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Ultrastructural Changes in a Cell Line from Plutella xylostella Treated with Bacillus thuringiensis Delta-endotoxin【Research report】

小菜蛾細胞株受蘇力菌內毒素處理精微構造之改變【研究報告】

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Abstract

Ultrastructural changes in a cell line, PX-1187, established from the diamondback moth, Plutella xylostella, treated with delta-endotoixn from Bacillus thuringiensis subsp. kurstaki (Btk) were observed using transmission electron microscopy. The normal PX-1187 cell has a large nucleus, several vesicles, intact mitochondria, Golgi complex and other organelles. When the cell were treated with delta-endotoxin for 30 min, the granules on mitochondrial cristae became separated from the inner membranes. The Golgi complex showed vacuoles and loose dictyosomes. After treating for 60 min, the cytoplasm was slightly vacuous, the mitochondrial cristae leaked, the dictyosomes showed damage and the cisternae were fused. After 90 min, parts of the cell membranes were perforated, the inner memberanes of the mitochondria lysed, and parts of the outer membranes disintegraterd. In addition, fusion of cisternae occurred and flocculent materials were seen in the Golgi complex. After 120 min, most cell membranes were destroyed, with the outer membranes of the mitochondria largely disintegrated. The Golgi complex exhibited lesions on its dictyosomes and fusion of flocculent materials. Cell lysis occurred by 150 min after treatment with delta-endotoxin.

摘要

由小菜蛾 (Plutella xylostella) 所建立之PX-1187細胞株經蘇力菌 (Bacillus thuringiensis subsp. kurstaki, Btk) 產生的δ-內毒素處理後，引起精微構造之改變，以穿透式電子影微鏡觀察。正常的PX-1187細胞具型大之細胞核、囊狀物數個、完整的粒線體、高基氏體及其它胞器。以δ-內毒素處理30分鐘後，粒線體內膜上之小顆粒有脫落的跡象；高基氏體四周形成一囊胞，膜狀物質較為鬆散，分泌物顆粒變小。處理60分鐘後，細胞質內空隙逐漸擴張，粒線體內膜已有破裂痕跡；高基氏體之膜狀物更疏鬆，分泌物顆粒數量減少。處理90分鐘後，部分細胞膜已有穿孔現象，粒線體內膜明顯破壞；高基氏體之膜狀物質已疏散斷裂，殘存少數分泌物顆粒；囊胞內模糊；細胞質內空隙繼續擴張。處理120分鐘，細胞膜大部分破壞，外膜崩解；粒線體外膜破裂，內膜已不存在；高基氏體之囊胞變大，只見不完整膜狀物質；細胞質內空隙變的更大。處理150分鐘後，細胞發生崩解。

Key words: Bacillus thuringiensis, Plutella xylostella, Diamondback moth, delta-endotoxin, PX-1187 cell line.

關鍵詞: 蘇力菌、小菜蛾、內毒素、PX-1187細胞株。

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Ultrastructural Changes in a Cell Line from *Plutella xylostella* Treated with *Bacillus thuringiensis* Delta-endotoxin

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ABSTRACT

Ultrastructural changes in a cell line, PX-1187, established from the diamondback moth, *Plutella xylostella*, treated with delta-endotoixn from *Bacillus thuringiensis* subsp. *kurstaki* (Btk) were observed using transmission electron microscopy. The normal PX-1187 cell has a large nucleus, several vesicles, intact mitochondria, Golgi complex and other organelles. When the cell were treated with delta-endotoxin for 30 min, the granules on mitochondrial cristae became separated from the inner membranes. The Golgi complex showed vacuoles and loose dictyosomes. After treating for 60 min, the cytoplasm was slightly vacuous, the mitochondrial cristae leaked, the dictyosomes showed damage and the cisternae were fused. After 90 min, parts of the cell membranes were perforated, the inner memberanes of the mitochondria lysed, and parts of the outer membranes disintegrated. In addition, fusion of cisternae occurred and flocculent materials were seen in the Golgi complex. After 120 min, most cell membranes were destroyed, with the outer membranes of the mitochondria largely disintegrated. The Golgi complex exhibited lesions on its dictyosomes and fusion of flocculent materials. Cell lysis occurred by 150 min after treatment with delta-endotoxin.

Key words: *Bacillus thuringiensis, Plutella xylostella,* Diamondback moth, delta-endotoxin, PX-1187 cell line.

小菜蛾細胞株受蘇力菌內毒素處理精微構造之改變

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摘　要

由小菜蛾（*Plutella xylostella*）所建立之PX-1187細胞株經蘇力菌（*Bacillus thuringiensis* subsp. *kurstaki*, Btk）產生的δ-內毒素處理後，引起精微構造之改變，以穿透式電子影微鏡觀察。正常的PX-1187細胞具型大之細胞核、囊狀物數個、完整的粒線體、高基氏體及其它胞器。以δ-內毒素處理30分鐘後，粒線體內膜上之小顆粒有脫落的跡象；高基氏體四周形成一囊胞，膜狀物質較為鬆散，分泌物顆粒變小。處理60分鐘後，細胞質內空隙逐漸擴張，粒線體內膜已有破裂痕跡；高基氏體之膜狀物更疏鬆，分泌物顆粒數量減少。處理90分鐘後，部分細胞膜已有穿孔現象，粒線體內膜明顯破壞；高基氏體之膜狀物質已疏散斷裂，殘存少數分泌物顆粒；囊胞內模糊；細胞質內空隙繼續擴張。處理120分鐘，細胞膜大部分破壞，外膜崩解；粒線體外膜破裂，內膜已不存在；高基氏體之囊胞變大，只見不完整膜狀物質；細胞質內空隙變的更大。處理150分鐘後，細胞發生崩解。

關鍵詞：蘇力菌、小菜蛾、內毒素、PX-1187細胞株。

Introduction

Cumulative information on pathological changes of insect tissues after ingesting parasporal crystals produced by *Bacillus thuringiensis* (Bt) has been available since Heimpel and August (1959) reported damage of midgut epithelium by this insecticidal protein. The solubilized Bt delta-endotoxin may cause swelling of the plasma membrane and organellar changes of midgut cells (Endo and Nishiitsutsuji-Uwo, 1980; 1981; Percy and Fast, 1983; Lane *et al.*, 1989; Mathavan *et al.*, 1989). Cell lysis of the midgut epithelium could possibly be due to changes in ion channels or ATP generation after treatment with delta-endotoxin (Fast el at., 1978; Harvey *et al.*, 1983; Himeno *et al.*, 1985; Gupta *et al.*, 1985). Molecular studies suggest that binding of endotoxin to receptors on the epithelial membrance of midgut may result in cell lysis caused by imbalance of osmotic pressure between both sides of the membrances or by other molecular interactions (Himeno *et al.*, 1985; Knowles and Ellar, 1986; McCarthy *et al.*, 1988). Murphy *et al.* (1976) first observed morphological changes causing swelling, lysis, and vesicle formation in several lepidopteran cell lines when treated with Bt endotoxin. However, electron microscopic observations on subcellular changes in insect cell lines treated with Bt endotoxin are not reported. Recently a PX-1187 cell line was established from embryos of the diamondback moth, *Plutella xylostella* (Lee and Hou, 1992). This line was observed susceptible to Bt endotoxin based on cytotoxicity (Chang, 1990) and was thus suitable for investigating the ultrastructural damage caused by delta-endotoxin at the subcellular level.

Materials and Methods

Purification of Bt parasporal crystal

B. thuringiensis subsp. *kurstaki* (HD-1) (Btk) was cultured in slant with nutrient agar (Difco). Mass culture of vegetable cells was carried out using 50 ml CHES medium under 200 rpm rotation

at 32℃ for 72 hr. Then, 10 ml of this culture was transferred to a flask containing 90 ml CHES medium and cultured for 24 hr. For purification of parasporal crystals, the culture was centrifuged at 4, 000 x g for 10 min. The precipitate was mixed with sterile distilled water, and the bacterial cells sonicated 3 times to release the crystals. The resulting solution was centrifuged again at 10,000 x g for 10 min to sediment the crystals. The spores and crystals were separated by centrifuging through a 67 / 72 / 87% sucrose discontinued gradient at 8,000 x g at 4℃ for 14 hr. The zone between 72 and 87% of the gradient contained the delta-endotoxin.

Solubilization of Bt parasporal crystal

The purified crystal preparation was incubated with 50 mM Na_2CO_3-$NaHCO_3$ solution containing 10 mM dithiothreithol, pH 9.5, at 37℃ for 1 hr, and the solution centrifuged at 10,000 x g for 10 min. The supernatant was treated with a half volume of saturated $(NH_4)_2SO_4$ for 10 min to precipitate proteins which were then isolated by centrifugation at 10,000 x g for 10 min and then mixed with 50 mM Tris-HCl buffer (pH 8.0). This solution served as the solubilized endotoxin. Protein content was determinded with phenol reagent (Lowry *et al.*, 1951). Before using, the endotoxin solution was incubated with trypsin (Sigma) (2:1, w / w) at 37℃ for 2 hr.

Cell line

The PX-1187 cell line established from embryos of *P. xylostella* by Lee and Hou (1992) was used throughout this study. The cells were cultured with TC-100 (Gibco) containing 10% fetal bovine serum (Gibco) and 0.35 g $NaHCO_3$, pH 6.2, 345-355 mOsm / kg.

Incubation of cells with delta-endotoxin

Fifteen ml of a cell suspension (2×10^5 cells / ml) per tube were centrifuged at 1,000 rpm for 10 min and washed twice with PBSA (phosphate buffered saline without $MgCl_2$ and $CaCl_2$) after discarding the supernatant. Then the cells were incubated with 5 ml fresh medium per tube, with (100 μg / ml) or without endotoxin, for 30, 60, 90, 120 or 150 min, and harvested by centrifuging at 1,000 rpm for 10 min. The pellets contained the toxin-treated or untreated cells.

Transmission electron microscopy

The toxin-treated or untreated cells were mixed with 5 ml 2.5% glutaraldehyde for 4 hr and centrifuged at 1,000 rpm for 10 min. The precipitate was washed 3 times with PBS and fixed with 5 ml 2% osmium tetraoxide for 2.5 hr. The fixed cells were solidified with sea plaque agarose at room temperature for 10-20 min, and cut into small cubes. For dehydration, the cubes were passed through the following steps: (1) 70, 80, and 90% ethanol; (2) a mixture of 95% ethanol and 95% acetone, (3) 95% acetone and (4) 100% acetone. The above dehydration was repeated twice at 10 min each. The cubes were embedded with Spurr's embedding medium. The resulting blocks were sectioned with an ultramicrotome (Sorvall MT2-B). The ultra-thin sections were placed on 200-mesh grids, and were stained with 25% uranyl acetate for 15 min and lead citrate for 9 min after treating with NaOH for absorbing CO_2. They were then observed under a JEOL-200CX transmission electron microsocope at 80 kv.

Results

Normal cells of PX-1187 line have large nuclei (Fig. 1A). They contain numerous intact mitochondria (Fig. 1B), membranous Golgi complexes with granular visicles and other cellular organelles in the cytoplasm (Fig. 1c). In addition, many vacuoles of various sizes were seen, and endoplasmic reticula (ER) were even-

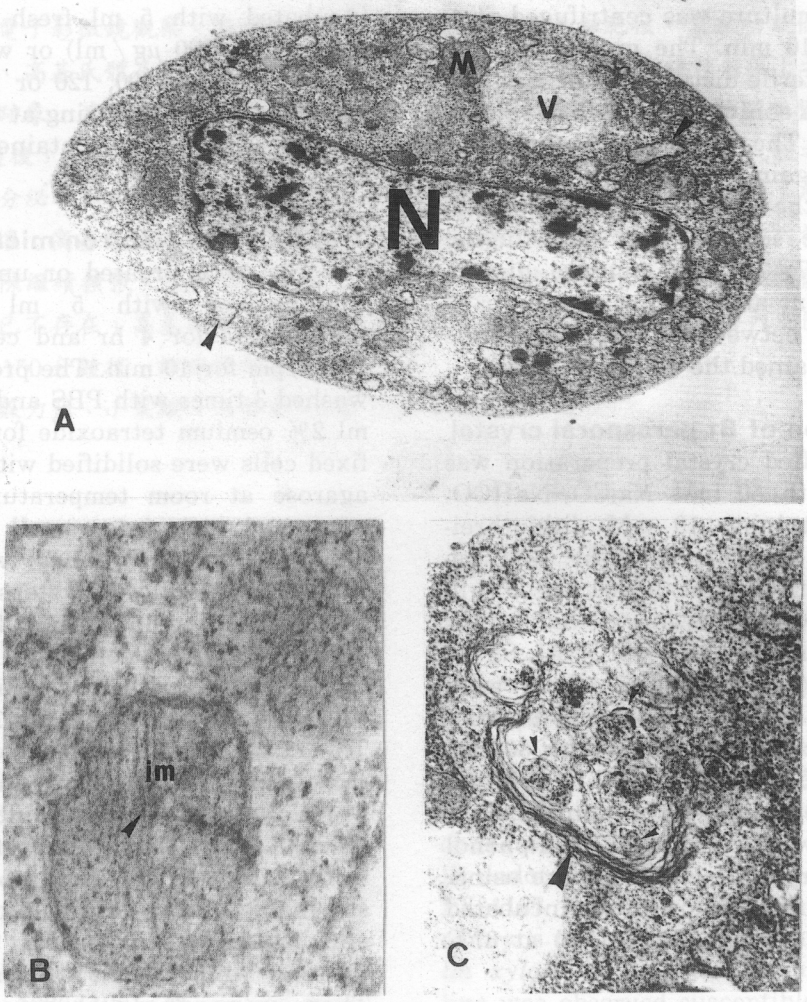

Fig. 1. The Ultrastructure of normal PX-1187 cells. A. A normal cell. N, nucleus; M, mitochondrion; v, vesicle; ER, endoplasmic reticulum (arrows). X12,000; B. The mitochondria showing inner membrane (im) with granules (arrow). X58,000; C. The Golgi complex showing cisternae (large arrow) and vesicles (small arrows). X28,000

ly distributed in cytoplasm (Fig. 1A).

After treatment with solubilized Btk endotoxin for 30 min, the nuclear chromatin masses became loosened, and the cytoplasm began to show destabilized areas (Fig. 2A). Separation of granules from mitochondrial cristae was observed (Fig. 2B). Vesicular structures and vacuoles were formed around the Golgi complex. Loose ER and granular cisternae

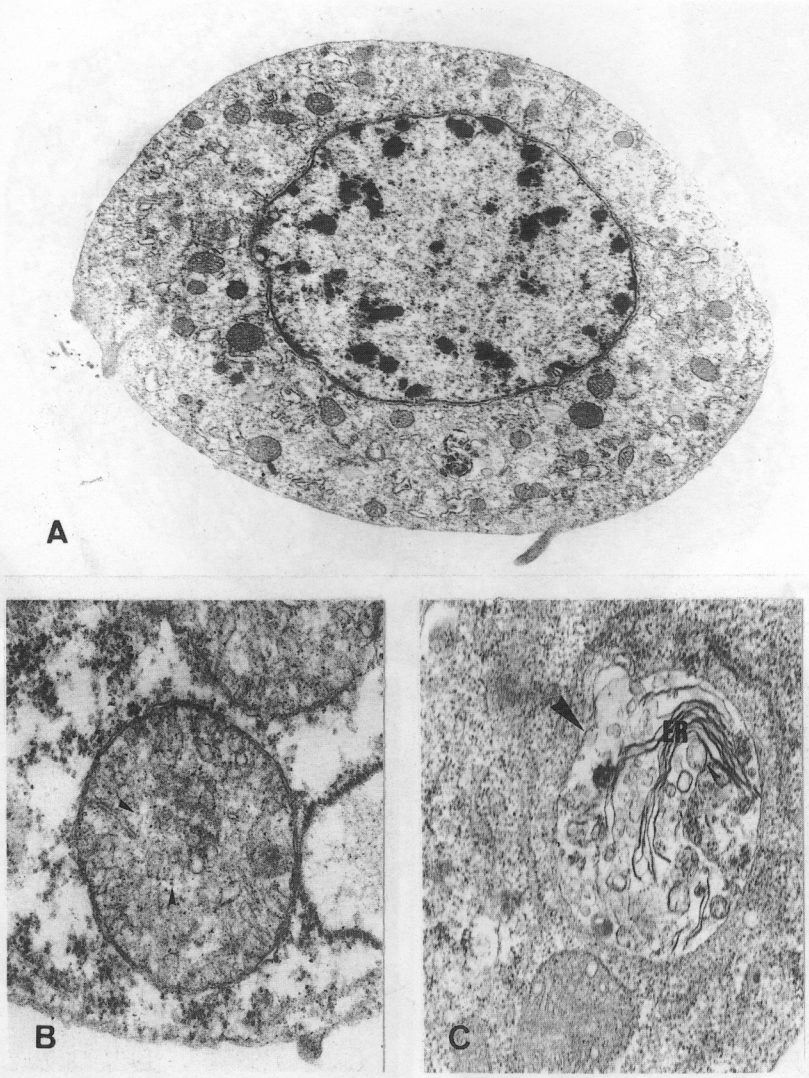

Fig. 2. PX-1187 cells treated with 100 μg / ml delta-endotoxin for 30 min. A. A treated cell. X12,000; B. A mitochondrion showing detachment of granules from inner membranes (arrow) and lysis of some inner membranes. X58,000; C. The Golgi complex (large arrow) with vacuoles, and shrunken granular cisternae (small arrows). X28,000

were shrunken, and the cytoplasmic membrane became distorted (Fig. 2C).

After treating PX-1187 cells with Btk delta-endotoxin for 60 min, the nucleus became severely affected, and the cytoplasm showed leaking and slightly vacuous areas (Fig. 3A). Lysis of the mitochondrial inner membrane occurred (Fig. 3B). The vesicles around the Golgi complex became loosened with their membranes

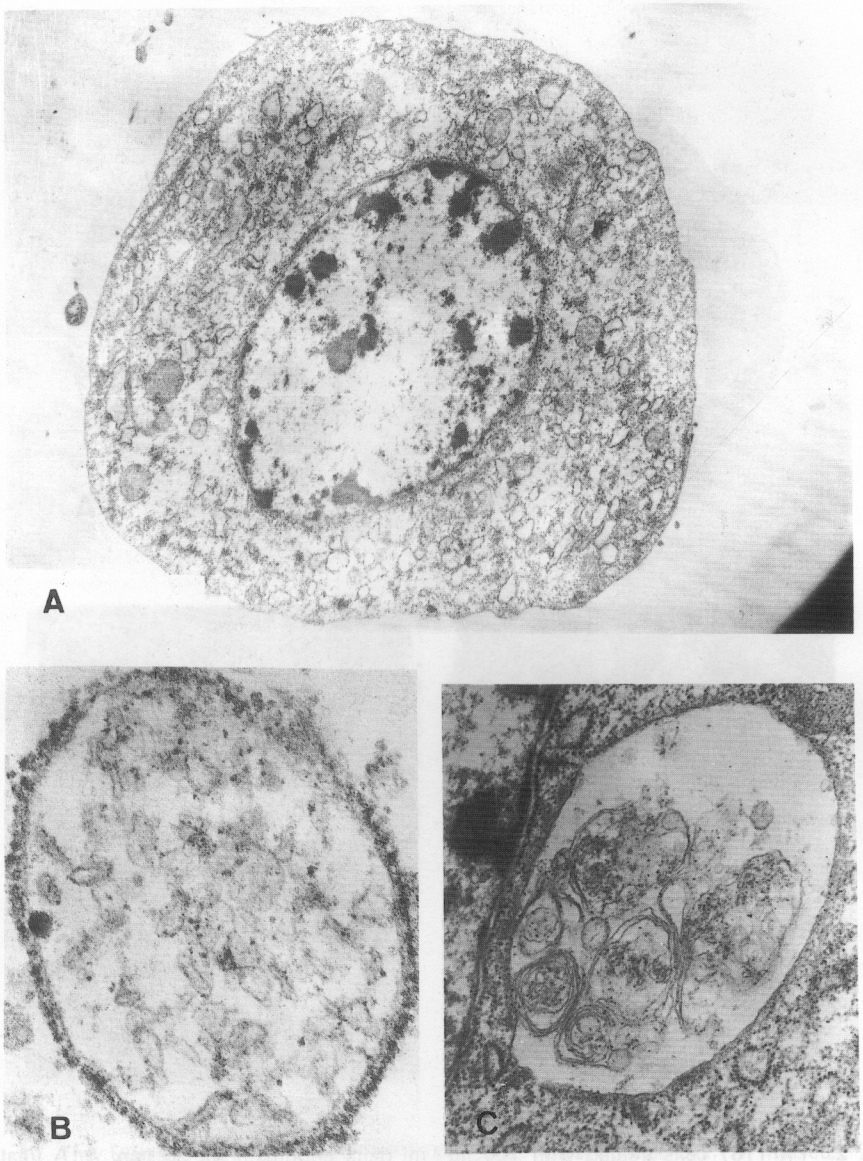

Fig. 3. PX-1187 cells treated with 100 μg / ml delta-endotoxin for 60 min. A. A treated cell. X12,000; B. Lysis of most inner membranes in a mitochondrion. X58,000; C. Vacuole enlargement with granular cisternal fusion in the Golgi complex. X28,000

datached and cisternae shrunken and fused. Damages to dictyosomes and ER's were also observable (Fig. 3C).

Nuclear changes in cultured cells exposed to endotoxin for 60 and 90 min were similar. However, parts of the plasma membrane were perforated and the intercellular space expanded at 90 min post-treatment (Fig. 4A). Inner membranes of mitochondria were largely lysed

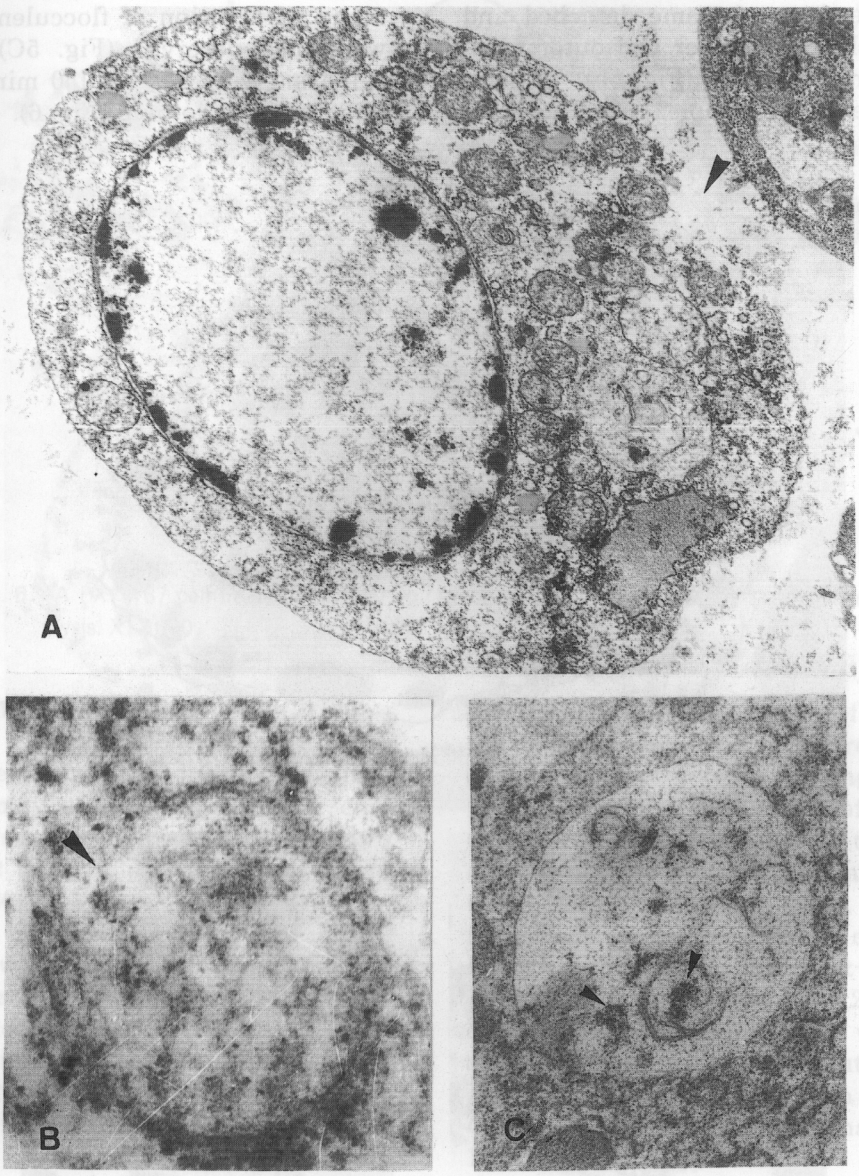

Fig. 4. PX-1187 cells treated with 100 μg / ml delta-endotoxin for 90 min. A. A treated cell showing perforation of the plasma membrane. X12,000; B. Lysis of inner membranes and disintegration of part of the outer membrtane (arrow) in a mitochondrion. X58,000; C. Fusion of granular cisternae and flocculent materials in the Golgi complex (arrows). X28,000

and parts of the outer membrances burst (Fig. 4B). Flocculent materials were seen in the Golgi complex along with fusion of granules on the cisternae (Fig. 4C).

Most of the plasma membranes of PX-1187 cells burst after treatment with Btk

delta-endotoxin for 120 min (Fig. 5A). The nuclear membrane became detached and both mitochondrial inner and outer membranes were destroyed (Fig. 5b). Vesicles around the Golgi complex were severely loosened, and lesions on dictyosomes along with fusion of flocculent materials were also observed (Fig. 5C). Complete cell lysis was seen by 150 min post-treatment with endotoxin (Fig. 6).

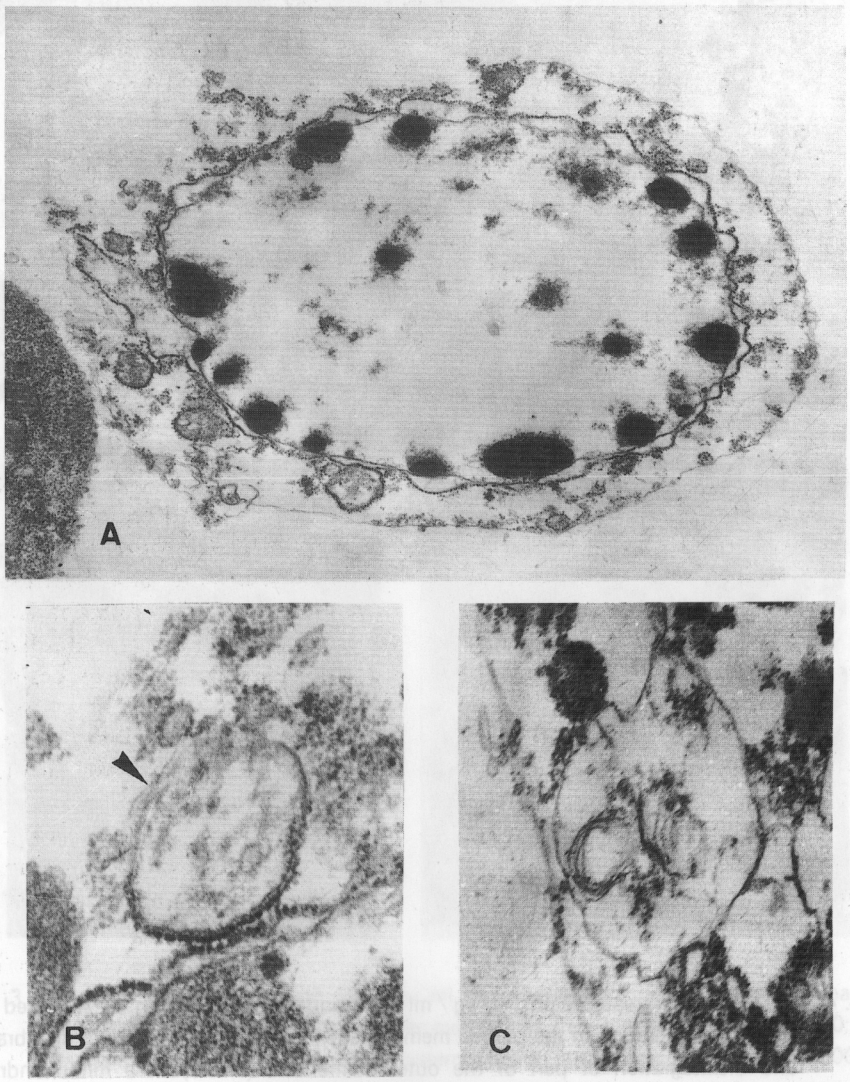

Fig. 5. PX-1187 cells treated with 100 μg / ml delta-endotoxin for 120 min. A. A treated cell showing disintegration of the plasma membrane. X12,000; B. Lysis of the inner membrane and the bursting of most of the outer membranes in mitochondria. X58,000; C. Lesion of ER and fusion of flocculent materials in the Golgi complex. X 58,000

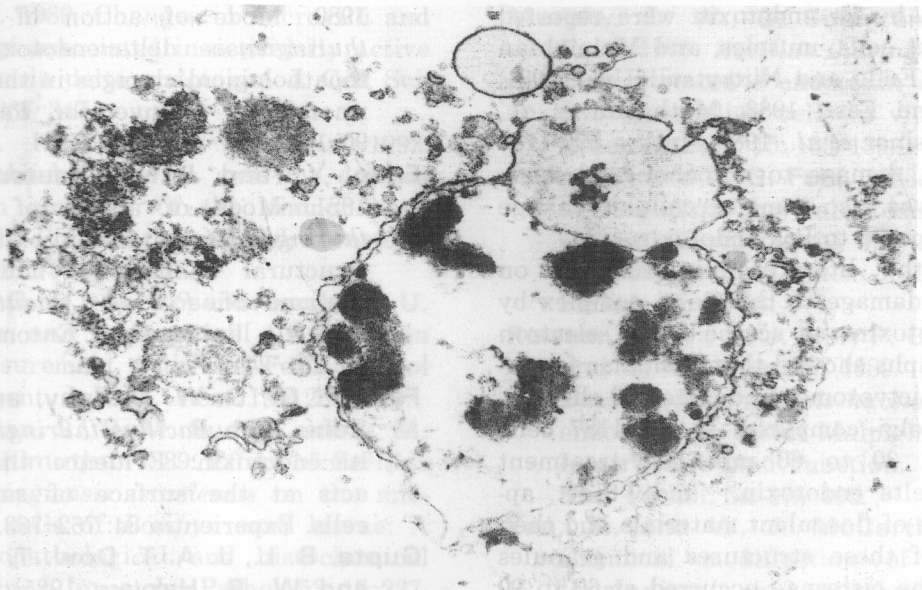

Fig. 6. A PX-1187 cell treated with 100 μg / ml delta-endotoxin for 150 min showing cell lysis. X12,000

Discussion

Delta-endotoxin produced by *B. thuringiensis* may cause morphological changes or lysis of cells cultured *in vitro* (Murphy *et al.*, 1976; Nishiitsutsuji-Uwo *et al.*, 1980). Electron microscopic observations by Nishiitsutsuji-Uwo *et al.* (1979) showed that the mitochondrial matrix became shrunken, the ER dilated and vacuole-like structures were formed after treating a *Trichoplsia ni*-derived cell line (TN-368) with endotoxin. The PX-1187 cell line established from *P. xylostella* was susceptible to Btk delta-endotoxin resulting in ca. 78% cytotoxicity after treatment with 80 μg / ml toxin based on ATP measurements (Chang, 1990). To ascertain the damage to the cell line, we used 100 μg / ml of the toxin for treatments. Yan and McCarthy (1991) reported 50% cell lysis caused by treatment with 3 to 10 μg / ml *B. t. thuringiensis* delta-endotoxin based on cell counts. However, cell count may not be as accurate as ATP measurements for determining the cytotoxicity of cells exposed to delta-endotoxin. The present study showed ultrastructureal changes in the PX-1187 cell line as a result of endotoxin treatment, causing damage to the nuclear membrane, ER, mitochondria, and the Golgi complex of these cells, The endotoxin-treated cells began to exhibit cellular lesion at 30 min post-treatment. Lysis of PX-1187 cells at 120-150 min after treatment is consistent with previous observations on midgut cells of *Bombyx mori* and Malpighian tubules of *Calpodes ethlius in vivo* (Mathavan *et al.*, 1989; Reisner *et al.*, 1989).

Cytotoxic swelling of the TN-368 line from *T. ni* after exposure to *B. t. aizawai* was induced by changing the Na^+ and K^+ flux and by stimulating Na^+ and K^+-ATPase in isotonic solution (Himeno *et al.*, 1985). The present results demonstrate that the membrane damages to cellular organelles of PX-1187 cells is consistent with the hypothesis of changes in permeability caused by Bt endotoxin in cultured cells. Subcellular lesions to mito-

chondria by Bt endotoxin were reported in midgut cells, muscles, and Malpighian tubules (Endo and Niitsutsuji-Uwo, 1981; Percy and Fast, 1983; Mathavan *et al.*, 1989; Resiner *et al.*, 1989). In the PX-1187 cell line, damage to mitochondrial membranes was also a major cellular change when exposed to Btk endotoxin.

In the literature, information on cellular damage to the Golgi complex by Bt endotoxin is scarce. Our electron micrographs showed that vesicular formation in dictyosomes and cisternal shrinkage in Golgi complexes in PX-1187 cells occurred 30 to 60 min post-treatment with delta-endotoxin, and that appearance of flocculent materials and then fusion of these structures and granules within the cisternae occurred at 60 to 90 min post-treatment. Apparently, the Golgi complex in addition to other organelles can be attacked by Bt endotoxin.

In summary, the PX-1187 cell line established from embryos of the diamondback moth, *P. xylostella*, is susceptible to Btk delta-endotoxin resulting in subcellular damage to plasma membranes, mitochondria, and the Golgi complex based on ultrastructural observations.

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