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# Proteomic Identification of a Novel Toxin Protein (Txp40) from *Xenorhabdus nematophila* and Its Insecticidal Activity against Larvae of *Plutella xylostella*

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**ABSTRACT:** For the identification of a novel insecticidal protein, a two-dimensional liquid chromatography (PF-2D) system was used in a quantitative proteomic analysis of *Xenorhabdus nematophila* CBNU strain isolated from entomophagous nematode *Steinernema carpocapsae*. Protein patterns obtained from minimum and maximum insecticidal activities during cultivation were contrasted, and a novel toxin protein (Txp40) was identified by MALDI-TOF/MS. The DNA sequence of the cloned toxin gene (1089 bp) has an open reading frame encoding 363 amino acids with a predicted molecular mass of 41162 Da. The *txp40* identified in this study is most closely related to the known *txp40* cloned from *X. nematophila* EB (ADQ92844) with 94.4% identical sequence residues. Following the expression of the newly identified toxin gene in *Escherichia coli*, the insecticidal activity of the recombinant toxin protein was determined against *Plutella xylostella* larvae; a 56.7% mortality rate was observed within 24 h.

**KEYWORDS:** *two-dimensional liquid chromatography, Steinernema carpocapsae, toxin gene (txp40), Xenorhabdus nematophila CBNU, insecticidal activity, Plutella xylostella*

## INTRODUCTION

Over the past few decades, the most successful microbial insecticides applied in the agricultural industries used the bacterium *Bacillus thuringiensis* (Bt) that produces insecticidal toxic proteins during sporulation.[1−4] However, the wide use of Bt on a large scale and the developments of transgenic plants expressing these toxin genes caused the appearance of insecticide-resistant population. More than 500 species of insects have evolved resistance to one or more conventional insecticides including insecticidal proteins derived from Bt.[5] Such phenomenon called for the isolation of novel insecticidal proteins from other microorganisms to regain control over diversified pest populations.[6]

*Xenorhabdus* species are Gram-negative enterobacteria, symbiotically associated with entomopathogenic nematodes of the family Steinernematidae that are used in the biological control of insect pests.[7] The nematodes destroy the insect host by releasing bacterial cells into the hemocoel and replicating themselves rapidly.[8] As the direct use of *Xenorhabdus* sp. for bioinsecticide is severely limited due to their vulnerability in water or soil for long periods, it is important that the insecticidal toxicity of these bacteria be reproduced in a heterologous host for their exploitation. Previous studies reported a toxin gene from *Xenorhabdus nematophila* that expresses high insecticidal activity against larvae of insects such as *Pieris brassicae* and *Galleria mellonella*, but little is known about the active ingredient responsible for such toxicity.[8−10]

Enhanced mass technology and the growing amount of protein sequence data make proteomics analysis a useful tool for the identification of new protein toxin genes. The classical approach in proteome study couples two-dimensional gel electrophoresis (2-DE) for protein separation with mass spectrometry analysis. Newly developed liquid-phase separation methods such as size exclusion chromatography, affinity chromatography, and ion-exchange chromatography exhibited practical difficulties due to the lack of isoelectric (p*I*) information and limited labeling efficiency.[11,12] Recently, a more convenient and rapid two-dimensional liquid chromatography separation (2-DL) method was developed to differentiate proteins and integrate information obtained by 2-DE. The ProteomeLab PF-2D platform (Beckman Coulter, Fullerton, CA) used for chromatofocusing separation and hydrophobic fractionation, as well as quantitative comparisons of various biological and clinical samples, works in full automation.[13−15] Compared to gel-based 2-DE, the PF-2D system offers

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excellent resolution with good reproducibility, high loading capacity, and improved detection of lower abundance proteins.[14,16,17] This study presents a new approach to *Xenorhabdus* sp. analysis by exploiting the applicability of PF-2D to the proteomic response analysis involving a *X. nematophila* CBNU strain isolated from a Korean insecticidal nematode *Steinernema carpocapsae*. The novel toxin gene (*txp40*) was successfully identified, cloned, and expressed into *Escherichia coli*. The insecticidal activity of the toxin gene product in *E. coli* that caused a rapid cessation of *Plutella xylostella* larvae is also reported.

## MATERIALS AND METHODS

**Sample Preparation for Two-Dimensional Liquid Chromatography (PF-2D) Analysis.** A symbiotic bacterium *X. nematophila* CBNU used in this study is isolated from the hemolymph of *P. xylostella* larva infected by the Korean insecticidal nematode *S. carpocapsae* (EcoBio Inc., 2008). The isolated *X. nematophila* CBNU strain was most closely related to *X. nematophila* ATCC 19061 with 98.6% 16S rRNA gene sequence similarity (data not shown). *X. nematophila* CBNU strain was grown in Luria−Bertaini medium (5 g/L yeast extract, 10 g/L tryptone, 10 g/L NaCl) for 72 h, at 28 °C, with shaking at 1800 rpm. During cultivation, the cell-free supernatants were collected every 12 h and insecticidal activity was monitored. Equal amounts of concentrated cell-free supernatants acquired at minimum and maximum toxicity (i.e., after 12 and 48 h of bacterial growth) were filtered through a 0.2 μm membrane. The proteins were precipitated with a PlusOne 2-D Clean-up kit (Amersham Biosciences, USA). The aggregated proteins were resolved using starting buffer solution included in the ProteomeLab kit. The protein concentration was determined using a Bio-Rad protein assay kit that is based on Bradford's procedure[18] (Bio-Rad, Richmond, CA, USA).

**PF-2D Analysis.** All buffers and columns used in this study were purchased from Beckman Coulter, and the ProteomeLab PF-2D system (Beckman Coulter) was used for the entire analysis. Prior to sample injection, protein concentrations were determined by the Bio-Rad assay and adjusted to a concentration of 1.5 mg/mL with first-dimension starting buffer. The first-dimension chromatofocusing was performed using a high-performance chromatofocusing (HPCF) column under ambient temperature at a flow rate of 0.2 mL/min. Before each run, the column was equilibrated with starting buffer (pH 8.5) until a stable baseline value of pH 8.3−8.5 was accomplished. Twenty minutes after the sample injection, the elution was started by switching to the elution buffer (pH 4.0). Proteins were eluted in the order of decreasing isoelectric point (p*I*). Protein peaks were monitored by absorbance at 280 nm. The first-dimension fractions were collected into 96-well plates at 0.3 pH intervals. The second-dimension separations, which involve reversed phase chromatography, were performed using a Beckman Coulter nonporous reverse phase (NPRP) column at 50 °C and a flow rate of 0.75 mL/min. The absorbance of proteins in the eluent was monitored at UV 214 nm. The column was first equilibrated with 10 column volumes of 100% solvent A (0.1% (v/v) trifluoroacetic acid (TFA) in water) prior to each sample injection. Two minutes after the injection of 200 μL of sample, bound proteins were eluted with 0−100% gradient of solvent B (0.08% (v/v) TFA in acetonitrile) for 30 min, followed by 100% solvent B for 5 min. The column was then re-equilibrated with 100% solvent A. The second-dimension fractions were collected in 96-well plates every 0.5 min. During PF-2D operation, protein peaks were analyzed with the integration tools provided in the 32 Karat software. The proteome profile obtained using the NPRP column was imported into ProteoVue for the final analysis with DeltaVue.

**MALDI-TOF Mass Spectrometric Analysis and Protein Identification.** Eluted fractions were evaporated to a final individual volume of 10 μL in a SpeedVac (Modulspin 40, BioTron, Korea). One molar $NH_4HCO_3$ and 10 mM DTT were added to each fraction to reach final concentrations of 100 and 1 mM, respectively. The resulting fractions were incubated at 60 °C for 10 min in an oven, and 1 μL of trypsin (100 ng/μL) in 50 mM $CH_3COOH$ was added. Digestion was performed at 37 °C for 24 h, and the fractions were evaporated to dryness in a SpeedVac. The dried tryptic digests were reconstituted with 2 μL of water and purified using ZipTipC18 (Millipore, Bedford, MA, USA). The purified peptides were eluted from the ZipTip directly onto a stainless steel plate with 1 μL of saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA) in 50% (v/v) acetonitrile (ACN) and 0.1% (v/v) TFA in water. Peptide samples were analyzed on a MALDI-TOF mass spectrometry system (AXIMA-CFR plus, Shimadzu, Japan), and the spectra were calibrated using a matrix and tryptic autodigestion ion peaks as internal standards. The peptide mass fingerprints were analyzed using the MASCOT program (http://www.matrixscience.com) and NCBI (http://www.ncbi.nlm.nih.gov/) database, where p*I* and molecular mass of protein data obtained were identified with a peptide of a similar mass (within 10 ppm range)

**Amplification of Identified Genes by PCR and Determination of Nucleotide Sequence.** According to the results of the MALDI-TOF mass spectrometric analysis and protein identification, three proteins (XptB1, XptC1, and txp40) were presumed to be present in *X. nematophila* CBNU. Primers for DNA amplification are designed on the basis of GenBank's nucleotide sequences of *xptB1*, *xptC1*, and *txp40*, respectively.[9,19] Three toxin genes were amplified with DNA polymerase (Takara, Osaka, Japan) and were cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA). The resulting plasmid was used to transform *E. coli* DH5α. The recombinant plasmids were then subjected to sequencing (Solgent, Korea).

**Cloning of a Novel Insecticidal *txp40* Gene.** A novel insecticidal *txp40* gene of 1089 bp isolated from *X. nematophila* CBNU strain was amplified by colony polymerase chain reaction (PCR) using the forward primer 5′-AGTGGATCCATGGTTAT-TAAACCCGTAA-3′ containing a *Bam*HI site and the reverse primer 5′-CATGCGGCCGCTTATATATTTTGGTAATG A-3′ containing a *Not*I site (the underlined sequences denote the restriction sites). The PCR amplification was carried out using a thermocycler (MyCycler thermal cycler, Bio-Rad). The amplified gene was digested with *Bam*HI and *Not*I. The digestion product was then ligated with the *Bam*HI and *Not* I double-digested pET-21a expression vector and transformed into *E. coli* BL21 (DE3) competent cells. The positive colonies with the *txp40* gene in the plasmid were confirmed by automated DNA sequencing.

**Heterologous Expression of the Recombinant Txp40 Protein.** *E. coli* BL21 (DE3) cells harboring pET-21 that contains a novel insecticidal *txp40* gene were inoculated into 5 mL of LB medium supplemented with 50 μg/mL ampicillin and then incubated overnight at 37 °C. The culture was diluted with 500 mL of LB (100-fold) medium containing 50 μg/mL ampicillin and further incubated at 37 °C up to a cell density of 0.6−0.8 absorbance at 600 nm. Expression of a novel insecticidal gene was induced by IPTG to a final concentration of 1 mM for 3 h. Recombinant *E. coli* cells cultured in 500 mL were centrifuged (6000 rpm, 10 min), washed three times with binding buffer (0.5 M NaCl, 20 mM Tris-HCl, 10 mM imidazole, pH 7.9), and suspended in 10 mL of binding buffer. After the addition of lysozyme and 1 mg/mL of binding buffer, the mixture was incubated on ice for 30 min. The suspension was disrupted by sonication on ice (20 s pulse with 10 s intervals for 30 min) using an ultrasonicator (Sonics, VCX-130, USA). The supernatants and pellets separately collected by 30 min of centrifugation at 10000*g* (RS-4S rotor, Kubota) were analyzed on 12% SDS-PAGE gels stained using Coomassie Brilliant Blue R-250. The clear supernatant was mixed with 5 mL of the $NI^{2+}$-nitrilotriacetate (NTA) resin (Qiagen, CA, USA) equilibrated with binding buffer. The mixture was mixed slowly for 1 h using a rotator at 4 °C. The resin/protein mixture was loaded into a column and thoroughly washed with washing buffer (0.5 M NaCl, 20 mM Tris-HCl, 20 mM imidazole, pH 7.9) and eluted with elution buffer (0.5 M NaCl, 20 mM Tris-HCl, 250 mM imidazole, pH 7.9). Recombinant insecticidal protein was eluted with a linear imidazole gradiant. Fractions containing $(His)_6$-tagged insecticidal protein were combined and dialyzed against water purified with slide-A-Lyzer Dialysis Cassette

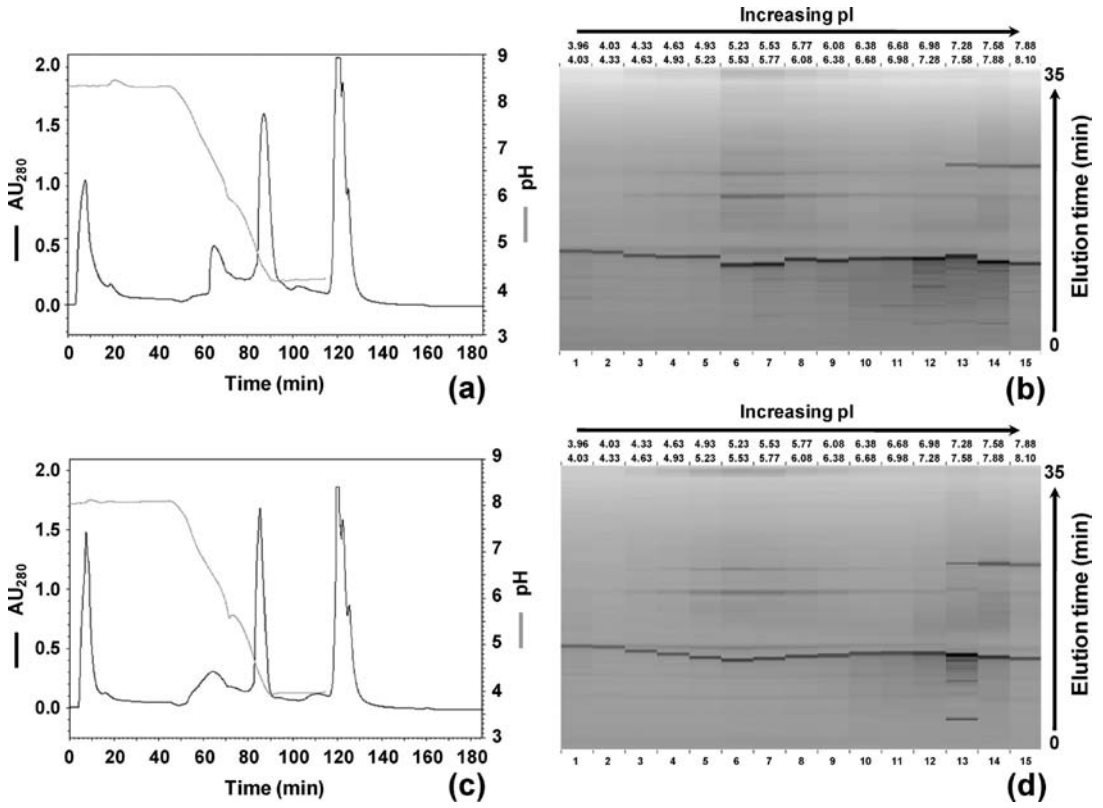


**Figure 1.** 2-DL pattern of cell-free protein present in *Xenorhabdus nematophila* CBNU: (a) first-dimension 280 nm absorbance and pH profiles for 12 h protein sample; (b) ProteoVue Map of the second-dimensional separation of 12 h protein sample; (c) first-dimension 280 nm absorbance and pH profiles for 48 h protein sample; (d) ProteoVue Map of the second-dimensional separation of 48 h protein sample.

(Pierece, Rockford, IL, USA). The protein concentration of purified six-histidine-novel insecticidal protein determined according to the Bradford method was subsequently subjected to reducing SDS-PAGE analysis.

**Measurement of Insecticidal Activity of Recombinant Toxin Protein.** To determine the insecticidal activity of purified recombinant insecticidal proteins, 3 $\mu$L of the purified proteins (1 g/L) dissolved in 10 mM phosphate buffer (pH 7.2) was injected through an intersegmental membrane of *P. xylostella* larvae. Prior to injection, larvae were incubated for 10 days at 25 °C on an artificial diet containing 10 parts wheat bran, 2 parts wheat germ, 1 part yeast, and 2 parts glycerol. A negative control, which contains 10 mM phosphate buffer (pH 7.2) was prepared and also injected to *P. xylostella* larvae using the same procedure. A minimum of 30 larvae were used for each test. The larvae injected with the recombinant insecticidal protein were incubated further for an additional 72 h. The mortality of *P. xylostella* larvae was monitored every 12 h for 3 days at 25 °C.

## RESULTS AND DISCUSSION

**Two-Dimensional Liquid Separation of Insecticidal Proteins by PF-2D and MALDI-TOF/MS Analysis of Second-Dimension Fractions.** The insecticidal activity of cell-free supernatants of *X. nematophila* CBNU strain measured every 12 h for 72 h revealed that the minimum and maximum insecticidal activities can be reached after 12 and 48 h of cultivation, respectively (data not shown). The ProteomeLab PF-2D system separates proteins according to their p*I* during its first-dimension analysis. The first-dimension chromatofocusing absorbance and pH profiles for 12 and 48 h protein samples (Figure 1, panels a and c, respectively) are very much similar to each other. During the first 10 min, the first major peak having a p*I* >8.5 was eluted. Between 50 and 115 min, chromatofocusing took place over decreasing pH gradient. Washing the column with high ionic strength solution created large peaks after 120 min. Fractions collected from the first dimension underwent second-dimension analysis, in which the chromatographic traces are organized according to p*I* range of the injected sample on the *x*-axis and retention time, that is, hydrophobicity (increasing acetonitrile concentration), on the *y*-axis. The conversion of data achieved using ProteoVue software is shown in Figure 1, panels b and d for 12 and 48 h protein samples, respectively. Different sets of protein appeared in the pH range of 3.96−8.10, mostly between 12 and 22 min, during a total 35 min of second dimension. Each protein band represents totals of 177 and 167 resolved proteins for 12 and 48 h samples, respectively. The comparative analysis performed using DeltaVue software revealed that 67 proteins from the 48 h cultivation group had higher abundance intensity as opposed to those cultivated within the 12 h period. Maximum insecticidal activity was exhibited after 48 h of cultivation. The differences in the total amount of secreted proteins over increasing cultivation period can lead to a conclusion that some toxin proteins that would induce higher levels of insecticidal activity are being produced by *X. nematophila* CBNU strain.

Whereas a gel-based 2-DE requires a complex pretreatment process that may involve inevitable contamination during the protein extraction, the ProteomeLab PF-2D platform has the benefit that intact peptides and proteins can be separated in water, acetonitrile, and TFA solvent systems that are directly compatible with offline MALDI-TOF mass spectrometric analysis. In this study, 67 protein peaks showing high absorbance intensity were collected for MALDI-TOF mass spectrometry. MALDI-TOF/MS analysis performed for each protein peak and subsequent peptide mass fingerprinting

**Table 1. MALDI-TOF/MS and in Silico Results of Identified Proteins Separated by PF-2D from *X. nematophila* CBNU**

| lane[a] | p*I* interval[a] | peak | gene name | protein name | p*I* | MW (Da) | accession no.[b] | sequence coverage[c] (%) |
|---|---|---|---|---|---|---|---|---|
| 5 | 4.83−5.13 | 1 | *xptC1* | XptC1 protein | 5.17 | 157736 | CAC38403 | 84 |
| 13 | 7.21−7.51 | 2 | *xptB1* | XptB1 protein | 7.05 | 110257 | CAC38402 | 77 |
| 14 | 7.51−7.81 | 3 | *txp40* | 40 kDa insecticidal toxin | 8.86 | 40850 | ABB29483 | 72 |

[a]Refer to Figure 1b,d. [b]Data from NCBI (http://www.ncbi.nlm.nih.gov/). [c]Mass tolerance in protein identification through PMF experiments was 10 ppm.

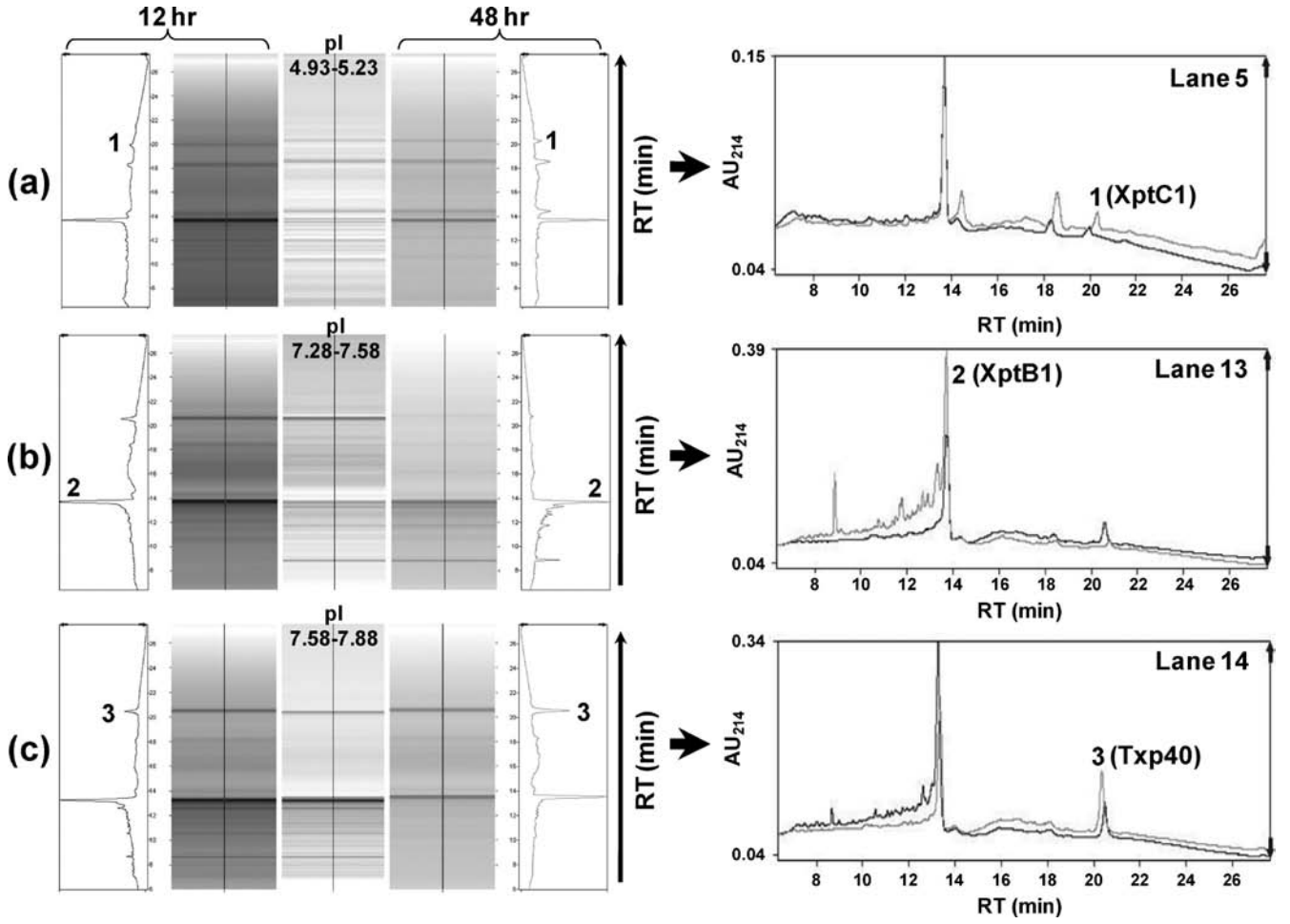


**Figure 2.** DeltaVue comparison of second-dimensional maps for 12 and 48 h protein samples: (a) protein samples of p*I* 4.93−5.23, corresponding to lane 5 in the ProteoVue Map; (b) protein samples of p*I* 7.28−7.58, corresponding to lane 13 in the ProteoVue Map; (c) protein samples of p*I* 7.58−7.88, corresponding to lane 14 in the ProteoVue Map.

achieved using NCBI database enabled identification of three toxin proteins, XptC1, XptB1, and Txp40, of 67 proteins, in which these toxin proteins showed 84, 77, and 72% respective homology with peptide sequences listed in the NCBI database. The results are summarized in Table 1. The p*I* values determined for these proteins are out of their intervals as experimental p*I* values of several proteins evaluated on the basis of 2-DL results are different from true p*I* values. In liquid chromatography systems p*I* is influenced by the concentrations of ions present in the liquid and adsorbed phase as well as the binding of a small part of the charged protein to the ion exchanger.[17] Three toxin protein bands portrayed by lanes 5, 13, and 14 of Figure 1, panels b and d, 2-DL maps were enlarged as shown in Figure 2, along with overlaying protein profiles. Peak 1 of lane 5 that eluted at 20.2 min was identified as XptC1 (Figure 2a). Figure 2b, lane 13, shows the elution of peak 2 or XptB1 at 14 min. Peak 3 or Txp40, lane 14, eluted at 20.4 min. Quantitative analysis performed with 32 Karat chromatography software revealed areas of peaks 1, 2, and 3 at each retention time were approximately 1.7-, 1.3-, and 3.1-fold greater, respectively, in the 48 h protein fraction than they were in the 12 h fraction, requiring further investigation of three toxin proteins.

**Cloning, Heterologous Expression, and Purification of a Novel *txp40* Gene Isolated from *X. nematophila* CBNU Strain.** Using MALDI-TOF/MS analysis, the amino acid sequences of three identified proteins being identical to sequences of insecticidal proteins from *X. nematopahila* recorded in GenBank (NCBI) were determined. To ascertain the sequence homology of each insecticidal gene present in *X. nematophila* CBNU strain, PCR was performed to amplify the full region using both ends of the gene-specific primers designed on the basis of GenBank's nucleotide sequences of *xptB1*, *xptC1*, and *txp40*. Then the PCR products were cloned

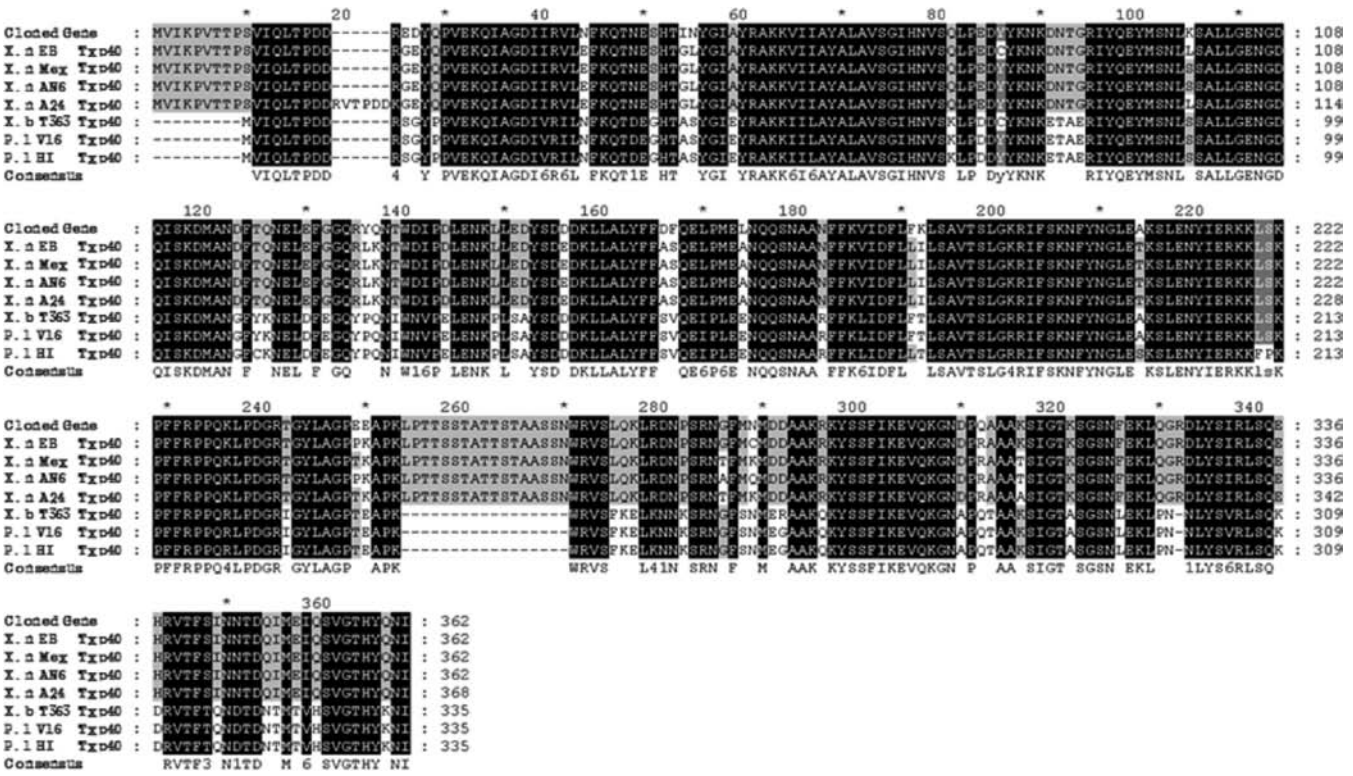

**Figure 3.** Protein sequence alignments of the identified toxin and the following previously reported proteins: *X. nematophila* EB (ADQ92844, "X. n EB Txp40"); *X. nematophila* Mex (ABB29483, "X. n Mex Txp40"); *X. nematophila* AN6 (ABB29484, "X. n AN6 Txp40"); *X. nematophila* A24 (ABB29480, "X. n A24 Txp40"); *X. bovienii* T363 (ABB29486, "X. b T363 Txp40"); *P. luminescens* V16 (ABB29487, "P. l V16 Txp40"); *P. luminescens* HI (ABB29485, "P. l HI Txp40").

into T-vector for sequence analysis. The sequences of *xptB1* and *xptC1* PCR products showed 100% homology with the nucleotide and amino acid sequences of GenBank's CAC38402 and CAC38403, respectively (data not shown). However, the sequence of the *txp40* PCR product showed 96.2 and 94.4% homology with the nucleotide and amino acid sequences of ADQ92844, respectively. The protein sequence alignment results revealed *txp40* had 94.4, 94.1, 94.1, 92.3, 71.8, 71.5, and 70.7% identical amino acid residues with *X. nematophila* EB (ADQ92844), *X. nematophila* Mex (ABB29483), *X. nematophila* AN6 (ABB29484), *X. nematophila* A24 (ABB29480), *P. luminescens* V16 (ABB29487), *X. bovienii* T363 (ABB29486) and *P. luminescens* HI (ABB29485), respectively (Figure 3). The nucleotide sequence of the *txp40* gene was deposited in the NCBI GenBank database under accession no. HQ658061. To determine the insecticidal activity of Txp40 against *P. xylostella* larvae, the *txp40* gene was cloned into expression vector pET-21a that was used for the transformation of *E. coli* BL21(DE3) cells, and the resulting vector was amplified by PCR. Agarose gel electrophoresis (Figure 4a) revealed a single PCR product that was released by *Bam*HI and *Not*I digestion and ligated to the pET-21a vector in the same restriction enzyme site. Recombinant plasmid was selected on the basis of the restriction digestion with *Bam*HI and *Not*I that released the 1089 bp *txp40* gene and 5443 bp pET-21a vector (Figure 4b). *E. coli* BL21 (DE3) cells were transformed with pET-21a::*txp40* to exhibit heterologous expression of *txp40*. Figure 5 shows a prominent band that was not found in the control, cells transformed without the insert, at about 40 kDa. The expression level of soluble 40 kDa insecticidal protein after 3 h of induction was about 15% of total 40 kDa insecticidal

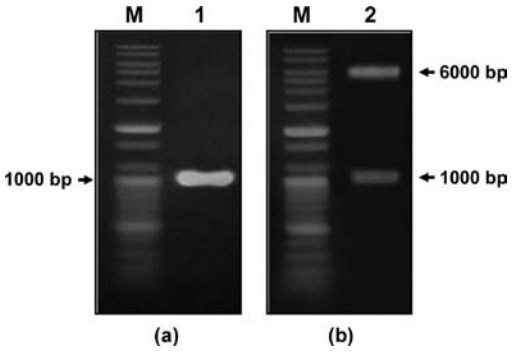


**Figure 4.** SDS-PAGE analysis of (a) *txp40* gene from *X. nematophila* CBNU loaded in lane 1 with DNA size marker in lane M (Bioneer catalog no. D-1035) and (b) pET-21::*txp40* digested with *Bam*HI and *Not*I loaded in lane 2 with DNA size marker in lane M.

protein in the soluble protein fraction, and >80% of expressed protein was soluble (lane 5 of Figure 5). When the recombinant 40 kDa insecticidal protein was purified by Ni-NTA resin, the purified 40 kDa insecticidal protein also showed a single protein band with an apparent MW of about 40 kDa on the SDS-PAGE (lane 7 of Figure 5). Finally, the insecticidal activity of purified 40 kDa protein was prepared to be measured.

**Insecticidal Efficiency of the Recombinant 40 kDa Insecticidal Protein.** The biological activity of the recombinant purified protein was tested by a direct injection assay, a method that delivers the toxin directly into the hemolymph. The insecticidal activity assay results indicated purified 40 kDa insecticidal Txp40 protein killed a high percentage of *P.*


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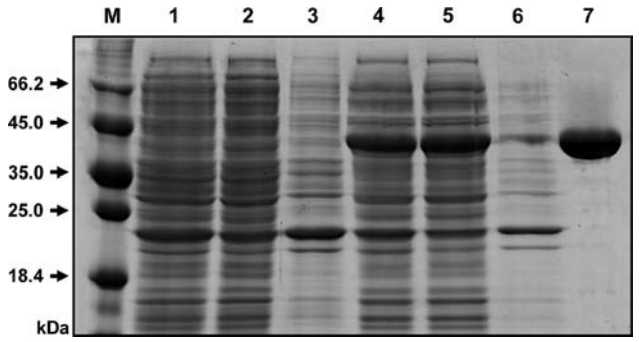


**Figure 5.** SDS-PAGE analysis of recombinant Txp40 protein from *E. coli* BL21 (DE3). Lanes: M, protein maker (Fermentas catalog no. SM0431); 1, total cell lysates of BL21(DE3) pET-21a; 2, soluble lysates of BL21(DE3) pET-21a; 3, insoluble lysates of BL21(DE3) pET-21a; 4, total cell lysates of BL21(DE3) pET-21a::*txp40*; 5, soluble lysates of BL21(DE3) pET-21a::*txp40*; 6, insoluble lysates of BL21(DE3) pET-21a::*txp40*; 7, 40 kDa insecticidal toxin.

*xylostella* larvae. Whereas 10 mM phosphate buffer at pH 7.2 used as a control for this experiment did not cause significant larval mortality, 40 kDa insecticidal protein killed 56.7% of the larvae under the identical experimental condition within 24 h (Table 2). The activity of recombinant 40 kDa insecticidal

**Table 2. Insecticidal Activities of the Recombinant Toxin Protein Tested against 30 Larvae of *P. xylostella***

| | negative control | | recombinant toxin protein | |
|---|---|---|---|---|
| incubation time (h) | no. of surviving larvae | mortality[a] (%) | no. of surviving larvae | mortality[a] (%) |
| 0 | 30 | 0 | 30 | 0 |
| 12 | 30 | 0 | 20 | 33.3 |
| 24 | 30 | 0 | 13 | 56.7 |
| 36 | 30 | 0 | 8 | 73.3 |
| 48 | 30 | 0 | 7 | 76.7 |
| 60 | 30 | 0 | 7 | 76.7 |
| 72 | 30 | 0 | 5 | 83.3 |

[a](Number of dead larvae)/(total number of larvae tested) × 100.

Txp40 protein against *P. xylostella* larvae was found to be dependent on the incubation time: 76.7 and 83.3% mortality at 48 and 72 h were observed, respectively. The insecticidal activity assay results were a clear indication of efficient insecticidal activity present in the recombinant 40 kDa insecticidal protein coded by the novel *txp40* gene.

In conclusion, the growing number of insecticide-resistant populations in agricultural industry demanded an inevitable quest for a novel insecticide. Proteomics analysis involving a two-dimensional liquid chromatography separation technique was proven useful at identifying a novel toxin protein. Additionally, insecticidal activity of *txp40* that increased over time indicated the potential of the toxin gene for possible application as a bioinsecticide with further research.

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### Notes

The authors declare no competing financial interest.

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