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# Molecular Cloning of Two cDNAs Encoding an Insecticidal Toxin from the Spider, *Araneus ventricosus*, and Construction of a Recombinant Baculovirus Expressing a Spider Toxin

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**We have cloned cDNAs encoding toxin from the spider, *Araneus ventricosus*, and constructed a recombinant baculovirus expressing the insecticidal toxin. The cDNAs encoding toxin were cloned from the cDNA library of *A. ventricosus*. Sequence analysis of the cDNAs encoding the toxin of *A. ventricosus* revealed that the 240 bp cDNA for AvTox-1 and 192 bp cDNA for AvTox-2 have an open reading frame of 80 and 64 amino acid residues, respectively. The deduced protein sequence of the toxin genes of AvTox-1 and AvTox-2 was aligned to that of the snack *Anemonia sulcata* and scorpion *Centruroides limpidus limpidus*, respectively. Northern blot analysis indicated that AvTox-2 toxin gene showed a fat body-specific expression pattern at the transcriptional level. Furthermore, we have explored the possibility of improving baculovirus by incorporating the *A. ventricosus* toxin gene into *Bombyx mori* nuclear polyhedrosis virus genome under the control of polyhedrin promoter. The AvTox-2 toxin gene was expressed as approximately 5.8 kDa band in the recombinant baculovirus-injected silkworm larvae. Bioassays with the recombinant virus expressing AvTox-2 on 5th instar silkworm larvae demonstrated a decrease in the time to kill ($LT_{50}$ 5.50 days) compared to wild-type BmNPV-K1 ($LT_{50}$ 6.72 days) in the injection of 10 viruses. These results indicate that *A. ventricosus* toxin is a novel member of the spider toxin family, suggesting that the toxin gene can be used in recombinant baculoviruses to reduce insect feeding damage and increase the speed of insect kill.**

**Key words** : Spider, Toxin, Baculovirus, Silkworm, Insecticide

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## Introduction

Baculoviruses have high potential as biological control agents for controlling insect pests. They have host specificity, stability, and safety. A drawback of baculovirus is that insect feeding is not arrested immediately following infection. Since the development of the baculovirus expression vectors expressing foreign genes (Smith *et al.*, 1983; Pennock *et al.*, 1984; Maeda *et al.*, 1985), there has been considerable interest in the insertion of target genes into the genome of baculoviruses to enhance their pesticidal efficacy (Maeda, 1995; Bonning and Hammock, 1996). Target genes that have been inserted for this purpose include those encoding for the *Buthus eupeus* insect toxin-1 (Carbonell *et al.*, 1988), the *Manduca sexta* diuretic hormone (Maeda, 1989), the *Bacillus thuringiensis* toxin (Merryweather *et al.*, 1990; Martens *et al.*, 1990), the *Heliothis virescens* juvenile hormone esterase (Hammock *et al.*, 1990; Bonning *et al.*, 1992; Eldridge *et al.*, 1992), the *Pyemotes tritici* TxP-1 toxin (Tomalski and Miller, 1991), and the *Androctonus australis* AaHIT toxin (Stewart *et al.*, 1991; Maeda *et al.*, 1991; McCutchen *et al.*, 1993).

Furthermore, insecticidal toxins from arthropod venoms have also been focused because these neurotoxic peptides have been proved to have a high insecticidal potency and great selectivity for insects. The peptides

have been identified from the venoms of several spiders (Adams *et al.*, 1989; Entwistle *et al.*, 1982; Krapcho *et al.*, 1995; Ornberg *et al.*, 1976; Quistad *et al.*, 1991; Skinner *et al.*, 1992). Most of these peptide toxins from the spiders appear to act by altering the function of presynaptic sodium or calcium channels, and are generally quite potent in insects (Adams *et al.*, 1989). The spider toxin genes have been also inserted into the baculovirus genome to produce enhanced insect viral pesticides (Hughes *et al.*, 1997; Krapcho *et al.*, 1995).

The spider, *Araneus ventricosus* is an abundant species in Korea (Kim *et al.*, 1997b). The body size of *A. ventricosus* is relatively larger than that of the other species. The mechanical properties of the web of the Korean native spiders, *A. ventricosus* and *Nephila clavata*, have been reported (Kim *et al.*, 1997a, b). However, the toxin gene on the spider *A. ventricosus* is not currently available. In this report, we present the cloning of the insecticidal toxin gene from the spider, *A. ventricosus*, construction of a recombinant baculovirus expressing the spider toxin, and its insecticidal effect on host larvae.

## Materials and Methods

### Preparation of spider

The spider *Araneus ventricosus* was collected at Kimhae, Kyungnam Province in Korea. The collected spider was directly used in this study.

### cDNA library screening, nucleotide sequencing and data analysis

A cDNA library (Chung *et al.*, 2001) was constructed from the whole body of *A. ventricosus*. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the expressed sequence tags (ESTs). For DNA sequencing, plasmid DNA was extracted by Wizard mini-preparation kit (Promega). Sequence of each cDNA clone was determined using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). The sequences were compared using the DNASIS and BLAST programs provided by the NCBI. GenBank, EMBL and SwissProt databases were searched for sequence homology using a BLAST algorithm program.

### RNA isolation and Northern blot analysis

Total RNAs were isolated from the whole body, cephalothorax, abdomen, fat body, silkgland, and mid gut of the *A. ventricosus* by using the Total RNA Extraction Kit (Promega, Madison, WI). The RNAs (10 μg/lane) from the *A. ventricosus* were denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a buffer containing 2 × PIPES, 50% formamide, 1% sodium dodecyl sulphate (SDS) and blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect the toxin gene transcripts was a 192 bp *A. ventricosus* toxin gene cloned in this study and labeled with [$\alpha$-$^{32}$P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA). After hybridization, the membrane filter was washed three times for 30 min each in 0.1% SDS and 0.2 × SSC (1 × SSC is 0.15 M NaCl and 0.015 M sodium citrate) at 65°C, and finally exposed to *X*-ray film.

### Cell culture and virus

The *Bombyx mori* (Bm5) cells were maintained at 27°C in TC100 medium (GIBCO BRL LIFE Technologies) supplemented with 10% fetal bovine serum (FBS, GIBCO BRL LIFE Technologies) as described by standard methods (O'Reilly *et al.*, 1992). Wild-type *Bombyx mori* nuclear polyhedrosis virus (BmNPV-K1) and recombinant BmNPV were propagated in Bm5 cells. The titer was expressed as plaque forming units (PFU) per ml (O'Reilly *et al.*, 1992).

### Construction of transfer vector

The 192 bp *A. ventricosus* toxin homologue gene cloned in this study was subcloned into pGem-T vector (Promega) to yield pGemT-AvTox2. The toxin homologue gene from pGemT-AvTox2 was digested with *Sac*I and *Kpn*I, and then inserted into the *SacI* and *Kpn*I sites of pBacPAK9 (Clontech, Palo Alto, CA) to produce transfer vector pBacPAK9-AvTox2. In pBacPAK9-AvTox2, the toxin homologue gene is under the control of the BmNPV polyhedrin promoter.

### Construction of recombinant virus

Cell culture dishes of thirty-five mm in diameter were seeded with $1.0 - 1.5 \times 10^6$ cells and incubated at 27°C for 1 hr to allow attachment. One microgram of bBpGOZA viral DNA (Je *et al.*, 2001), one μg of pBacPAK9-AvTox2 in 20 mM HEPES buffer and sterile water were mixed up to 50 μl in a polystyrene tube. Fifty microliter of Lipofectin™ (100 μg/ml, GIBCO BRL LIFE Technologies, Gaithersburg, MD) was gently mixed with the DNA solution and the mixture was incubated at room temperature for 30 min. The cells were washed twice with 2 ml serum-free TC100 medium and refed with 1.5 ml serum-free TC100 medium. The Lipofectin-DNA complexes were added dropwise to the medium covering the cells while the dish was gently swirled. After incubation at 27°C for 5 hrs, TC100

medium containing antibiotics and 10% FBS was added to each dish and incubation at 27°C was continued. At 5 days postinfection (p.i.), the supernatant was harvested, clarified by centrifugation at 2,000 rpm for 5 min, and stored at 4°C before plaquing on Sf9 cells. Recombinant BmNPV was plaque-purified on 6-well plates seeded with $1.5 \times 10^6$ Bm5 cells as described (O'Reilly *et al.*, 1992). Cells were visualized by inverted phase contrast microscopy (Olympus, Tokyo, Japan).

**SDS-Urea gel electrophoresis**

Fifth-instar silkworm larvae were individually mock-injected or injected with the wild-type BmNPV and recombinant BmNPV of $1 \times 10^1$ PFU. After injection, hemolymph from larvae was respectively harvested at 1, 2, 3, 4, and 5 days p.i. For SDS-Urea gel electophoresis (Laemmli, 1970) of hemolymph, uninfected hemolymph and hemolymph from larvae injected with wild-type BmNPV or recombinant BmNPV were diluted with PBS (140 mM NaCl, 27 mM KCl, 8 mM $Na_2HPO_4$, 1.5 mM $KH_2PO_4$, pH 7.4) and mixed with protein sample buffer. The total hemolymph proteins were subjected to 15% SDS-Urea gel electophoresis anlysis.

**Bioassay**

The recombinant of recombinant BmNPV was determined against the fifth-instar silkworm larvae. To determine the median lethal time ($LT_{50}$) measurement, sixty fifth-instar larvae were injected with recombinant BmNPV ($1 \times 10^1$ PFU). After injection, the silkworm larvae were maintained with fresh mulberry leaves and checked at frequent intervals. Dead larvae were removed and the cause of death diagnosed by appearance and microscopic examination. $LT_{50}$ calculations were made with the vistat program (Boyce Thompson Institute, Ithaca, New York).

## Results and Discussion

**Cloning, sequence analysis, and characterization of the cDNA encoding A. ventricosus toxin homologue**

Construction of cDNA library was prepared from the whole body of the spider, *A. ventricosus*. The sequencing of randomly selected clones harboring cDNA inserts was performed to generate the *A. ventricosus* ESTs. Of these ESTs, two exhibited similarity to the reported toxins. The complete DNA sequence of cDNAs encoding a putative

```
(A)    1 ATGCCTTTCCACTACTTTCCCAAACTGAATCCTATAACCTGGATCCCTGGATGGATT
(B)  1  M  P  F  H  Y  F  P  K  L  N  P  I  T  W  I  P  G  W  I

    58  CCTGGTTTGGGTAAAGATAGATGCCTTCTTCCGAAGGTAACAGGACCTTGCAAAGCT
    20  P  G  L  G  K  D  R  C  L  L  P  K  V  T  G  P  C  K  A

   115  AGTCTGACCCGTTACTATTATGACAAGGACACCAAAGCATGCGTGGAATTCATCTAC
    37  S  L  T  R  Y  Y  Y  D  K  D  T  K  A  C  V  E  F  I  Y

   172  GGTGGCTGCAGAGGAAACAGGAACAATTTCAAACGGAAAGATGAGTGCGAAAAAGCC
    56  G  G  C  R  G  N  R  N  N  F  K  R  K  D  E  C  E  K  A

   229  TGCACGGATCACTAA
    75  C  T  D  H  *

(C)                                        *        *              *
 AvTox  1  MPFHYFPKLNPITWIPGWIPGLGIDRCLLPKVTGPCKASLTRYYYDKDTKACV
  AsKC  1                        INKD-CLLPMDVGRCRASHPRYYYNSSSKRCE
  AsKC  2                        INKD-CLLPMDVGRCRARHPPYYYNSSSRRCE
  AsKC  3                        INGD-CELPKVVGRCRARFPRYYYNLSSRRCE

                 *              *  *
 AvTox  1  EFIYGGCRGNRNNFKRKDECEKACTDH
  AsKC  1  KFIYGGCRGNANNFHTLEECEKVCGVR
  AsKC  2  KFIYGGCRGNANNFITKKECEKVCGVR
  Askc  3  KFIYGGCGGNANNFHTLEECEKVCGVRS
```

**Fig. 1.** The nucleotide (A) and deduced amino acid (B) sequences of *A. ventricosus* toxin homologue gene (AvTox-1). The start codon of ATG is boxed and the termination codon is underlined. The deduced amino acid sequence of *A. ventricosus* toxin homologue gene (AvTox-1) is aligned with *A. sulcata* toxin gene (C). The six conserved cysteine residues are asterisked. The GenBank accession number is AY091482.

```
(A)   1 ATGGCACTTGCACTGCTGGGATTAACTATTAAACCCGAACATGTACCGGAAGGCACC
(B)   1 M  A  L  A  L  L  G  L  T  L  K  P  E  D  V  P  E  G  T

     58 GGGAAAGCTGTTGCAGATGTGGAGGCGCTGGCATGCGATCCGGCCCAATGTATGAGG
     20 G  K  A  V  A  D  V  E  A  L  A  C  D  P  A  Q  C  M  R

    115 TCCTGTCCCTTCAATCCCTTCTTAAATCAGTACGGAGGAATTTGTAAAAACGGCCAA
     39 S  C  P  F  N  P  F  L  N  Q  Y  G  G  I  C  K  N  G  Q

    172 TGCGTATGCGTAAAACCTTCTTGA
     58 C  V  C  V  K  P  S*

(C)                                                *     *  *
    AvTox 2 MALALLGLTLKPEDVPEGTGKAVADVEALIC-DPAQCMRSCPFNPFLNQYGGI
C.l.limpides                              ITINVKCTSPQQCLRPCK-DRFGQHAGGK
              *     * *
    AvTox 2 CKNGQCVCVKPS
C.l.limpides CINGKCKC
```

**Fig. 2.** The nucleotide (A) and deduced amino acid (B) sequences of *A. ventricosus* toxin homologue gene (AvTox-2). The start codon of ATG is boxed and the termination codon is underlined. The deduced amino acid sequence of *A. ventricosus* toxin homologue gene (AvTox-2) is aligned with *C. limpidus limpidus* toxin gene (C). The six conserved cysteine residues are asterisked. The GenBank accession number is AY091483.

member of the toxin gene family was analyzed. Two cDNAs showed homology to *Anemonia sulcata* kalicludine toxin gene (Schweitz *et al.*, 1995) and *Centruroides limpidus limpidus* potassium-channel blocking toxin (Martin *et al.*, 1994), which we designated AvTox-1 (Fig. 1) and AvTox-2 (Fig. 2), respectively.

The complete DNA sequence of AvTox-1 revealed that the 240 bp cDNA has an open reading frame of 80 amino acid residues (Fig. 1A and B). A multiple sequence alignment of the deduced amino acid sequence of AvTox-1 gene with related toxin sequences is shown in Fig. 1C. This alignment illustrates that AvTox-1 shows similarity to the snake *A. sulcata* kalicludine gene. The predicted protein of AvTox-1 exhibits six cysteine residues. The six cysteine residues in the AvTox-1 are completely conserved at the homologous locations in the *A. sulcata* kalicludine. Considering the amino acid sequence of *A. sulcata* kalicludine, AvTox-1 suggests the toxin being consisted of a propeptide of 20 amino acids and a mature protein of 60 amino acids. The predicted molecular mass of the mature toxin from the deduced amino acid sequence is approximately 6.6 kDa.

The complete DNA sequence of AvTox-2 revealed that the 192 bp cDNA has an open reading frame of 64 amino acid residues (Fig. 2A and B). A multiple sequence alignment of the deduced protein sequence of AvTox-2 gene with related toxin sequences is shown in Fig. 2C. This alignment illustrates that AvTox-2 shows similarity to the scorpion *C. limpidus limpidus* potassium-channel blocking toxin gene. The predicted amino acid of AvTox-2 exhibits six cysteine residues. These are conserved at the homologous locations in the *C. limpidus limpidus* potassium-channel blocking toxin. Considering the amino acid sequence of *C. limpidus limpidus* potassium-channel blocking toxin, AvTox-2 suggests the toxin being consisted of a propeptide of 23 amino acids and a mature protein of 41 amino acids.

The hydropathy plot of AvTox-1 and AvTox-2 are pre-

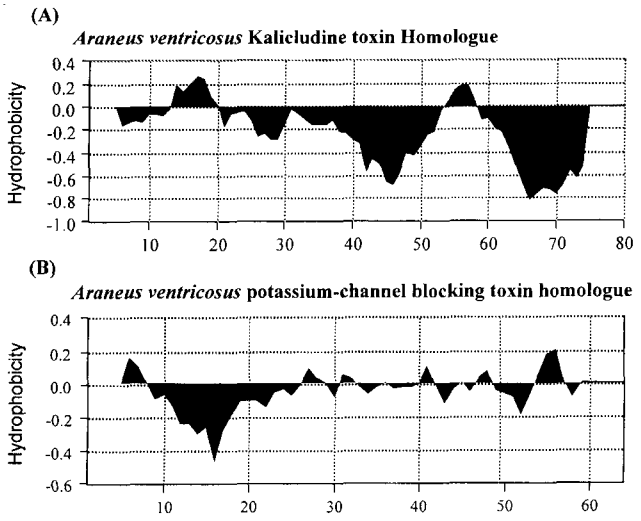


**Fig. 3.** The hydropathy profile of *A. ventricosus* toxin homologue genes, AvTox-1 (A) and AvTox-2 (B). Hydropathic analysis was done as described by Kyte and Doolittle (1982).

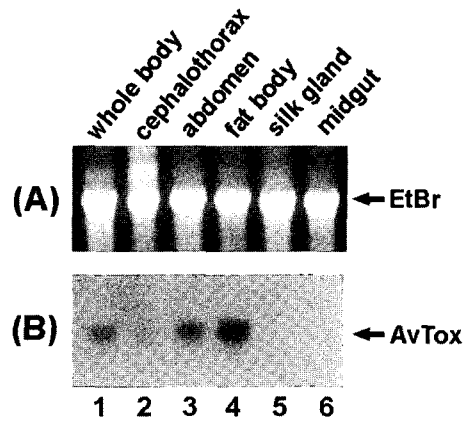


**Fig. 4.** Northern blot analysis of the *A. ventricosus* toxin homologue gene (AvTox-2). Total RNAs were isolated from the whole body (lane 1), cephalothorax (lane 2), abdomen (lane 3), fat body (lane 4), silk gland (lane 5), and mid gut (lane 6). The RNAs were separated by 1.0% formaldehyde agarose gel electrophoresis (A), transferred on to a nylon membrane, and hybridized with the radiolabelled 192 bp *A. ventricosus* toxin homologue gene (AvTox-2) (B). Transcripts are indicated on the right of panel by an arrow.

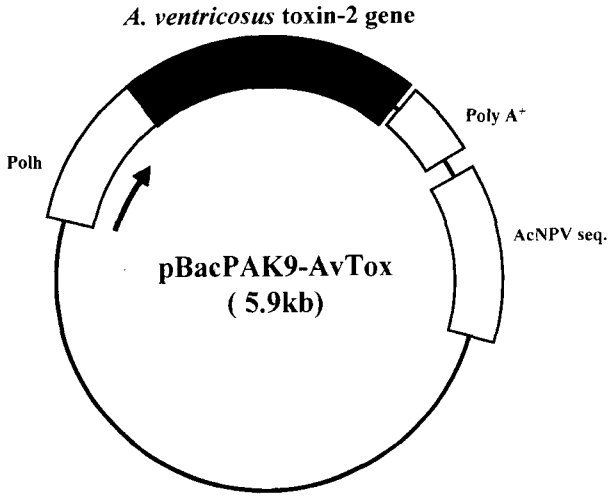


**Fig. 5.** Structure of the transfer vector used to generate the recombinant baculovirus. The transfer vector pBacPAK9-AvTox-2 was constructed by insertion of the *A. ventricosus* toxin homologue gene into pBacPAK9, under the control of BmNPV polyhedrin promoter (Polh). The arrow shows the direction of transcription.

sented in Fig. 3. It shows that the profile of AvTox-1 is significantly different from AvTox-2, although two toxins are derived from the same organism of *A. ventricosus*.

To confirm the expression pattern of the AvTox-2 gene at the transcriptional level, the Northern blot analysis was carried out using the mRNA prepared from a few tissues such as whole body, cephalothorax, abdomen, fat body, silk gland, and mid gut (Fig. 4). A hybridization signal was detected as a single band in the mRNA from whole body and abdomen as positive controls and fat body. The result of the Northern hybridization exhibits the expression of the AvTox-2 gene only in the fat body.

### Expression of the AvTox-2 protein in baculovirus-infected silkworm larvae

To assess AvTox-2 gene, the 192 bp AvTox-2 cDNA was inserted into baculovirus transfer vector. The baculovirus transfer vector was used to generate recombinant virus expressing AvTox-2 as described in Fig. 5. Transfer vector pBacPAK9-AvTox-2 was constructed by digestion of pBacPAK9 with *Sac*I and *Kpn*I, and ligation with AvTox-2 gene under the control of BmNPV polyhedrin promoter. Recombinant BmNPV (BmNPV-AvTox-2) was produced in insect Bm5 cells by cotransfection with wild-type BmNPV DNA (Je *et al.*, 2001) and the pBacPAK9-AvTox-2 transfer vector containing the AvTox-2 gene.

To examine the expression of AvTox-2 gene by recombinant virus in silkworm larvae, the protein synthesis in hemolymph injected with recombinant virus was analyzed

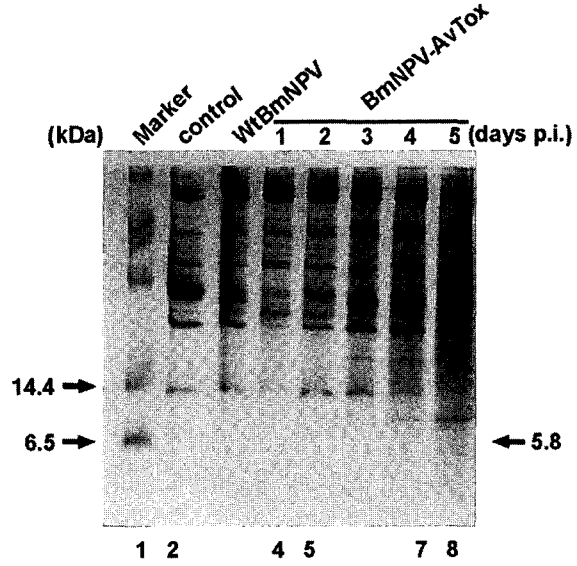


**Fig. 6.** SDS-Urea gel electrophoresis analysis of *A. ventricosus* toxin expression of recombinant baculovirus in silkworm larvae. Fifth-instar silkworm larvae were mock-injected (lane 2) or injected with wild-type BmNPV (lane 3) and recombinant BmNPV (lanes 4, 5, 6, 7, and 8) of 1 $10^1$ PFU. Hemolymph from larvae injected with virus was respectively harvested at 1 (lane 4), 2 (lane 5), 3 (lanes 3 and 6), 4 (lane 7), and 5 (lane 8) days p.i. Total hemolymph proteins were subjected to 15% SDS-Urea gel electrophoresis anlysis. The solid arrow on the right of panel indicates the toxin band of 5.8 kDa. Molecular weight standards were used as size marker (lane 1).

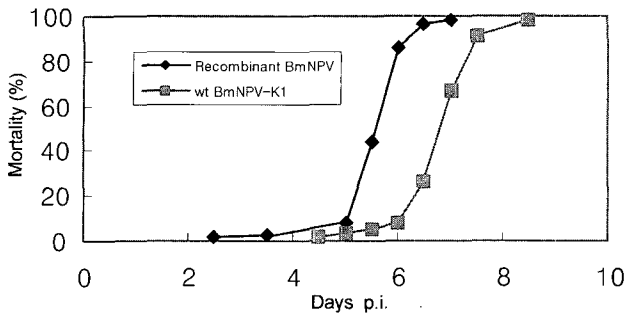


**Fig. 7.** Mortality of the silkworm injected with recombinant baculovirus and wild type BmNPV. Each the 5th instar silkworm larva was injected with virus of $1 \times 10^1$ PFU.

**Table 1.** $LT_{50}$ values for recombinant baculovirus and wild type BmNPV in silkworm larvae*

| Virus | $LT_{50}$ |
|---|---|
| Recombinant baculovirus | 5.50 |
| Wild-type BmNPV | 6.72 |

*Each the 5th instar silkworm larva was injected with virus of $1 \times 10^1$ PFU.

by SDS-Urea gel electrophoresis (Fig. 7). The AvTox-2 expressed by the AvTox-2 gene was present as a band of about 5.8 kDa in the hemolymph from larvae injected with recombinant virus, but not in the hemolymph from larvae injected with wild-type BmNPV or mock-injected larvae.

### Toxicity of a recombinant baculovirus expressing AvTox-2 protein

To determine the toxicity of a recombinant baculovirus expressing AvTox-2, toxicity of a recombinant baculovirus was evaluated against fifth-instar silkworm larvae and compared with wild-type BmNPV. Accumulative mortality of the silkworm injected with recombinant baculovirus is shown in Fig. 7. In the treatment of recombinant baculovirus, dead larvae were firstly observed from 2.5 days p.i., and the mortality was finally 100% at 6.5 days p.i. In the treatment of wild-type BmNPV, on the other hand, dead larvae were firstly observed from 4.5 days p.i., and the mortality was finally 100% at 8.5 days p.i.

The improved insecticidal activity of the recombinant baculovirus expressing AcTox-2 was demonstrated by reduction in $LT_{50}$ value (Table 1). The $LT_{50}$ value for recombinant baculovirus was significantly reduced to 5.50 days compared with 6.72 days in the wild-type BmNPV, potentializing the toxin as an effective insecticide. Two spider toxin genes from *Diguetia canities* and *Tegenaria agrestis* have been inserted previously into the baculovirus to produce enhanced viral pesticides and reported a reduction in the $LT_{50}$ upon insertion of genes encoding for spider toxins (Krapcho *et al.*, 1995; Hughes *et al.*, 1997).

In conclusion, we report gene nucleotide sequence of *A. ventricosus* toxin genes and construction of recombinant baculovirus expressing the spider toxin. In this study we demonstrated that the recombinant baculovirus expressing AvTox-2 is more effective in killing of insects than the wild-type virus.

## References

Adams, M. D., E. E. Herold and V. J. Venema (1989) Two classes of channel-specific toxins from funnel web spider venom. *J. Comp. Physiol. A* **164**, 333-342.

Bonning, B. C. and B. D. Hammock (1996) Development of recombinant baculoviruses for insect control. *Annu. Rev. Entomol.* **41**, 191-210.

Bonning, B. C., M. Hirst, R. D. Possee and B. D. Hammock (1992) Further development of a recombinant baculovirus insecticide expressing the enzyme juvenile hormone esterase from *Heliothis virescens*. *Insect Biochem. Mol. Biol.* **22**, 453-458.

Carbonell, L. F., M. R. Hodge, M. D. Tomalski and L. K. Miller (1988) Synthesis of a gene coding for an insect-specific scorpion neurotoxin and attempts to express it using baculovirus vectors. *Gene* **73**, 409-418.

Chung, E. H., K. S. Lee, J. H. Han, H. D. Sohn and B. R. Jin (2001) Analysis of expressed sequence tags of the spider, *Araneus ventricosus*. *Int. J. Indust. Entomol.* **3**, 191-199.

Eldridge, R., D. R. O'Reilly, B. D. Hammock and L. K. Miller (1992) Insecticidal properties of genetically engineered baculoviruses expressing an insect juvenile hormone esterase gene. *Appl. Environ. Microbiol.* **58**, 1583-1591.

Entwistle, I. D., R. A. W. Johnstone, D. Medzihradszky and T. E. May (1982) Isolation of a pure toxic polypeptide from the venom of the spider *Phoneutria nigriventer* and its neurophysiological activity on an insect femur preparation. *Toxicon* **20**, 1059-1067.

Hammock, B. D., B. C. Bonning, R. D. Possee, T. N. Hanzlik and S. Maeda (1990) Expression and effect of the juvenile hormone esterase in a baculovirus vector. *Nature* **344**, 458-461.

Hughes, P. R., H. A. Wood, J. P. Breen, S. F. Simpson, A. J. Duggan and J. A. Dybas (1997) Enhanced bioactivity of recombinant baculoviruses expressing insect-specific spider toxins in lepidopteran crop pests. *J. Invertebr. Pathol.* **69**, 112-118.

Je, Y. H., J. H. Chang, M. H. Kim, J. Y. Roh, B. R. Jin and D. R. O'Reilly (2001) The use of defective *Bombyx mori* nucleopolyhedrovirus genomes maintained in *Escherchia coli* for the rapid generation of occlusion-positive and occlusion-negative expression vectors. *Biotech. Lett.* **23**, 1809-1817.

Kim, J. P., Y. B. Lee, S. J. Jang and M. A. Kim (1997a) The

studies of properties of matter and chemical qualitative analysis on web of *Nephila clavata* L. KOCH, 1878. *Korean Arachnol.* **13**, 63-65.

Kim, J. P., Y. B. Lee, S. J. Jang and M. A. Kim (1997b) The studies of properties of matter and chemical qualitative analysis on web of *Araneus ventricosus* L. KOCH, 1878. *Korean Arachnol.* **13**, 87-91.

Krapcho, K. J., R. M. Kral Jr., B. C. Vanwagenen, K. G. Eppler and T. K. Morgan (1995) Characterization and cloning of insecticidal peptides from the primitive weaving spider *Diguetia canities*. *Insect Biochem. Molec. Biol.* **25**, 991-1000.

Kyte, J. and R. F. Doolittle (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

Laemmli, U. K. (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Maeda, S., T. Kawai, M. Obinata, H. Fujiwara, T. Horiuchi, Y. Saeki, Y. Soto and M. Furusawa (1985) Production of human interferon in silkworm using a baculovirus vector. *Nature* **315**, 592-594.

Maeda, S. (1995) Further development of recombinant baculovirus insecticides. *Curr. Opin. Biotech.* **6**, 313-319.

Maeda, S. (1989) Increased insecticidal effect by a recombinant baculovirus carrying a synthetic diuretic hormone gene. *Biochem. Biophys. Res. Commun.* **165**, 1177-1183.

Maeda, S., S. L. Volrath, T. N. Hanzlik, S. A. Harper, K. Majima, D. W. Maddox, B. D. Hammock and E. Fowler (1991) Insecticidal effects of an insect-specific neurotoxin expressed by a recombinant baculovirus. *Virology* **184**, 777-780.

Marthens, J. W. M., G. Honee, D. Zuidema, J. W. M. van Lent, B. Visser and J. M. Vlak (1990) Insecticidal activity of a bacterial crystal protein expressed by a recombinant baculovirus in insect cells. *Appl. Environ. Microbiol.* **56**, 2764-2770.

Martin, B. M., A. N. Ramirez, G. B. Gurrola, M. Nobile, G. Prestipino and L. D. Possani (1994) Novel K(+)-channel-blocking toxins from the venome of the scorpion *Centruroides limpidus limpidus* Karsch. *Biochem. J.* **304**, 51-56.

McCutchen, B. F., P. V. Choudary, R. Crenshaw, D. Maddox, S. G. Kamita, N. Palekar, S. Volrath, E. Fowler, B. D. Hammock and S. Maeda (1991) Development of a recombinant baculovirus expressing an insect-selective neurotoxin: potential for pest control. *Bio/Technology* **9**, 848-853.

McMaster, G. K. and G. G. Carmichael (1977) Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.

Merryweather, A. T., U. Weyer, M. P. G. Harris, M. Hirst, T. Booth and R. D. Possee (1990) Construction of a genetically engineered baculovirus insecticides containing the *Bacillus thuringiensis* ssp. *kurstaki* HD-73 delta endotoxin. *J. Gen. Virol.* **71**, 1535-1544.

O'Reilly, D. R., L. K. Miller and V. A. Luckow (1992) Baculovirus Expression Vectors: A Laboratory Manual. W. H. Freeman & Co., New York.

Ornberg, R. L., T. Smyth Jr, and A. W. Benton (1976) Isolation of a neurotoxin with a presynaptic action from the venom of the black widow spider (*Latrodectus mactans* Fabr.). *Toxicon* **14**, 329-333.

Pennock, G. D., C. Shoemaker and L. K. Miller (1984) Strong and regulated expression of *Escherichia coli* beta-galactosidase in insect cells with a baculovirus vector. *Mol. Cell. Biol.* **4**, 399-406.

Quistad, G. B., C. C. Reuter, W. S. Skinner, P. A. Dennis, S. Suwanrumpha and E. W. Fu (1991) Paralytic and insecticidal toxins from the funnel web spider, *Hololena curta*. *Toxicon* **29**, 329-336.

Schweitz, H., T. Bruhn, E. Guillemare, D. Moinier, J. M. Lancelin, L. Beress and M. Lazdunski (1995) Kalicludines and kaliseptine. Two different classes of sea anemone toxins for voltage sensitive K+ channels. *J. Biol. Chem.* **270**, 25121-25126.

Skinner, W. S., P. A. Dennis, J. P. Li and G. B. Quistad (1992) Identification of insecticidal peptides from venom of the trap-door spider, *Aptostichus schlingeri* (Ctenizidae). *Toxicon* **30**, 1043-1050.

Smith, G. E., M. D. Summers and M. J. Fraser (1983) Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Mol. Cell. Biol.* **3**, 2156-2165.

Stewart, L. M. D., M. Hirst, M. L. Ferber, A. T. Merryweather, P. J. Cayley and R. D. Possee (1991) Construction of an improved baculovirus insecticide containing an insect-specific toxin gene. *Nature* **352**, 85-88.

Tomalski, M. D. and L. K. Miller (1991) Insect paralysis by baculovirus-mediated expression of a mite neurotoxin gene. *Nature* **352**, 82-84.