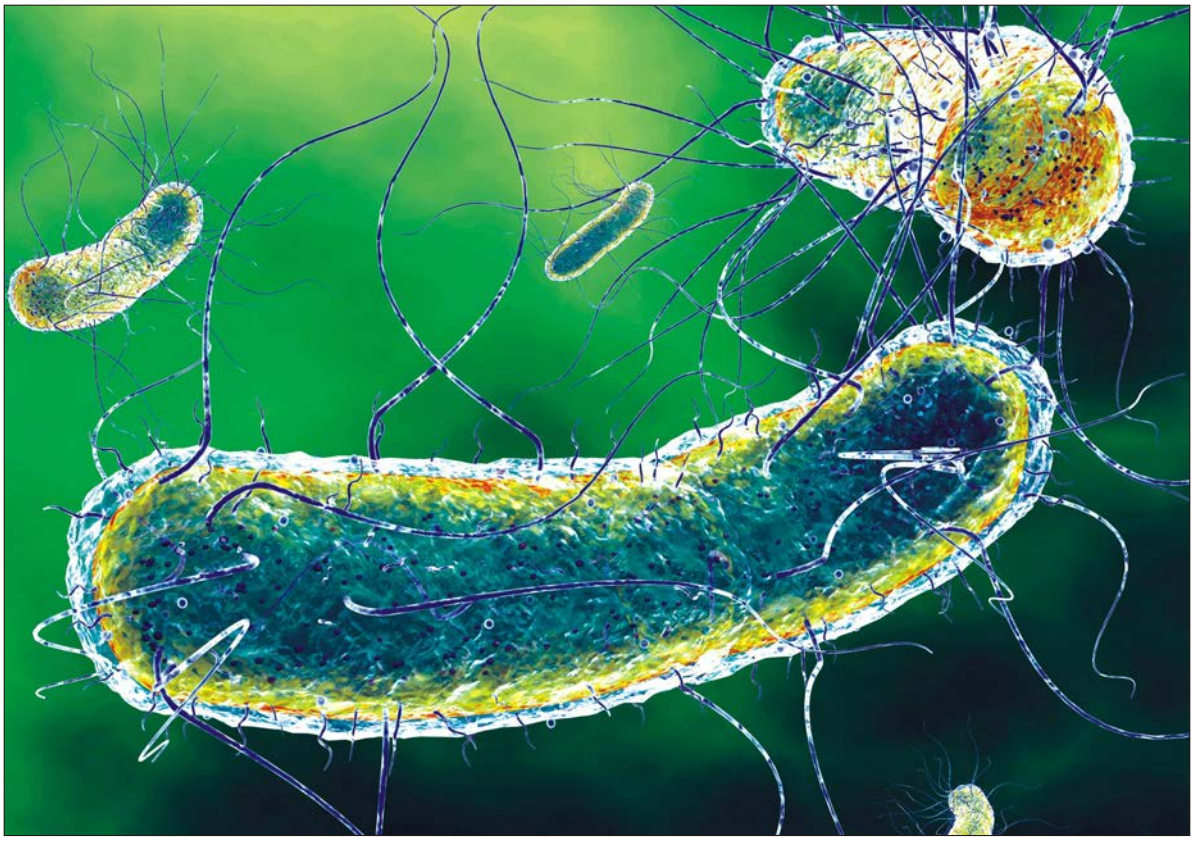

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Effect of *Bacillus thuringiensis* var. *israelensis* Endotoxin on the Intermediate Snail Host of *Schistosoma japonicum*

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Abstract: *Bacillus thuringiensis* demonstrates a very wide spectrum of biological activities. It is gaining widespread acceptance in the control of pests and vectors of diseases. Few reports have described the responses of the intermediate host of Schistosomes to *B. thuringiensis* toxins. This preliminary study showed the effect of *Bacillus thuringiensis israelensis* endotoxin on the intermediate host of *Schistosoma japonicum* under laboratory conditions. The alkali soluble endotoxin protein of two *B. thuringiensis israelensis* strains; (977) and (NRRL HD-522) were bioassayed for its toxicity against non-infected Chinese *Oncomelania* snails then analyzed using SDS-PAGE. The toxin concentration of 20 ng mL^{-1} from both strains resulted in 0% mortality after 48 h, but the concentration of 0.9 μg mL^{-1} resulted in 70% mortality after 24 h. Bioassays indicated that non-infected Chinese *Oncomelania* snails are susceptible for the soluble protein of the test strains at high concentrations only. SDS-PAGE polypeptide pattern of the soluble proteins investigated against snails, with a predominant protein bands at about 66, 53, 35 and 28 kDa. The soluble proteins of the test strains have molluscicidal activity towards non-infected Chinese *Oncomelania* snails at high concentrations only.

Key words: Bacteria, *Bacillus thuringiensis*, endotoxin, snail host

INTRODUCTION

Bacillus thuringiensis (*B. thuringiensis*) is a Gram-positive bacterium that forms a parasporal crystal during the stationary phase of its growth cycle. *B. thuringiensis* was initially characterized as an insect pathogen and its insecticidal activity was attributed largely or completed (depending on the insect) to the parasporal crystal. More than 200 crystal protein genes (encoding Cry and Cyt proteins) have been described (Salem *et al*., 2006).

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B. thuringiensis demonstrates a very wide spectrum of biological activities. It is gaining widespread acceptance in the control of pests and vectors of diseases. Since the discovery and identification of *Bacillus thuringiensis* subsp. *Israelensis* (serotype H14) in 1978, this agent has been developed as an alternative tool for mosquito control (De Barjac, 1978). Schistosomiasis is a parasitic disease transmitted to man and other mammals via snails. *B. thuringiensis israelensis* is used in localities where mosquito or blackfly larvae are present together with snail vectors of schistosomes (Horák *et al*., 1996). Few reports have described the responses of the intermediate host of *Schistosoma mansoni* to *B. thuringiensis* strains and other bacterial species (Osman *et al*., 1992; Weiser *et al*., 1992; De Oliveira *et al*., 2004). It was also reported that China started the production of Jie-Jue-Ling, a *B. thuringiensis israelensis* preparation controlling mosquito larvae as early as in 1979 and efforts were made to assess the toxicity and field efficiency of *B. thuringiensis israelensis* in mosquito (Yu and Wang, 1987; Yu and Shen, 1990).

However, exploration of biological factors for control of *Oncomelania* snails that act as intermediate host of *Schistosoma japonicum* in Asia is not developed. To our knowledge, no reports have described the responses of *Oncomelania* snails to *B. thuringiensis* strains.

In this study, the proteins of two *B. thuringiensis israelensis* strains tested against non-infected Chinese *Oncomelania* snails under laboratory conditions and analyzed using SDS-PAGE.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Two *B. thuringiensis* strains were used in this study. *B. thuringiensis israelensis* (977) was kindly supplied by Prof. Priest, FG, Heriot University, England). *B. thuringiensis israelensis* (NRRL HD-522) was kindly supplied by Prof. Nakamura, LK, United State Department of Agriculture. All experiments of this study have been carried during the year 2006. These strains were cultured in NB (nutrient broth) medium for 5 days at 30°C under continuous shaking at 200 rpm.

Preparation of Soluble Toxin Protein

Spore-crystal complex was suspended in 0.1 M Na_2CO_3 buffer (pH 10) (De León and Ibarra, 1995). The suspension was incubated for 3 h at 37°C under continuous shaking at 200 rpm, followed by centrifugation at 12000 rpm for 30 min. Supernatant was dialyzed against deionized water overnight at 4°C. Protein concentration was determined by measurement the absorbance at 280 nm and the protein analyzed using 10% SDS-polyacrylamide gel according to previous report (Laemmli, 1970).

Oncomelania Hupensis Snail Bioassay

The intermediate host of *Schistosoma japonicum* was collected from Xichang City of Sichuan Province in China. To detect possible infections by *Schistosoma japonicum*, snails were kept in 200 mL jars in tap water and under light for 3 h. The water was examined under light microscope for the existence of cercariae. The snails were allowed to adapt to laboratory conditions for 1-3 weeks.

The soluble toxin proteins were added to 20 mL dechlorinated tap water to a final concentration of about 20 ng mL^{-1} and 0.9 μg mL^{-1} for both tested strains, immersion of groups of 10 snails of uniform size in these concentrations for 24 h. And then, the snails were washed with and reimmersed in dechlorinated tap water to recover them for 24 h. Two

replicates were tested for each strain. The mortality percentage calculated as the number of dead snails to the starting number. The sample was replaced by dechlorinated tap water in the control group.

RESULTS AND DISCUSSION

It was observed that the concentration of the soluble protein was an important factor in determining their toxicity against snails. Where the low toxin concentration of 20 ng mL^{-1} for both strains (977) and (NRRL HD-522), resulted in 0% mortality after 48 h, but the high concentration of 0.9 μg mL^{-1} resulted in 70% mortality after 24 h. SDS-PAGE polypeptide pattern of the soluble proteins investigated against snails, with a predominant protein bands at about 66, 53, 35 and 28 kDa for *B. thuringiensis israelensis* (977) and (NRRL HD-522), (Fig. 1).

In our previous studies, it has been shown that *B. thuringiensis* strain that has been selected for being toxic against *Biomphalaria alexandrina* snails (Salem, 2004).The spore and crystal suspensions of the same strain was highly toxic (80% mortality) for both non-infected field collected and laboratory-reared *Biomphalaria alexandrina* snails in Egypt. The same strain and other *B. thuringiensis* standard strains were preliminary bioassayed for toxicity against infected, non-infected Chinese *Oncomelania* snails and the emitted cercariae (Salem *et al*., 2006). The new isolate reacted positively to the universal primers specific for the entire coding regions of 7 cry genes including cry1, cry3 and cry4. Moreover, the results of SDS-PAGE displayed common protein bands at about 66, 43 and 35 kDa in the protein patterns of strains from different serovars as well as the new isolate in addition to the distinct protein bands for each strain.

In the present study, bioassays indicated that non-infected Chinese *Oncomelania* snails are susceptible for the soluble protein of the test strains at high concentration only.

The dose which cause 70% mortality in this study is greater than those seen with mosquitoes and this variation is presumably may be due to individual species sensitivity (Tyrell *et al*., 1979).

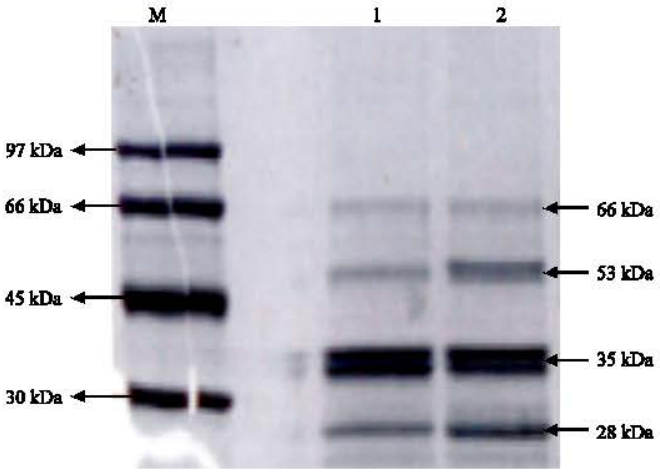

Fig. 1: SDS-PAGE of alkali soluble protein of two *B. thuringiensis israelensis* strains: Lane M: Protein molecular weight marker; Lanes 1 and 2: Strains 977 and HD-522, respectively

The molluscicidal activity of alkali-soluble protein from *B. thuringiensis israelensis* strains may attributed to a 66 kDa protein alone or in combination with other obtained polypeptides. It has been reported that purified crystal proteins of *Bacillus thuringiensis* var. *israelensis* by FPLC on a Mono Q column yielded 130, 65, 28, 53, 30-35 and 25 kDa proteins and all the purified proteins killed *Aedes aegypti* larvae after citrate precipitation, but the 65 kDa protein was the most toxic. The precipitated mixture of 27 and 130 kDa proteins was almost as toxic as solubilized crystals. Moreover, the activated form (25 kDa) of the 27 kDa protein was generally cytotoxic with the lowest LCs0 values *in vitro* assays against a range of insect cell lines while they found the activated forms of the 130 and 65 kDa protoxins (53 and 30-35 kDa proteins, respectively) were much more specific than the 25 kDa protein in their action on dipteran cells reported that a 65 kDa protein responsible for mosquitocidal activity (Chilcott and Ellar, 1988). Other researchers separated three major protein components, 130, 65 and 26 kDa from solubilized *Bacillus thuringiensis* subsp. *israelensis* crystal by gel filtration and the results showed that bioassays of mixtures of the individual toxins towards mosquito larvae revealed a number of synergistic interactions which explained in part why the native crystal is considerably more toxic than any of the individual toxins (Park *et al*., 2001). The dipteran-specific insecticidal protein Cry4A is produced as a protoxin of 130 kDa in *Bacillus thuringiensis* subsp. *israelensis*. Yamagiwa *et al*. (1999) processed it into the two protease-resistant fragments of 20 and 45 kDa through the intramolecular cleavage of a 60 kDa intermediate *in vitro* and *in vivo*. The functional properties of the two fragments, GST (glutathione S-transferase) fusion proteins of the 60 kDa intermediate showed that neither the GST-20 kDa fusion protein (GST-20) nor the GST-45 kDa fusion protein (GST-45) was actively toxic against mosquito larvae of *Culex pipiens*, whereas the GST-60 kDa intermediate fusion protein (GST-60) exhibited significant toxicity.

Based on the data presented here, it could be concluded that the soluble proteins of the test strains have molluscicidal activity towards non-infected Chinese *Oncomelania* snails at high concentrations only and the molluscicidal activity of alkali-soluble protein from *B. thuringiensis israelensis* strains may attributed to a 66 kDa protein alone or in combination with other obtained polypeptides.

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