**RESEARCH LETTER**

*Photorhabdus luminescens* PirAB-fusion protein exhibits both cytotoxicity and insecticidal activity

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**Abstract**

The binary toxin ‘*Photorhabdus* insect-related’ proteins (PirAB) produced by *Photorhabdus luminescens* have been reported to possess both injectable and oral activities against a range of insects. Here, PirAB-fusion protein was constructed by linking *pirA* and *pirB* genes with the flexible linker (Gly4Ser)_3 DNA encoding sequence and then efficiently expressed in *Escherichia coli*. To better understand the role of PirAB toxin played in the process of invasion, its cytotoxicity against insect midgut CF-203 cells was investigated. Application of purified PirAB-fusion protein as well as PirA/PirB mixture caused loss of viability of CF-203 cells after 24 h incubation. CF-203 cells treated by PirAB-fusion protein displayed morphological changes typical of apoptosis, such as cell shrinkage, cell membrane blebbing, nuclear condensation and DNA fragmentation. Moreover, PirAB-fusion protein also exhibited injectable insecticidal activity against *Spodoptera exigua* larvae. The bodies of *S. exigua* fourth-instar larvae injected with PirAB-fusion protein turned completely black. Thus, we concluded that PirAB-fusion protein possessed similar biological activity (cytotoxicity and insecticidal activity) to PirA/PirB mixture, which would enable it to be used as an efficient agent for pest control.

**Introduction**

*Photorhabdus luminescens* is a gram-negative proteobacterium that forms a ‘symbiosis of pathogens’ with insect-pathogenic nematodes. It colonizes the intestine of infective entomopathogenic nematodes from the genera *Heterorhabditis* (Waterfield et al., 2009). The nematodes invade larvae of susceptible insects and release bacteria from their intestines into the insect hemocoel which proliferate rapidly and kill the insect host by producing a number of toxins. Four main classes of toxins have been characterized in *P. luminescens* so far (Dowling & Waterfield, 2007). Members of the first class, toxin complexes (Tcs), are orally toxic to caterpillar pests and have recently been used to create insect-resistant transgenic plants (Waterfield et al., 2001). Members of the second class, ‘make caterpillars floppy’ toxins (Mcf), cause damage of the insect midgut after injection into larvae with loss of body turgor and a ‘floppy’ phenotype of the caterpillars (Daborn et al., 2002). *Photorhabdus* virulence cas-ettes (PVC) are the third class of toxins, which are functional homologs of the *Serratia entomophila* anti-feeding toxins and encode physical structures that resemble bacteriocins (Yang et al., 2006). Fourth, and most recently, the ‘*Photorhabdus* insect-related’ proteins (PirAB) have been shown to be binary toxins with both injectable and oral activity against some insects (Waterfield et al., 2005).

PirAB toxins are encoded at two distinct loci in the *P. luminescens* TT01 genome, *plu4093/plu4092* and *plu4437/plu4436*, each with a pair of proteins encoded at each locus (Duchaud et al., 2003). Histological examination of *Plutella xylostella* larvae fed with recombinant *Escherichia coli* expressing PirA/PirB proteins reveals gross abnormalities of the midgut epithelium, with profound swelling and shedding of the apical membranes (Blackburn et al., 2006). PirAB toxins also show larvicial activity against mosquito larvae *Aedes aegypti* and *Aedes albopictus* (Ahantarig et al., 2009). Both PirA and PirB components are necessary for injectable activity against
caterpillars of the wax moth *Galleria mellonella*. Injection of either PirA or PirB alone into *G. mellonella* is not associated with any mortality. In contrast, a combination of individual PirA and PirB preparations exhibits full activity against this insect. Similar gene pairs are also found in the genome of *Photorhabdus asymbiotica* and a *pirB* homolog has appeared in the genome of *Yersinia intermedia* (Wilkinson et al., 2009).

*Photorhabdus luminescens* entering in the hemolymph compartment have to face the insect immune system, consisting of several defensive mechanisms that parallel many aspects of the vertebrate innate immune system. Cellular immune response, which is mediated by circulating hemocytes, comes into play immediately after the penetration of a foreign body into the insect hemocoel (Ribeiro & Brehelin, 2006). To survive and propagate in the insect hemocoel, *P. luminescens* has to produce factors which can cope with cellular immune effectors of susceptible insects. Actually, most of above mentioned toxins have been reported to possess cytotoxicity against insect hemocytes. Tc toxins inhibit phagocytosis of bacteria when added on insect hemocyte monolayers. The biologically active components of the Tc toxins have recently been characterized in insect hemocyte monolayers. The ADP-ribosyltransferases, which modify actin and Rho components of the Tc toxins have recently been characterized in insect hemocyte monolayers. The biologically active components of the Tc toxins have recently been characterized as ADP-ribosyltransferases, which modify actin and Rho GTPases (Lang et al., 2010). Mcf toxin causes apoptosis in both the insect hemocytes and the insect midgut epithelium. Insect hemocytes treated with Mcf in the cell culture media die via membrane blebbing, and midgut epithelial cells of injected insects also show rapid blebbing and TUNEL-positive nuclei (Dowling et al., 2004). Injection of PVCs destroys insect hemocytes, which undergo dramatic actin cytoskeleton condensation (Yang et al., 2006). However, the cytotoxicity of PirAB toxins against insect cells has not been studied until now. To better understand the role that PirAB toxin plays in the process of bacterial invasion, we determined the cytotoxicity of PirAB toxin in insect midgut CF-203 cells and investigated its probable mechanism. Meanwhile, PirAB-fusion protein was constructed and its cytotoxicity and insecticidal activity were compared with PirA/PirB mixture.

**Materials and methods**

**Bacterial strains and plasmids**

*Escherichia coli* DH5α was used as the host for recombinant DNA cloning. *Escherichia coli* Rosetta (DE3) was used as the host for expression of PirA (Plu4093), PirB (Plu4092) and PirAB-fusion protein. *Photorhabdus luminescens* TT01 was grown in Luria–Bertani (LB) medium at 30 °C and all *E. coli* strains were grown in LB medium at 37 °C. Plasmid pUC19 (Ampicillin resistance; Invitrogen) was used as cloning vector and pETDuet-1 (Ampicillin resistance; Novagen) was used as expression vector.

**Construction of PirAB toxin expression plasmids**

Total DNA extracted from *P. luminescens* TT01 was used as template for amplification of *plu4092* and *plu4093* (GenBank accession no. BX571865). Oligos used in this study are listed in Supporting Information, Table S1. Oligo pairs EPirA-F/EPirA-R and EPirB-F/EPirB-R were used to amplify *plu4093* and *plu4092*, respectively. PCR products of *plu4093* were double-digested by NdeI/XhoI and cloned into pETDuet-1 to generate plasmid pET-pirA. PCR products of *plu4092* were double-digested by PstI/HindIII and then cloned into pETDuet-1 and pET-pirA separately to generate plasmid pET-pirB and pET-pirAB, respectively.

For PirAB fusion expression, oligo pairs FPirA-F/FPirA-R and FPirB-F/FPirB-R were used to amplify *plu4093* and *plu4092*, respectively. The encoding sequence of flexible linker (Gly4Ser)3 was incorporated into the oligo FpirB-R. PCR products of *plu4093* were double-digested by HindIII/SphI and cloned into pUC19 to generate pUC-pirA. PCR products of *plu4092* were double-digested by PstI/SphI and cloned into pUC-pirA to generate pUC-pirAB. Then PstI/HindIII fragment from pUC-pirAB containing *plu4093*-linker-*plu4092* chimeric gene was cloned into pETDuet-1 to generate PirAB-fusion expression plasmid pET-FpirAB. All the constructs were confirmed by DNA sequencing.

**Analysis of PirAB toxin expression in Rosetta (DE3)**

Plasmid pET-pirA, pET-pirB, pET-pirAB, and pET-FpirAB were transformed into Rosetta (DE3) separately and resultant strains were designated as Rosetta (pirA), Rosetta (pirB), Rosetta (pirAB) and Rosetta (FpirAB), respectively. Recombinant strains were grown in LB medium supplemented with ampicillin (100 μg mL−1) at 37 °C to an OD600 nm of 0.6–0.8. Then isopropyl-beta-D-thiogalactopyranoside (IPTG) was added at a final concentration of 1 mM to induce the protein expression. After IPTG induction for 4 h, aliquots of 1 mL culture were sampled and harvested by centrifugation (10 000 g, 1 min). Pellets were washed three times with distilled water and suspended in 0.1 mL lysis buffer (50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole, pH 8.0). The suspension was lysed by sonication and centrifuged at 10 000 g for 2 min. The supernatant was collected and 10-μL aliquots were taken for SDS-PAGE analysis.
A candidate band of PirAB-fusion protein on SDS-PAGE gel was excised, in-gel-digested (Rana singh & Akhurst, 2002), and then analyzed by nano-LC-MS/MS using an LTQ XL mass spectrometer (Thermo Fisher, San Jose, CA).

Soluble proteins were directly purified on 1 mL HisTrap TM HP prepacked column (GE Healthcare), using an AKTA Purifier system (GE Healthcare). The column was equilibrated in His A buffer (20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4). Proteins were eluted using a step gradient up to 0.5 M imidazole in His A buffer. Solutions were then dialyzed extensively against 0.9% NaCl before dialysis against deionized water for further study. The protein content was determined using the Bio-Rad Bradford reagent.

**Cytotoxicity assay**

The effect of PirAB toxin on cell viability was determined against FPMI-CF-203/2.5 (CF-203). The CF-203 cell line, originating from the midgut of the spruce budworm (*Choristoneura fumiferana; Lepidoptera, Tortricidae*) was the kind gift of Prof. Guido F. Caputo (Natural Resource Canada). CF-203 was cultured in Insect-Xpress medium (BioWhittaker, Cambrex Bioscience, Walkersville, MD) supplemented with 2.5% heat-inactivated fetal bovine serum (Sigma-Aldrich, Bornem, Belgium) at 27 °C (Vandenborre *et al.*, 2008).

Wells of a 96-well microtiter plate were loaded with 100 μL of cell suspension and exposed to different concentrations of toxins or deionized water in the control treatment. The plates were incubated at 28 °C for 2 days. Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. The absorbance was read at 490 nm using an enzyme-linked immunosorbent assay plate reader. Cell viability was calculated as mean absorbance of the toxin group divided by the control. For each concentration, three replicates were performed and each experiment was repeated twice. Cell morphological changes were analyzed using an inverted light microscope (Leica DML; Leica Microsystems S.p.A, Milan, Italy).

**Confocal fluorescence microscopy**

PirAB toxin-treated CF-203 cells were seeded in 2 mL Insect-Xpress culture medium and incubated at 28 °C for 12 h. The coverslip was then turned upside down on a glass slide containing 0.25 μg mL⁻¹ Mitotrack Red and incubated at the room temperature for 20 min. For tubulin labeling, the cells were incubated in phosphate-buffered saline (PBS) containing 0.5% Triton X-100 at room temperature for 10 min. After washing with PBS, cells were incubated at room temperature for 1 h with PBS containing 2% BSA and then placed upside down on the coverslip on a glass slide containing 5 μg mL⁻¹ mouse anti-α-tubulin-Alexa 488 (green; Invitrogen) and incubated at room temperature for 1 h. After washing four times, the cells were incubated at room temperature for 20 min in 5 μg mL⁻¹ 4',6-diamino-2-phenylindole dihydrochloride (DAPI). After washing extensively with PBS, a fluorescence quencher was added dropwise to seal the tablet. Deionized water-treated CF-203 cells were used as negative control. Confocal images were acquired using a Zeiss LSM 510 META (Germany).

**Hoechst 33342 and propidium iodide co-staining**

Cell viability and nuclear morphology were assessed by the Hoechst 33342 and propidium iodide co-staining method (Yuan *et al.*, 2002). The Apoptosis and Necrosis Assay Kit (Beyotime Institute of Biotechnology, Hai Men, China) was used according to the manufacturer’s instructions.

**DNA fragmentation analysis**

CF-203 cells treated with PirAB toxin or deionized water (negative control) were collected after 24 h. Cells were homogenized by freezing and thawing several times and mixed in DNA extraction buffer (10 mM Tris–HCl, 150 mM NaCl, 10 mM EDTA-NaOH, 0.1% SDS, pH 8.0) on ice. Homogenized cells were treated with 20 mg mL⁻¹ RNaseat 37 °C for 30 min. Subsequently, 100 mg mL⁻¹ of proteinase K was added and cells were incubated at 50 °C for 60 min. DNA was extracted using a standard phenol-chloroform extraction method. DNA samples were analyzed using 2% agarose gel.

**Insect bioassay**

The oral toxicity of PirAB toxin was tested against *Helicoverpa armigera* and *Spodoptera exigua*. The diet of insects was prepared using the method described previously (Hu *et al.*, 2009). Different amounts (1, 5, 10, 20, and 50 μg) of PirAB toxin preparations were applied to 1-cm³ disks of artificial diet. Treated food blocks were allowed to dry for 30 min. Three first-instar larvae were placed on each of 10 food blocks per treatment before incubation at 25 °C for 7 days. The bioassay was performed three times. The percent mortality of larvae and the weight of surviving larvae were then recorded.

Injectable toxicity of PirAB toxin against *S. exigua* was also tested. For each treatment, PirAB toxin preparations were injected directly into the insect hemocoel in a cohort of six-fourth-instar larvae from the third abdominal foot. Deionized water was used as negative control.
After injection, larvae were incubated at 28 ± 1 °C on an artificial diet and monitored over 7 days for any deleterious effects. The bioassay was performed three times.

**Results**

**PirAB toxins are expressed as soluble proteins in Rosetta (DE3)**

The theoretical molecular weight (MW) of 419-amino acid protein PirB (encoded by pla4092) is 46.2 kDa and the theoretical MW of PirA (encoded by pla4093) which consists of 133 amino acids is 14.8 kDa. When compared with un-induced cultures (Fig. 1, lanes 1 and 3), prominent bands of c. 48 and 17 kDa were found in the supernatants of induced cultures of Rosetta (pirB) and Rosetta (pirA), respectively (Fig. 1, lanes 2 and 4). Both bands were present in the supernatant of induced cultures of Rosetta (pirAB; Fig. 1, lane 6). This demonstrated that both PirA and PirB could be expressed as soluble proteins in Rosetta (DE3), regardless of whether they were expressed separately or co-expressed.

To express PirAB-fusion protein, the encoding sequence of flexible linker (Gly-Ser)3 was inserted between pla4092 and pla4093 genes (Fig. 2a) and the chimeric gene was introduced into Rosetta (DE3). A prominent band of c. 64 kDa was present in the supernatant of induced cultures of Rosetta (PpirAB; Fig. 2b, lane 2). When this band was excised, in-gel-digested and analyzed by mass spectrometer, both PirA and PirB proteins were identified (Fig. 2c and d). This clearly demonstrated that PirAB-fusion protein was efficiently expressed as a soluble fraction in Rosetta (DE3).

**PirAB toxin causes loss of viability in insect midgut CF-203 cells**

Application of PirA/PirB mixture or PirAB-fusion protein to CF-203 cells resulted in rapid loss of cell viability after 24 h of incubation based on the MTT test. Treatment with the lowest concentration (0.025 μM) of PirAB-fusion protein or PirA/PirB mixture resulted in around 20% loss of cell viability. Cytotoxicity increased along with the increase in the amount of PirAB toxin. Both PirA-fusion protein and PirA/PirB mixture at ≥ 0.2 μM caused 90–100% inhibitory effect (Fig. 3). A 50% effective concentration (EC50) was estimated to be 0.082 μM (0.06–0.09 μM) and 0.074 nM (0.06–0.1 μM) for PirAB-fusion protein and PirA/PirB mixture, respectively. Overlapping 95% confidence intervals indicated that PirAB-fusion protein and PirA/PirB mixture possessed a comparable level of cytotoxicity against CF-203 cells.

**Morphological changes in midgut CF-203 cells caused by PirAB toxin**

PirA alone or low concentrations (≤ 0.2 μM) of PirB alone did not show any morphological changes or loss of cell viability in CF-203 cells (Fig. 4b), similar to the control (Fig. 4a). However, even at the lowest concentration, after 24 h incubation, both PirA/PirB mixture and PirAB-fusion protein could cause CF-230 cell shrinkage, membrane blebbing and formation of surface blisters (Fig. 4c and d). After incubation for another 24 h, cytotoxic effects of PirAB toxin on CF-203 cells were more severe, as the dead cells had degenerated to form debris in the culture (Fig. 4e and f). A high concentration of PirB (≥ 0.4 μM) alone also exhibited slight cytotoxicity against CF-203 cells.

Cell damage induced by PirAB toxin was further investigated using a Zeiss laser scanning confocal microscope. The control cells displayed strong green fluorescence (microtubules) around the nuclei (strong blue fluorescence; Fig. 5, upper panel). In contrast, cells treated with PirAB-fusion protein or PirA/PirB mixture resulted in a dramatic decrease of green fluorescence (Fig. 5, lower panel). In addition, the blue fluorescence of treated cells became much denser than that of the control cells. This demonstrated that treatment of CF-203 cells with PirAB-fusion protein and PirA/PirB mixture resulted in a notable decrease of cellular tubulin and nuclear condensation. Again, these phenomena were not observed when PirA alone or low concentrations of PirB alone were applied (data not shown). The density and distribution of red

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**Fig. 1.** SDS-PAGE analysis of PirAB toxin expressed in Rosetta (DE3). Supernatants of induced or un-induced cell lysates were separated on 10% SDS-PAGE. M, marker; lane 1, Rosetta (pirB) without induction; lane 2, Rosetta (pirB) induced with IPTG; lane 3, Rosetta (pirA) without induction; lane 4, Rosetta (pirA) induced with IPTG; lane 5, Rosetta (pirAB) without induction; lane 6, Rosetta (pirAB) induced with IPTG. Arrows indicate the expression of PirB or PirA protein. Both PirA and PirB proteins were expressed as soluble proteins in Rosetta (DE3).
fluorescence between control cells and treated cells did not show any significant difference, which indicated that PirAB toxin did not target mitochondria of CF-203 cells. PirAB toxin exhibits cytotoxicity on midgut CF-203 cells, probably via apoptosis.

We next investigated the possible mechanisms responsible for the cytotoxicity of PirAB toxin on CF-203 cells using an Apoptosis and Necrosis Assay Kit. The intact membrane of live cells excludes charged cationic dyes, such as trypan blue, propidium and ethidium, and short incubation with these dyes results in selective labeling of dead cells, whereas live cells show minimal dye uptake. Most of the CF-203 cells treated with 0.1 μM PirAB-fusion protein showed strong blue fluorescence and weak red fluorescence. Conversely, weak blue fluorescence and weak red fluorescence were detected in control cells (Fig. S1).

Agarose gel electrophoresis of DNA extracted from CF-203 cells treated with 0.1 μM PirAB-fusion protein or 0.1 μM PirAB-fusion protein showed a clear ladder pattern of the DNA (i.e. DNA fragmentation; Fig. 6, lane 1). In contrast, control cells (treated with deionized water) showed no DNA fragmentation (Fig. 6, lane 2). Therefore, we hypothesized that the cytotoxicity exhibited by PirAB toxin on midgut CF-203 cells is probably via apoptosis.

PirAB-fusion protein exhibits injectable insecticidal activity against S. exigua larvae

Within the tested concentrations (1, 5, 10, 20 and 50 μg mL⁻¹), neither PirAB-fusion protein nor PirA/PirB mixture exhibited oral toxicity against H. armigera or S. exigua larvae. However, even at the lowest dose (500 ng), injection of PirA/PirB mixture or PirAB-fusion protein caused the death of S. exigua fourth-instar larvae after 12 h, and most of these individuals injected with PirAB-fusion protein turned completely black (Fig. S2). Injection of PirA or PirB alone did not result in any mortality or growth inhibition of S. exigua fourth-instar larvae, even after 7 days.
Discussion

*Photorhabdus luminescens* proliferates in the hemolymph before the insect dies and must therefore be able to survive vigorous attack of the insect immune response (Silva et al., 2002). Cell-mediated immunity comes into play immediately after the insect hemocoel is penetrated by a foreign body, resulting in the clearance of non-pathogenic bacteria from the hemolymph within hours. As hemocytes are the immunocytes of insects, toxins that exhibit...
cytolytic effect on immunocytes and are probably involved in immunosuppression (Ribeiro & Brehelin, 2006). The above-mentioned Tc toxins, Mcf toxins and PVCs produced by P. luminescens have been reported to have a cytolytic effect on insect immunocytes and are thought to be necessary for protecting P. luminescens from the insect immune response. Here we report that PirAB, another major class of toxins produced by P. luminescens, also exhibited cytotoxicity against insect midgut CF-203 cells. After exposure to PirAB toxin for 24 h, CF-203 cells displayed the typical apoptotic morphological characteristics such as cell shrinkage, cell membrane blebbing and nuclear condensation. One of the most typical characteristics of apoptosis involves modification in the nucleus where DNA fragmentation due to inter-nucleosomal cleavage of chromosomal DNA takes place (Nagata, 2000). Our results showed DNA fragmentation in midgut CF-203 cells treated with PirAB toxin for 24 h. Our results suggest that PirAB toxin probably exhibited cytotoxicity against CF-203 cells via apoptosis. Whether PirAB toxin induced apoptosis in CF-203 cells dependent on caspase activation needs further study.

Confocal microscopy revealed that PirAB toxin caused a notable decrease of cellular tubulin in CF-203 cells. Microtubules, principal components of cytoskeleton, play an important role in many cellular processes, including nucleic and cell division, organization of intracellular structure and intracellular transport (Archuleta et al., 2011). As a result, the microtubule is a prime target for pathogens and their virulence factors. Mouse macrophages treated with Bacillus anthracis lethal toxin induced a notable decrease in the level of cellular tubulin and altered the stability of the microtubule network (Chandra et al., 2005). Treatment of human colonocytes with Clostridium difficile Toxin A resulted in tubulin deacetylation and subsequent microtubule depolymerization (Nam et al., 2010). The precise point at which PirAB toxin targets microtubules of CF-203 cells remains unclear. Although high concentration of PirB alone has a cytolytic effect on CF-203 cells, both components are necessary to restore the full cytotoxicity of PirAB toxin. Further studies are necessary to examine the cytotoxicity of PirAB against insect hemocytes and to elucidate its precise role in immunosuppression.

The coding sequences of PirA and PirB proteins were located closely in the chromosome of P. luminescens and separated by a 29-bp DNA fragment. As gene fusion/fission is a major contributor to the evolution of multidomain bacterial proteins (Pasek et al., 2006), we wanted to test whether pla4093-pla4092 underwent a fission event. With this aim, we constructed pla4093-linker+pla4092 chimeric gene and expressed PirAB-fusion protein in E. coli. Direct fusion of proteins without a linker may lead to many undesirable outcomes, including misfolding of the fusion proteins, low yield in protein production or impaired bioactivity. Thus, many empirical linkers with various sequences and conformations for the construction of recombinant fusion proteins have been designed. The most commonly used flexible linkers have sequences primarily consisting of stretches of Gly and Ser residues ('GS' linker). An example of the most widely used flexible linker has the sequence of (Gly-Gly-Gly-Gly-Ser)n (Chen et al., 2012). Here, we chose to insert a flexible linker sequence (Gly7Ser)3 between PirA and PirB to maximize the opportunity for both components to fold into their native three-dimensional structures. PirAB-fusion protein exhibited cytotoxicity similar to that of the PirA/PirB mixture. PirAB-fusion protein also has injectable activity against S. exigua larvae and most of these individuals became completely black. When the organisms invade, prophenoloxidase is released from blood cells and PO is activated via complex enzymatic cascades to produce melanin; through regulation of coagulation or melanization the invaders are therefore limited and the melanin pigmentation is maintained on the insect larvae epidermis (Castillo et al., 2011).

In conclusion, we assayed the cytotoxicity of PirAB toxin against insect midgut CF-203 cells. PirAB toxin exhibits cytotoxicity against CF-203 cells probably via apoptosis, as PirAB toxin-treated CF-203 cells displayed morphological changes typical of apoptosis, such as cell shrinkage, cell membrane blebbing, nuclear condensation and DNA fragmentation. We also constructed PirAB-fusion protein exhibiting a biological activity similar to that of the PirA/PirB mixture.

![Fig. 6. DNA fragmentation in midgut CF-203 cells after treatment with 0.1 μM of PirAB-fusion protein for 24 h. M, DNA molecular weight standard; lane 1, DNA isolated from treated CF-203 cells; lane 2, DNA isolated from control CF-203 cells (treated with deionized water).](image)
Acknowledgements

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Fluorescence detection of midgut CF-203 cells treated with PirAB toxin by using Apoptosis and Necrosis Assay Kit.

**Fig. S2.** The injectable activity of PirAB-fusion protein against *S. exigua* larvae.

**Table S1.** Oligos.