HISTOPATHOLOGICAL EFFECTS OF A PROTEIN COMPLEX PURIFIED FROM XENORHABDUS NEMATOPHILA ON THE MIDGUT OF HELICOVERPA ARMIGERA LARVAE

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Abstract

Entomopathogenic nematodes in the field carry symbiotic bacteria (Xenorhabdus or Photorhabdus) into the host insect through a natural orifice or the body wall and then release the symbiotic bacteria into the blood cavity of the insect. The symbiotic bacteria multiply and release a variety of active substances, including insecticidal proteins, which kill the host insect rapidly. In this study, a protein complex (named Xnpt) with insecticidal activity was isolated from X. nematophila HB310 strain using methods of salting out and native polyacrylamide gel electrophoresis (PAGE). Six polypeptides ranging 50~250 kDa were well separated from Xnpt protein by sodium dodecyl sulfate (SDS)-PAGE. Xnpt showed growth inhibition effect on the neonates of Helicoverpa armigera and destroyed the excised peritrophic membrane of H. armigera. The histopathology of Xnpt to H. armigera fourth-instar larvae was studied by dissecting and olefin slice of the midgut. The midgut tissues of the larvae began to change after treated with Xnpt (500 ng/mL) orally in 6 hours. The forepart of the peritrophic membrane began to fracture, and the midgut cells extended. The epithelium was decomposed gradually, and the midgut tissues were loose or disordered. The peritrophic membrane disappeared at 12 h but appeared again at 72 h following transient or sublethal exposure to the toxin. The histological analysis of H. armigera larvae midgut showed that Xnpt has extensive histopathological effects on the host tissues.

Introduction

Xenorhabdus spp. and Photorhabdus spp. are symbiotically associated with nematodes of the families, Steinernematidae and Heterorhabditidae, respectively. Entomopathogenic nematodes such as Steinernematidae and Heterorhabditidae carry symbiotic bacteria into the blood cavity of host insect and then release the symbiotic bacteria. The bacteria produce toxins to overcome immune response of insect hosts and kill their hosts. A family of oral and injectable insecticidal toxins produced by Xenorhabdus and Photorhabdus has been identified (Blackburn et al. 1998). Bowen et al. (1998) isolated several kinds of Tc from Photorhabdus luminescens W14 and reported that the histopathology of the Manduca sexta midgut following oral Tca treatment was very similar to that described for the δ-endotoxins from Bacillus thuriqiensis (Bowen et al. 1998). It implies that these bacteria have the potential to be developed as insecticidal agents. Xenorhabdus nematophila HB310 was isolated from Steinernema carpocapsae HB310. We isolated a toxin complex with oral activity from X. nematophila HB310 and described the influence of the toxin protein on the Helicoverpa armigera larvae.

Methods

H. armigera larvae and X. nematophila HB310 were obtained from the Pest Biocontrol Insectary, Hebei Agricultural University. Toxin complex was obtained using the methods such as salting out and native-PAGE from the cells of X. nematophila HB310 (Wang et al. 2005). H. armigera neonates and fourth-instar larvae were placed in the wells of a 24-well cell-culture plate filled with diet and held in an incubator at 26±1°C. The diet was either treated with phosphate (PBS) buffer as an untreated control, or with toxin complex (protein concentration 5.19 μg /g diet). Symptoms of toxicity were noted, and survivors were weighed 120 h after the initiation of the bioassay. Three replicates were used for each treatment, with 72 total insects per treatment.

Fourth-instar larvae of H. armigera were transferred to the artificial diet treated with 20 μL of toxin complex (51.9 μg/mL). The peritrophic membranes (PMs) were obtained by dissecting the treated larvae midguts at 6, 12, 24, 36, 48, 60, 72, and 96 h. The difference between control and treatment was observed at the same period of time. Ten insects were dissected per treatment.

The midguts from the fourth-instar larvae of H. armigera were dissected and immediately fixed in Bouin's fluid. The fixed larvaal midguts were then embedded in paraffin, and 5 μm sections were cut. The sections were stained with eosin and hematoxylin and mounted with glycerol for microscope imaging.

Results and Discussion

We isolated every protein band from the native-PAGE spectrum of X. nematophila HB310 intracellular protein extracts (Fig 1). Bioassay results indicated that the oral insecticidal activity of the second protein band was higher than that of other bands to H. armigera neonates. This protein was named as Xnpt complex (Fig.1). In the SDS-PAGE spectrum, this protein complex was separated to more bands. Xnpt complex showed strong growth inhibition effect on the neonates of H. armigera. After 5 days of feeding, the larva that fed toxin were considerably smaller compared to the larvae in control (Table 1).

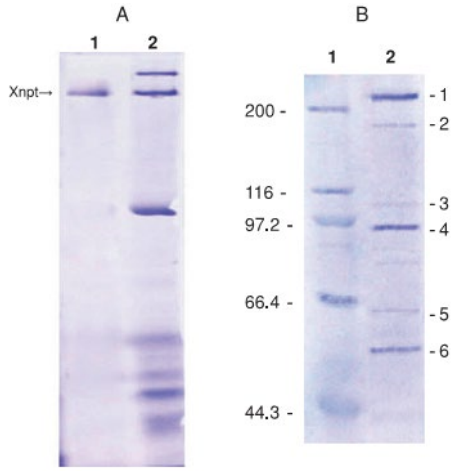

Fig. 1. Native-PAGE and SDS–PAGE analysis of Xnpt complex

A: 1: the arrow indicates Xnpt complex. 2: multi-bands of crude intracellular protein.
B: 1: molecular mass marker. 2: Xnpt complex was separated into six bands.

Table 1. Oral toxicity of Xnpt complex against H. armigera larvae (120 h)

Sample	Average weight	
	Neonate	Fourth-instar larvae
CK	9.37 ± 2.20 a*	285.6 ± 4.60 a
Xnpt complex	0.33 ± 0.01 d	133.5 ± 2.95 b

*Means followed by the same letter do not differ significantly at α= 0.05.

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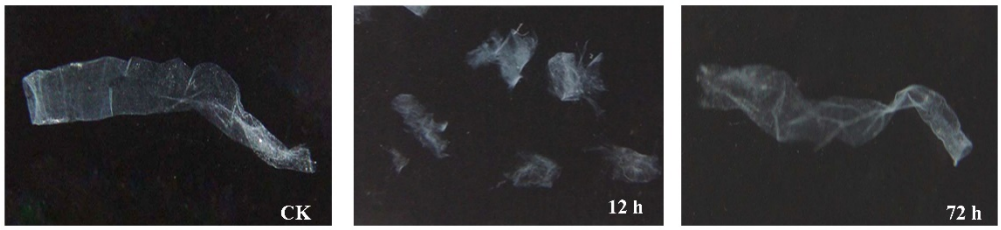

Fig. 2. The effects of Xnpt complex on the peritrophic membrane (PM) of *H. armigera*
CK: control;12h: PM after 12 h on the treated diet; 72h: PM after 72 h on the treated diet.

In the control group, the peritrophic membrane (PM) of H. armigera was complete, translucent and elastic. After 6 h of exposure to the toxin, the PMs color turned to milk-white. After 12 h, PMs ruptured into several fragments in water

(Fig. 2). However, recovery of the PMs back to the complete structure as well as transparent membrane clarity was observed after 72 h of treatment (Fig. 2).

The columnar cells were ellipsoidal and arranged closely, and PMs could be recognized clearly in control (Fig. 3). After being exposed to toxin-treated diet for 12 h, the columnar cells of treatment swelled apically and began to extrude large cytoplasmic vesicles into the gut lumen. PMs disappeared completely (Fig. 3) and although the gut epithelium was still disorganized, the PMs reappeared at 72 h (Fig.3).

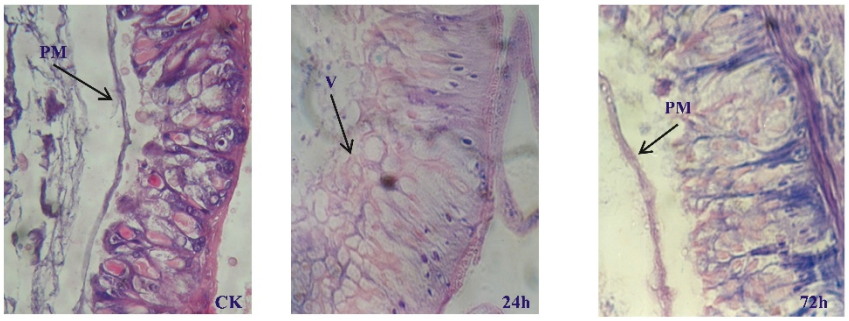

Fig. 3. The histopathological effects of Xnpt complex on the midgut of *H.armigera* (400×).
CK: control; 24h: after 24 h of exposure to Xnpt; 72h: after 72 h of exposure to Xnpt. PM: peritrophic membrane; V: vesica.

Summary

In our results, Xnpt complex with insecticidal activity was isolated from X. nematophila HB310. Xnpt showed strong growth inhibition effect on the neonates of H. armigera. The histopathological results show that the action target of the toxin complex is the midgut epithelium in H. armigera, which acted in the same fashion as tca against Manduca sexta (ffrench-Constant and Bowen, 1999; Blackburn et al., 1998) and δ-endotoxins and Vip3A from B. thuringiensis (Aronson et al., 2001). The PMs serve as the first line of defense in the midgut, so the PMs had begun to occur transformation at 6 h after being fed with toxin-treated diet and were broken into pieces after 12 h. Then, the toxin penetrated the midgut epithelial cells and continued to destroy the cells. When the insects were subjected to transient or sublethal exposure to the toxin, the epithelial cells were gradually restored and excreted, and the PMs were renewed with the disappearance of the toxin activity. Xnpt complex has high oral toxicity against a wide range of insects. Hence, it has the potential to be used as a bacterial insecticide or as an alternative to Bt for transgenic deployment.

References

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