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Cloning and expression analysis of a predicted toxin gene from *Photorhabdus* sp. HB78

Mei LI[1,2], Lihong QIU[1]*, Yi PANG[1]

[1]State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, China; [2]University of Electronic Science and Technology of China Zhongshan Institute, Zhongshan 528402, China

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Abstract - The genome of the insect pathogen *Photorhabdus luminescens* TT01 strain contains multiple genes predicted to encode toxins. One of these, *plu0840*, has 55% sequence identity with an enterotoxin from *Aeromonas hydrophila*. In order to further investigate this gene, we successfully cloned the complete *plu0840* from *Photorhabdus* sp. HB78 and expressed it as a GST-Plu0840 fusion protein in *Escherichia coli* BL21(DE3) using pGEX-4T-1 as a vector. Most of GST-Plu0840 was insoluble and sequestered into inclusion bodies. The inclusion bodies were harvested and dissolved. The resultant protein was cleaved and purified from the GST-tag. Oral bioassay showed that Plu0840 inhibited growth of *Spodoptera litura* and *Spodoptera exigua* larvae.

Key words: cloning, enterotoxin, expression, *Photorhabdus*, toxicity, toxin gene/*plu0840*.

INTRODUCTION

Photorhabdus are Gram-negative bacteria belonging to the family of *Enterobacteriaceae* (Poinar *et al.*, 1977). The bacteria symbiotically associate with entomopathogenic nematodes (EN) and are released into the insect haemocoel upon nematode invasion. The insect is then killed mainly by toxins produced by the bacteria. The bacteria continue to replicate within the insect cadaver and the nematodes feed on the bacterial-insect medium within the dead or dying insect (Dunphy and Webster, 1988). Analysis of the genome of *Photorhabdus luminescens* TT01 identified more predicted toxin genes than any other bacteria sequenced to date, such as, the toxin complex (*tc*) or *tc-like* genes, haemolysin A, chitinase, Rtx (repeats-in-toxin)-like toxin, and -endotoxin (Duchaud *et al.*, 2003). Tc or Tc-like toxin is a high molecular mass toxin complex consisting of about 10 polypeptides ranging from 30 to 200 kDa in size, which is secreted from the bacteria and requires proteolytic processing for its activity. Multiple *tc* genes have been identified in several *Photorhabdus* species (Blackburn *et al.*, 1998; Bowen and Ensign, 1998; Ffrench-Constant and Bowen, 2000; Waterfield *et al.*, 2005a), and *tc*-like genes have also been identified in insect-associated bacteria *Xenorhabdus nematophila* (Morgan *et al.*, 2001; Sergeant *et al.*, 2003). Tc toxins are often located in the pathogenic island and toxic to insects by either ingestion or injection. Besides Tc toxin, several other toxins have also been identified and examined, such as Mcf and Pir from *Photorhabdus* (Brillard *et al.*, 2002; Daborn *et al.*, 2002; Waterfield *et al.*, 2005b), A24tox, Xh1A and insecticidal pilin subunit from *X. nematophila* (Brown *et al.*, 2004; Khandelwal *et al.*, 2004; Cowles and Goodrich-Blair, 2005).

Sequence analysis showed that an ORF in the *P. luminescens* TT01 genome, *plu0840*, have 55% sequence identity with an enterotoxin Ast from *Aeromonas hydrophila*, which played an important role in *A. hydrophila*-induced gastroenteritis in a mouse model (Sha *et al.*, 2002). In order to investigate the role of *plu0840* in the pathogenicity of *Photorhabdus* species against insect hosts, we successfully cloned it from *Photorhabdus* sp. HB78, a native strain of *Photorhabdus* and expressed it in *Escherichia coli*. The oral and haemocoel toxicity of the purified Plu0840 protein against insect hosts were also tested for the first time.

MATERIALS AND METHODS

Bacterial cultures and growth conditions. The symbiotic bacteria used in this study were listed in Table 1. The symbiotic bacteria were preserved in 15% glycerol solution in liquid nitrogen. The bacteria were streaked from stock cultures on nutrient agar supplemented with 0.025% (w/v) bromothymol blue and 0.004% (w/v) triphenyl tetrazolium chloride (NBTA). Broth cultures were grown from a single primary phase colony in LB medium at 28 °C on a shaker at 200 rpm to OD_{600} of 1.5. *E. coli* DH5α and *E. coli* BL21(DE3) (Novagen, USA) were cultured at 37 °C for sequencing, cloning and expression, respectively.

* Corresponding author. Phone: +86-20-84113009;
Fax: +86-20-84037472; E-mail: qiulh@mail.sysu.edu.cn

TABLE 1 - Bacteria strains used for high toxicity screening against *Helicoverpa armigera*

Species/isolate No.	EN	Isolated from
Photorhabdus temperata GZa139	*Heterorhabditis* sp.	Guizhou province, China
Photorhabdus sp. HB78	*Heterorhabditis* sp.	Hebei province, China
Photorhabdus sp. YNa125	*Heterorhabditis* sp.	Yunnan province, China
Photorhabdus sp. Hmeg	*Heterorhabditis megidis*	Yunnan province, China
Xenorhabdus sp. YNc215	*Steinernema* sp.	Yunnan province, China
Xenorhabdus sp. YNb28	*Steinernema* sp.	Yunnan province, China
Xenorhabdus sp. YNa192	*Steinernema akhursti*	Yunnan province, China
Xenorhabdus sp. GDc345	*Steinernema* sp.	Guangdong province, China
Xenorhabdus innexi Scap	*Steinernema scapterisci*	Uruguay
Xenorhabdus sp. GDh7	*Steinernema aciari*	Guangdong province, China
Xenorhabdus sp. YNa111	*Steinernema ceratophorum*	Yunnan province, China
Xenorhabdus sp. GZb26	*Steinernema ceratophorum*	Guizhou province, China

Screening of symbiotic bacteria with high toxicity against *Helicoverpa armigera*.
Aliquots of 1 ml bacterial culture were collected after broth cultures were grown at 28 ºC to OD_{600} of 1.5. For each sample, cells in the cultures were settled by centrifugation (10000 *x g*, 1 min). The supernatant was condensed to 200 µl, and then mixed to 1.5 g artificial diet. The cell sediment was washed two times by PBS and lysed via ultrasonication (200-300 W, 6 x 10 s, 10 s pause), then mixed to 1.5 g artificial diet. About 50 mg artificial diet containing supernatant or sediment of symbiotic bacteria and 1 *H. armigera* neonate were transferred to each well of a 24-well tissue culture plate. The plate was sealed and incubated at 28 °C. The mortality of the larvae was recorded 4 days after treatment. Thirty larvae were tested and the experiment was repeated 3 times.

Cloning and sequencing of the complete *plu0840* gene. Genomic DNA was isolated from the highly toxic symbiotic bacteria and used as the template for PCR. The following primers were designed based on the sequence of *P. luminescens* TT01 *plu0840* ORF (GenBank access no.: BX571861): plu0840-F:5'-GGATCCATGAAGGA AACTGCT-GTG-3', plu0840-R:5'-GTCGACTTATTTCGATGGGGT-CAAAG-3'; (the underlined sequences denoted the restriction sites). PCR reactions were carried out in a total volume of 50 µl with the following reagents: 20 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgSO_4$, 10 mM $(NH_4)_2SO_4$, 0.25 mM dNTPs, 0.2 µM of each primer, *Taq* DNA polymerase (Takara, Japan). PCR cycling was as follows: 35 cycles of 94 ºC (30 s), 53 ºC (30 s), 72 ºC (2 min). The resultant PCR products were gel purified and ligated into pMD18-T (Takara). The resultant plasmids were named pMDT-plu0840 and were transformed into *E. coli* DH5α. Three clones were sampled randomly and the inserted DNA fragment was sequenced.

Bioinformatics. The sequence of *plu0840* was compared with the homologous sequences of *P. luminescens* TT01. The predicted amino acid sequence and characteristics of *plu0840* from *Photorhabdus* sp. HB78 were also compared to those of *plu0840* from *P. luminescens* strain TT01. The search for *plu0840* gene homology sequences in the non-redundant nucleotide and protein databases were performed using various BLAST programs (version 2.2.6) available via NCBI (www.ncbi.nlm.nih.gov/BLAST/). Motif and fold recognition analysis of the predicted protein sequence was performed using programs such as InterProScan (www.ebi.ac.uk/interpro/) and CDD (conserved domain database), and the Structure Prediction Metaserver (bioinfo.pl/Meta/pdb-test.pl/). The amino acid composition of the sequence was compared with bacterial sequences available at EBI (www.ebi.ac.uk/proteome/). The sequence was also analysed using programs available via ExPASY (kr.expasy.org/tools/), including SignalP (version 3.0).

Expression of pGEX-plu0840. pMDT-plu0840 was digested with *Bam*H? and *Sal*? (Takara). The 1920bp inserted fragment was then cloned into expression vector pGEX-4T-1, named pGEX-plu0840 and then transformed into *E. coli* BL(DE3) (Novagen). The inserted DNA fragment was sequenced again. *E. coli* DE3 cells harbouring pGEX-plu0840 were grown in LB medium with ampicillin (100 mg/l) at 37 ºC to OD_{600} of 0.6-0.8. Then isopropyl-β-D-thiogalactopyranoside (IPTG) was added at a concentration of 1 mM to induce the expression of the protein. Aliquots of 1 ml bacterial culture were collected 3 h after induction and the cells in the cultures were harvested by centrifugation (10000 *x g*, 1 min). The cell pellet was resuspended in distilled water for SDS-PAGE analysis.

Fusion protein solubility analysis and transmission electron microscopic observation.
E. coli BL(DE3) cells harbouring pGEX-plu0840 were grown in LB broth with ampicillin (100 mg/l) at 37 ºC to OD_{600} of 0.6-0.8. Aliquot of 2 ml bacteria culture was sampled 3 h after IPTG induction and the cells were harvested by centrifugation (10000 *x g*, 1 min). A half of the resultant cell pellet was washed 3 times with distilled water and then resuspended in 0.1 ml distilled water. About 5 µl resultant suspension was transferred onto a copper grid with formvar membrane, negatively stained with 3% phosphotungstic acid and observed under a transmission electron microscope (JEM-100CX/II, Japan). The remaining cell pellet was suspended in 0.1 ml lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0) and then cells were lysed by sonication (200-300 W, 6 x 10 s , 10 s pause). The resultant solution was centrifuged at 10000 *x g* for 2 min. Aliquot of 10 µl supernatant was taken for SDS-PAGE. The cell sediment was washed three times with distilled water, resuspended in 0.1 ml lysis buffer and 10 µl aliquot was taken for SDS-PAGE.

Dissolution and purification of the fusion proteins. *Escherichia coli* DE3 (pGEX-plu0840) and *E. coli* BL21(DE3) (pGEX-4T-1) were inoculated into 50 ml LB medium with ampicillin (100 mg/l) and then incubated at 200 rpm at 37 ºC. IPTG was added to the culture when OD_{600} increased to 0.6-0.8 and then incubated for another 3 h to induce protein synthesis. The cells in the cultures were harvested by centrifugation at 4 ºC. Of the resultant cell pellet, 1.5 g were resuspended in 20 ml buffer A (50 mmol/l Tris-HCl, 0.5 mmol/l EDTA, 50 mmol/l NaCl, 5% glycerol, 0.2 mmol/l DTT, pH 7.9) and then lysed via ultrasonication (200-300 W, 10 x 30 s, 30 s pause). The lysate was centrifuged at 10000 *x g* for 10 min and the supernatant was transferred to another tube. Buffer A (18 ml) and 20% sodium deoxycholate (DOC, 2 ml) were added to the sediment and incubated at room temperature for 10 min. The suspension was centrifuged at 4 ºC, 10000 *x g* for 10 min. and the supernatant was discarded. Buffer A (19.7 ml) and 20% sarkosyl (SKL, 0.3 ml) were added into the sediment and mixed thoroughly using a blender. The solution was incubated at room temperature for 30 min and then centrifuged at 10000 *x g* for 10 min. The supernatant containing the target protein GST-Plu0840 was harvested and the concentration was determined by Coomassie protein assay protocol (BioRad). The protein solution was then diluted with buffer A to a terminal concentration of 0.1 mg/ml and 0.03% SKL was added into the solution. The resultant solution was dialysed in buffer A for 8 h at a ratio of 200 ml protein solution to 2 litre buffer A and the process was repeated once. The resultant protein solution was condensed to about 20 ml in PEG20000 and centrifuged at 10000 *x g* for 10 min. To remove any insoluble substances and impurity, the soluble fusion protein was purified using MicroSpin GST Purification Module (Amersham, USA) as described by the manufacturer. The resultant protein solution was analysed by SDS-PAGE.

Cleavage and purification of Plu0840 from GST-Plu0840. Cleavage of GST-Plu0840 was performed at 22 ºC for 16 h with 1 unit thrombin protease (Amersham) per 100 µg fusion protein. The result was checked by SDS-PAGE. The GST was removed from cleaved GST-Plu0840 using MicroSpin GST Purification Module (Amersham) as described by the manufacturer, briefly, added up to 600 µl from resultant cleaved GST-Pit and GST solution to the Glutathione Sepharose 4B MicroSpin column. Room temperature for 10min to ensure optimal binding of GST to the matrix, then spin at 735 *x g* for 1 min, collect each flow-through. Added the flow-through to a new column to purify once more, the flow-throughs was Plu0840 protein.

GST harvested from *E. coli* DE3 cells transformed with pGEX-4T-1 was used as a control. Because most GST produced in *E. coli* DE3 (pGEX-4T-1) cells was soluble, GST was harvested and purified from the cell lysate supernatant using the MicroSpin GST Purification Kit. Thrombin protease (1 unit to 100 µg GST) was added in the GST solution at 22 ºC for 16 h. GST was purified from cleaved GST by MicroSpin GST Purification Kit.

In order to remove thrombin protease, dialysis was performed in PBS for 8 h at 4 °C and condensed in PEG20000. The resultant solution contained Plu0840 and GST, respectively.

Bioassays of Plu0840 protein. The oral insecticidal activity of Plu0840 was tested against the first-instar larvae of *Spodoptera litura* and *Spodoptera exigua*. Plu0840 or GST (control) was mixed in the artificial diet of the tested insects at a dose of 25 µg Plu0840 per gram of the artificial diet. Another untreated control was designed at the same time. About 0.1 g artificial diet and 1 larva were transferred to each well of a 12-well tissue culture plate; each larva received a maximum of 2.5 µg Plu0840 during a four day period. The plate was sealed and incubated at 28 °C. The mortality and body weight of the larvae were recorded 4 days after treatment. Thirty larvae were tested and the experiment was repeated 3 times.

Haemocoel insecticidal activity of Plu0840 was determined against larvae of both *Galleria mellonella* (fifth or early sixth-instar) and *S. litura* (fourth-instar). Aliquot of 5 µl solution containing 100 ng Plu0840 or 100 ng GST was injected into each larva. The larvae were then incubated at 28 °C. The changes in morphology and behaviours of the larvae were monitored and the mortality was recorded 5 days after treatment for *G. mellonella* and *S. litura*, respectively.

RESULTS

Screening of symbiotic bacteria with high toxicity against *Helicoverpa armigera*

The results of screening of high toxicity symbiotic bacteria showed that *Photorhabdus* sp. HB78 and *Xenorhabdus* sp. YNc215 had high toxicity against *H. armigera* (Table 2), so these two strains were used for amplification of the target gene.

TABLE 2 - Bacteria strains used for high toxicity screening against *Helicoverpa armigera*

Species/isolate No.	Average mortality (%)	
	Supernatant	Cell sediment
Photorhabdus temperata GZa139	29.23 ± 1.67*	6.67 ± 0.45
Photorhabdus sp. HB78	91.80 ± 1.71	28.97 ± 2.71
Photorhabdus sp. YNa125	70.63 ± 2.75	18.55 ± 1.14
Photorhabdus sp. Hmeg	35.83 ± 2.89	33.50 ± 1.73
Xenorhabdus sp. YNc215	100	29.20 ± 1.26
Xenorhabdus sp. YNb28	20.83 ± 0.58	29.17 ± 1.44
Xenorhabdus sp. YNa192	79.17 ± 3.82	66.67 ± 1.44
Xenorhabdus sp. GDc345	62.50 ± 2.50	37.83 ± 0.58
Xenorhabdus innexi Scap	70.83 ± 3.81	75.00 ± 2.64
Xenorhabdus sp. GDh7	51.17 ± 1.89	25.33 ± 2.02
Xenorhabdus sp. YNa111	37.50 ± 2.50	33.33 ± 2.25
Xenorhabdus sp. GZb26	54.83 ± 1.04	41.67 ± 2.89
Escherichia coli	11.10 ± 1.65	7.50 ± 1.05
LB	6.83 ± 0.50	6.83 ± 0.50

* Standard error of the mean. Values are an average from three independent experiments; each experiment was performed on 30 larvae.

TABLE 3 - BLAST results of the predicted amino acid sequence of HB78 *plu0840*

Purification step	Total volume (ml)	Total protein (mg)	Total activity (mU)	Specific activity (mU/mg)	Yield (%)	Purification fold
Hypothetical protein	*Photorhabdus luminescens* TT01	CAE13135	1-639	1-639	95	97
Enterotoxin	*Aeromonas hydrophila*	AAL73391	31-638	19-626	56	74
Hypothetical protein SKA34	*Photobacterium* sp. SKA34	ZP_01161106	11-638	8-630	54	71
Hypothetical protein	*E.coli* CFT073	NP_753099	14-633	42-660	48	67
Hypothetical protein YfreA	*Yersinia frederiksenii*	ZP_00827944	23-637	12-627	48	66
Hypothetical protein YintA	*Yersiniaintermedia*	ZP_00831931	30-637	6-614	48	67
α-N-acetylgalactosaminidas	*Clostridium perfringens*	AAM55479	237-626	221-627	26	45
Sialidase-like	*Clostridium perfringens*	BAB80970	237-630	482-910	26	44
α-Galactosidase	*Streptomyces coelicolor*	NP_624603	249-320	173-242	29	47
Glycoside hydrolase	*Solibacterusitatus*	ZP_00524012	192-319	271-405	26	29

Cloning and sequencing of *plu0840*

DNA fragment with a size similar to the expected size of *plu0840* was successfully amplified from *Photorhabdus* sp. HB78 by PCR and cloned into *E. coli* using the methods described. Sequencing results showed the inserted DNA fragments in three clones of *E. coli* DH5α (pMDT-plu0840), and three clones of *E. coli* DE3 (pGEX-plu0840) had identical sequences.

Bioinformatic analysis of *plu0840*

Blast searches for homology sequences of *plu0840* from *Photorhabdus* sp. HB78 in the nucleotide and protein databases showed that it was homologous to Plu0840, a hypothetical protein in the related bacterium *P. luminescens* TT01, which shared 96% nucleotide and 95% predicted amino acid sequence identity (Fig. 1) (Table 3). The second (56% identity out of a region of 608 amino acids compared) and third highest (54% identity out of a region of 628 amino acids compared) were enterotoxin of *A. hydrophila* and a hypothetical protein SKA34 of *Photobacterium* sp. SKA34, respectively (Table 3). Analysis of the amino acid sequence using InterProScan showed a (trans) glycosidase domain presented in Plu0840 from 225 to 481 amino acids and a probable 21 amino acids transmembrane-region presented in the sequence of the first 31 amino acids of Plu0840; CDD-Search showed a conserved domain of Gal (α-galactosidase) with responsibility for carbohydrate transportion and metabolism. The predicted protein contained a cleavage site of signal peptide between the 31st and 32nd amino acid (SignalP).

Expression of GST-Plu0840 in *Escherichia coli* BL21(DE3) and fusion protein solubility analysis

The GST-Plu0840 fusion protein was successfully expressed in *E. coli* BL21(DE3) using the protocol described. A prominent band of about 95 kDa was visible by SDS-PAGE analysis in LB cultures of *E. coli* BL21(DE3) (pGEX-plu0840) induced by IPTG (Fig. 2), which was found neither in the uninduced cultures nor in the control (*E. coli* BL21(DE3) transformed with pGEX-4T-1, data not shown). The size of the 95 kDa protein band was a little smaller than the predicted size (98 kDa) of the expressed protein. Most of fusion protein GST-Plu0840 expressed in *E. coli* DE3 (pGEX-plu0840) was insoluble (Fig. 3). Transmission electron microscopic observation showed the expressed protein sequestered into inclusion bodies (data not shown).

Dissolution, cleavage of the fusion protein and purification of Plu0840 from cleaved GST-Plu0840

The results of SDS-PAGE showed that the insoluble fusion protein GST-Plu0840 expressed in *E. coli* BL21(DE3) (pGEX-plu0840) was successfully dissolved and purified using the protocols described. Two bands with their sizes in accordance with those of GST and Plu0840 were visualized by SDS-PAGE (Fig. 4 lane 2) after GST-Plu0840 was cleaved by thrombin. Plu0840 and GST were purified successfully (Fig. 4 lane 5 and 6) by the protocols described.

Bioassays of Plu0840 protein

Oral activity of Plu0840 showed that the average body weight of the treated first-instar larvae of either *S. litura* or *S. exigua* fed with Plu0840 (treatment) was significantly (Pair T-test, $P < 0.01$) lower than that of GST control and untreated larvae (Table 4). Haemocoel activity of Plu0840 showed no significant increase on mortality of the mature larvae of either *G. mellonella* or *S. litura.*

DISCUSSION

Plu0840 is a predicted ORF in the genome of the insect pathogenic bacterium *P. luminescens* TT01 strain. Plu0840 was expected to be a toxin because its predicted amino acid sequence shared 55% sequence identity to an enterotoxin of *A. hydrophila*. Sha *et al*. (2002) demonstrated that the enterotoxins of *A. hydrophila* evoked diarrhoea in a murine model by developing various combinations of enterotoxin gene-deficient mutants using maker-exchange mutagenesis. However, *Photorhabdus* are insect pathogenic bacteria associated with entomopathogenic nematodes

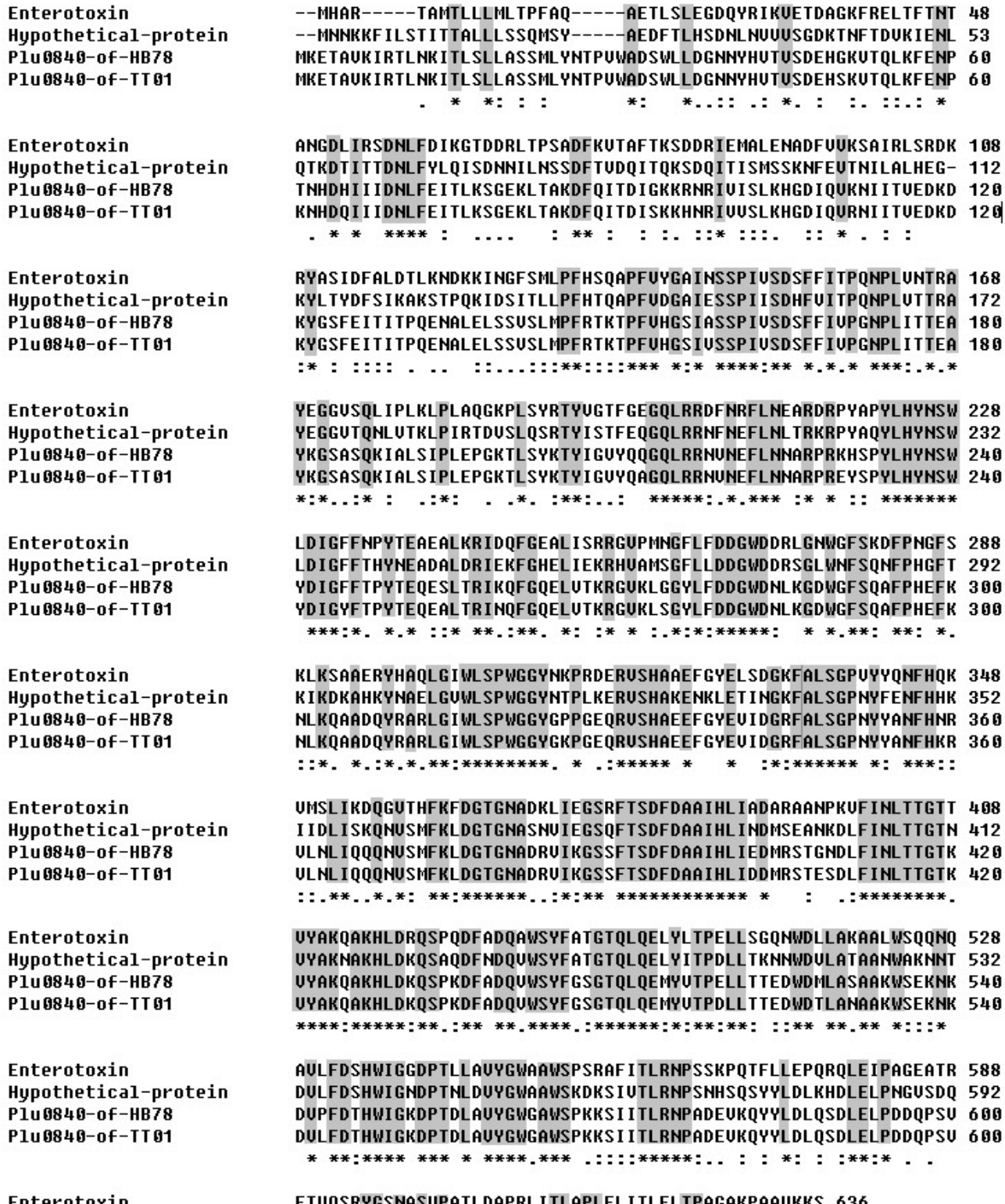

FIG. 1 - Multiple sequence alignment of Plu0840 homologues, using CLUSTAL W, version 1.83. The Plu0840 homologues consist of the Plu0840 protein from *Photorhabdus* sp. HB78, the hypothetical protein Plu0840 from *Photorhabdus luminescens* TT01 (accession No. CAE13135), the enterotoxin from *Aeromonas hydrophila* (accession No. AAL73391), and the hypothetical protein of *Photobacterium* (accession No. ZP-0116116). Identical residues are indicated by shaded boxes and symbols "*", two identical residues out of four amino acids are marked by symbols ":", and similar amino acids are marked by symbols ".".

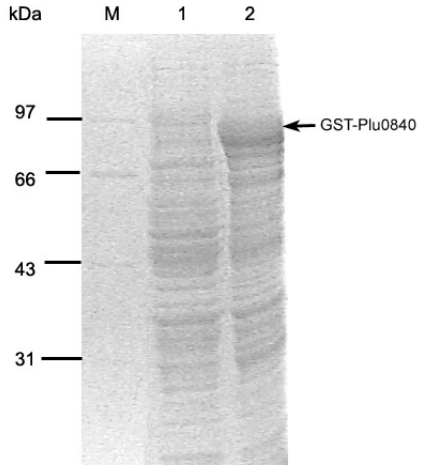

FIG. 2 - SDS-PAGE analysis of the fusion protein GST-Plu0840 expressed in LB cultures of *Escherichia coli* BL21(DE3) (pGEX-plu0840). M: protein molecular weight standards; lane 1: LB culture of DE3 (pGEX-plu0840) without induction, lane 2: LB culture of DE3 (pGEX-plu0840) induced with IPTG (1 mM) for 3 h.

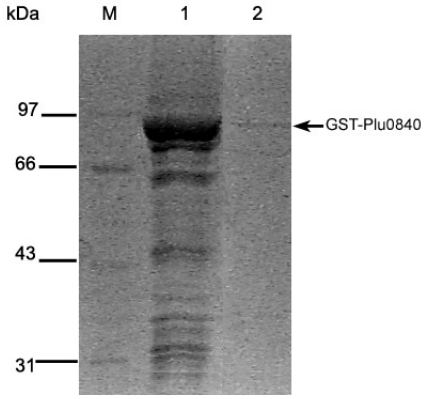

FIG. 3 - Solubility of GST-Plu0840 expressed in *Escherichia coli* BL21(DE3) (pGEX-plu0840). M: protein molecular weight standards, lane 1: GST-Plu0840 in the sediment of cell lysate, lane 2: GST-Plu0840 in the supernatant of cell lysate.

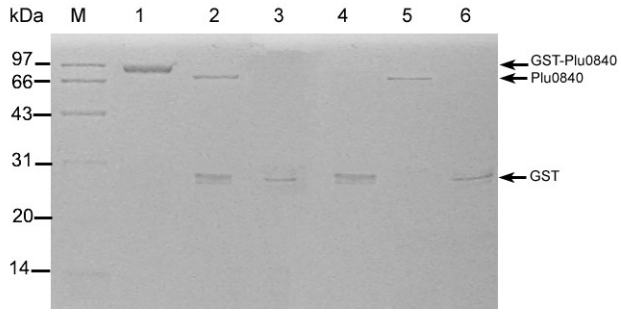

FIG. 4 - SDS-PAGE analysis of the Plu0840 and GST followed by the dissolution, purification and cleavage protocols described. M: protein molecular weight standards; lane 1: GST-Plu0840, lane 2: cleaved GST-Plu0840, lane 3: GST, lane 4: cleaved GST, lane 5: purified Plu0840 from cleaved GST-Plu0840, lane 6: purified GST from cleaved GST.

TABLE 4 - Oral bioassay of Plu0840 against the first-instar larvae of *Spodoptera exigua* and *Spodoptera litura*

	Average body weight (mg/larva)	
	Spodoptera exigua	*Spodoptera litura*
Plu0840	38.9 ± 1.7[a]*	23.9 ± 1.5[c]
GST	50.8 ± 1.3[b]	32.1 ± 1.9[d]
Untreated larvae	50.7 ± 0.7 [b]	33.5 ± 1.2[d]

* Standard error of the mean. Values are an average from three independent experiments; each experiment was performed on 30 larvae. Numbers with different superscript letters indicated the significance of their difference $P < 0.01$, and those with the same letter indicated $P > 0.05$.

of the genus *Heterorhabditis* (Forst *et al.*, 1997). Entomopathogenic nematodes are currently used as biopesticides to control insect pests in a number of countries, including the United States and Australia. Extensive laboratory tests showed that *Photorhabdus* had no pathogenicity to endotherm such as chicken, mice, rabbits, pigs etc. (Poinar *et al.*, 1982; Obendorf *et al.*, 1983). More than 50 years of field application of entomopathogenic nematodes for controlling insect pests also showed that EN and their symbiotic bacteria are safe to human and other vertebrates and EN-based biopesticides were exempted from registration in many countries, including USA and all European countries. *Photorhabdus* bacteria are released from the gut of their nematode vector into the insect heamocoel and killed the insect host by producing a range of toxins (Ffrench-Constant *et al.*, 2003). Scientists are attempting to develop insect-resistant transgenic crops by using insecticidal toxin genes derived from *Photorhabdus* spp. (Ffrench-Constant *et al.*, 2000). Therefore, it is expected that plu0840 is an insecticidal toxin instead of a toxin against endotherm.

In the present study, we cloned the complete gene *plu0840*, expressed it in *E. coli* and showed that the resulted protein Plu0840 inhibited growth of *S. litura* and *S. exigua* via oral ingestion, but it had no haemocoel insecticidal activity against *G. mellonella* and *S. litura*. This is the first experimental examination of this gene. Although the oral toxicity of Plu0840 to the target insects tested is weak, sequence analysis showed that the first 31 amino acids of Plu0840 are likely to be a signal peptide and it was not removed in the Plu0840 used for bioassay in this study, which might affects its insecticidal activity. It is worth to examine the insecticidal activity of the Plu0840 without signal peptide.

Besides being an insecticidal toxin, sequence analysis showed that Plu0840 contains a conserved (trans)glycosidase domain, indicating that it might also play a role in carbohydrate transport and metabolism. α-Galactosidases catalyze the hydrolysis of α-1,6-linked α-galactose residues from oligosaccharides, such as melibiose (galactose-α-1,6-glucose), raffinose (galactose-α-1,6-sucrose), and stachyose (galactose α-1,6-raffinose) and from polymeric galactomannans (Luonteri *et al.*, 1998). Some α-galactosidases are also known to catalyze transgalactosylation, especially at a high concentration of substrate. α-Galactosidases have been isolated from a variety of eukaryotes and bacteria (Fridjonsson *et al.*, 1999).

Whether Plu0840 has a function of glycosidase needs further examination.

In conclusion, we successfully cloned a predicted toxin gene *plu0840* from HB78, expressed it in *E. coli* and demonstrated that the resultant protein Plu0840 had weak oral toxicity against *S. litura* and *S. exigua*. The results obtained from this study laid a foundation for further study on the function of Plu0840 from *Photorhabdus* and the pathogenic mechanism of the toxin against insect pests.

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