Int. J. Indust. Entomol. Vol. 9, No. 2, 2004, pp. 217~223

International Journal of *Industrial Entomology*

Characterization of the *v-cath* Gene of *Bombyx mori* Nuclear Polyhedrosis Virus K1

Kwang Sik Lee, Jianhong Li†, Yeon Ho Je[1], Soo Dong Woo[2], Hung Dae Sohn and Byung Rae Jin*

College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea.
[1]School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea.
[2]Department of Plant Medicine, Chungbuk National University, Cheongju 361-763, Korea.

(*Received 5 October 2004; Accepted 16 November 2004*)

A cathepsin L-like cysteine protease, *v-cath*, encoded by the baculovirus has been shown to play a role in host liquefaction. We have identified a *v-cath* gene in the silkworm virus, *Bombyx mori* nuclear polyhedrosis virus (BmNPV) K1 strain. The 969 bp *v-cath* has an open reading frame of 323 amino acids. A putative cleavage site and catalytic sites were conserved in BmNPV-K1 *v-cath*. The predicted three-dimensional structure of BmNPV-K1 *v-cath* revealed that the overall fold of BmNPV-K1 *v-cath* is similar to that of other proteases of the papain family. The deduced amino acid sequence of BmNPV-K1 *v-cath* showed 98% and 97% protein sequence identity to BmNPV T3 strain and to *Autographa californica* nuclear polyhedrosis virus, respectively. The BmNPV-K1 *v-cath* differed at 4 amino acid positions from BmNPV T3. The *v-cath* gene in BmNPV-K1 genome is located on the *Eco*RV 6 kb and *Xho*I 9 kb fragments. Northern hybridization analysis of BmNPV K1 *v-cath* gene revealed that it is expressed late in infection.

Key words: Baculovirus, *Bombyx mori* nuclear polyhedrosis virus, Cathepsin L-like cysteine protease (*v-cath*), Insect cells

*To whom correspondence should be addressed.
College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea. Tel: +82-51-200-7594; Fax: +82-51-200-7594; E-mail: brjin@daunet.donga.ac.kr

†Present address: College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P. R. China

Introduction

Baculoviruses possess a large circular DNA genome that replicates in the nuclei of infected cells. During infection, baculovirus genes are expressed in a highly regulated cascade in which early gene expression and viral replication are essential for late and very late gene expression. The baculovirus-infected cells begin to produce budded virus and then, in the late and very late phases of infection, make occlusion-derived virions and polyhedra.

The dispersal of polyhedra and the horizontal transmission of baculovirus infection are facilitated by the liquefaction of the host tissues (Volkman and Keddie, 1990). The host liquefaction depends on the presence of two viral gene products: a chitinase, chiA (Hawtin *et al.*, 1997) and a cathepsin L-like cysteine protease, *v-cath*. A previous report revealed the absence of liquefication in host tissues infected with chiA-deficient mutants, indicating that *v-cath* is not processed properly in the absence of chiA (Hom and Volkman, 2000). The *v-cath* is synthesized as an inactive proenzyme and is activated by proteolytic removal of the inhibitory propeptide yielding the mature protease during infected cell death (Hom *et al.*, 2002).

The *v-cath* encoded by the baculovirus has been shown to play an essential role in the liquefaction of host tissues during a viral infection (Slack *et al.*, 1995; Lanier *et al.*, 1996; Hawtin *et al.*, 1997; Hom and Volkman, 2000; Hom *et al.*, 2002). The *v-cath* has been implicated in the liquefaction process in *Bombyx mori* nuclear polyhedrosis virus (BmNPV)-infected insect larvae (Ohkawa *et al.*, 1994) and a closely related *v-cath* gene in *Autographa californica* nuclear polyhedrosis virus (AcMNPV), with the same function in virus-infected *Trichoplusia ni*, has also been identified (Rawlings *et al.*, 1992; Slack *et al.*, 1995). In addition, the *v-cath* genes from baculovirus also have been identified in *Choristoneura fumiferana* NPV (Hill *et*

al., 1995) and *Orgyia pseudotsugata* NPV (Ahrens *et al.*, 1997). Insects infected with virus mutants lacking either the *chiA* or *v-cath* gene remained intact several days after death (Hawtin *et al.*, 1997).

AcNPV and BmNPV are extensively studied members of baculovirus. These NPVs have been utilized in the studies of virus genetic structure, gene expression, development of baculoviruses as expression vectors of foreign genes, and genetically modified virus insecticides (King and Possee, 1992; O'Reilly *et al.*, 1992; Ayres *et al.*, 1994; Gomi *et al.*, 1999). In BmNPV, T3 strain has been studied extensively (Gomi *et al.*, 1999; Maeda, 1984; Maeda *et al.*, 1985), but Korean strain K1, which is slightly different from the BmNPV T3 in viral genome, is not well understood. The polyhedrin (Woo *et al.*, 1995), *p10* (Kang *et al.*, 1997), *vlf-1* (Park *et al.*, 2000), *ie1* (Park *et al.*, 2001a), *egt* (Park *et al.*, 2001b), and *p35* (Lee *et al.*, 2001) genes from BmNPV-K1 were identified and developed into polyhedrin gene- and *p10* gene-based expression vectors (Woo *et al.*, 1995; Kang *et al.*, 1997).

In this study, we report the characterization of the cathepsin L-like cystein protease, *v-cath* gene from BmNPV-K1. The sequence of BmNPV-K1 *v-cath* presented here was aligned to that of AcNPV (Rawlings *et al.*, 1992; Slack *et al.*, 1995) and BmNPV T3 (Ohkawa *et al.*, 1994). We also examined the *v-cath* mRNA expression in BmNPV-K1-infected *B. mori* cells and the localization of *v-cath* gene in BmNPV-K1 genome.

Materials and Methods

Cells and virus

The *Bombyx mori* 5 (Bm5) (Grace, 1962) cells were grown at 27°C in TC-100 medium (GIBCO BRL LIFE Technologies) supplemented with 10% fetal bovine serum (FBS; GIBCO BRL LIFE Technologies) (O'Reilly *et al.*, 1992). Wild-type *Bombyx mori* nuclear polyhedrosis virus K1 (BmNPV-K1) (Woo *et al.*, 1995; Kang *et al.*, 1997; Park *et al.*, 2000) was propagated in Bm5 cells. The titer was expressed as plaque forming units (PFU) per ml (O'Reilly *et al.*, 1992).

Viral genome isolation and PCR

Polyhedra and viral DNA were obtained from Bm5 cells by standard methods (O'Reilly *et al.*, 1992). Viral DNAs were used as templates. The *v-cath* gene was amplified from viral DNAs using the primers 5-GGAAACGGC-GACCACCAACCACAA-3 and 5-TTAATAAATGACT-GCAGTAGACGC-3, annealing to the translation start region and translation termination region, respectively (Rawlings *et al.*, 1992; Ohkawa *et al.*, 1994; Slack *et al.*, 1995). After 35-cycle amplification (94°C for 1 min; 55°C for 1 min; 72°C for 1 min), PCR product was analyzed by 1% agarose gel electrophoresis.

DNA sequencing and data analysis

The PCR product was purified with PCR purification kit (QIAGEN) following manufacturers instruction and then cloned into pGem-T vector (Promega, Madison, WI). The deletion mutants of *v-cath* gene were constructed using an Exo Mung Bean Deletion Kit (Stratagene, La jolla, CA). DNA sequencing was performed 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 (www.ncbi.nlm.nih.gov/BLAST). MacVector (ver. 6.5, Oxford Molecular Ltd) was used to align the amino acid sequences of *v-cath* genes.

Protein structure modeling

To generate structural models, amino acid sequences of BmNPV-K1 *v-cath* cDNA were submitted to Swiss-Model (Schwede *et al.*, 2003) using the First Approach Method set at default parameters. Homology models were generated using the known structure of the *Homo sapiens* cathepsin F (Protein Data Bank code No. 1m6d), *H. sapiens* cathepsin K (Protein Data Bank code No. 7pck), and *H. sapiens* cathepsin K (Protein Data Bank code No.1by8). Swiss-Pdb viewer version 3.7 was used to generate a three-dimensional image.

Southern blot analysis

Viral DNAs digested with *Eco*RV and *Xho*I were electrophoresed through a 1.0% agarose gel as described previously (O'Reilly *et al.*, 1992). The DNA from the gel was transferred onto a nylon blotting membrane (Schleicher & Schuell, Dassel, Germany) and hybridized at 42°C with a probe in a hybridization buffer containing 5 × SSC, 5 × Denhardts solution, 0.5% SDS, and 100 μg/ml denatured salmon sperm DNA. The 969 bp BmNPV *v-cath* gene was labeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL) using the Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, CA) for use as a probe for hybridization. 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 exposed to autoradiography film.

RNA isolation and Northern blot analysis

Total cellular RNA was isolated from mock-infected or wild-type BmNPV-infected Bm5 cells. A total of 1×10^6

cells per 35-mm-diameter dish was infected at a multiplicity of infection of 5 PFU per cell. Cells were collected at 12, 24, 36, 48, 60, and 72 hrs postinfection (p.i.). Total cellular RNA was isolated using Total RNA extraction kit (Promega). Total cellular RNA (10 μg per lane) from the infected cells was denatured by glyoxalation (McMaster and Carmichael, 1977), transferred onto a nylon blotting membrane (Schleicher & Schuell) and hybridized at 42°C with a probe in a hybridization buffer. Hybridization condition, fragment labeling, and filter washing were as described for the Southern blot analysis.

Results and Discussion

Cloning, sequencing, and molecular modeling of BmNPV-K1 *v-cath*

When the nucleotide sequences of the BmNPV T3 (Gomi *et al.*, 1999) and AcNPV genomes (Ayres *et al.*, 1994) were compared, ORFs were highly conserved (over 90% identity). The average amino acid sequence identity between homologous ORFs was about 93% (Gomi *et al.*, 1999). To identify *v-cath* gene in BmNPV-K1, therefore, we have employed PCR by designing primer set based on the conserved region of *v-cath* of AcNPV and BmNPV T3 (Fig. 1A). The expected amplified PCR product was amplified in BmNPV-K1 (Fig. 1B). As shown in Fig. 1, the molecular size of the product in BmNPV-K1 was identical to that expected. The PCR product for sequencing was cloned.

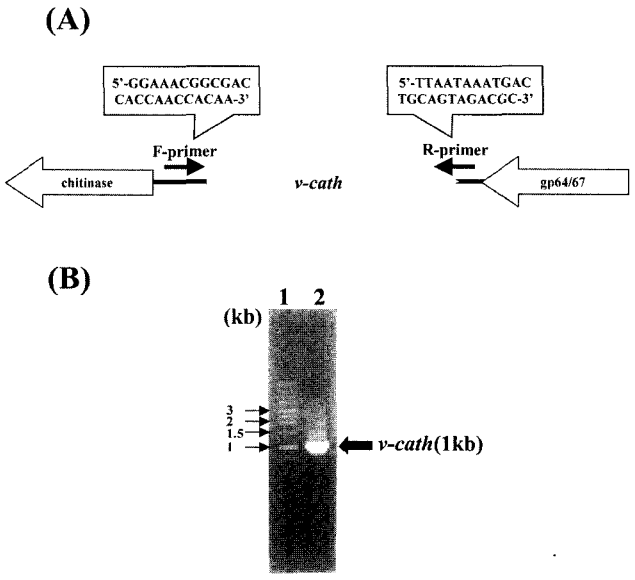

Fig. 1. PCR of *v-cath* gene from BmNPV-K1. The PCR primers for identification of BmNPV-K1 *v-cath* were based on the previously identified *v-cath* of AcNPV and BmNPV T3 (A). The amplified PCR product was analyzed by 1% agarose gel electrophoresis (B). Lane 1, molecular size marker; lane 2, BmNPV-K1 *v-cath*. Arrow indicates the amplified *v-cath* from BmNPV-K1.

The nucleotide sequence of PCR product was analyzed and its amino acid was deduced. As the result of the complete nucleotide and deduced amino acid sequences (GenBank accession number; AY817140) in Fig. 2, the *v-cath* of 969 bp has an open reading frame of 323 amino acids with a predicted molecular weight of about 36 kDa.

The deduced amino acid sequence of BmNPV-K1 *v-cath* was compared with those of baculoviruses including AcNPV and BmNPV T3, respectively (Fig. 3). Based on reported post-translational cleavage sites in propapain and procathepsin L (Gal and Gottesman, 1988; Vernett *et al.*, 1990), a putative cleavage site was previously identified at Pro^{113} in AcMNPV *v-cath* (Slack *et al.*, 1995) and C. fumiferana MNPV (Hill *et al.*, 1995). This residue is very well conserved in BmNPV-K1 *v-cath* as well as in other baculovirus *v-cath* aligned. The amino acids which con-

```
1   ATGAACAAAATTTTGTTTTATTTGTTTGTGTACGCCGTTGTAAAGAGCGCGGCCTACGAT
1    M  N  K  I  L  F  Y  L  F  V  Y  A  V  V  K  S  A  A  Y  D

61  CCTTTGAAAGCGCCTAATTATTTTGAAGAATTTGTTCATCGATTCAACAAAAATTATAGT
21   P  L  K  A  P  N  Y  F  E  E  F  V  H  R  F  N  K  N  Y  S

121 AGCGAAGTTGAAAAATTGCGAAGATTCAAAATTTTCCAACACAATTTAAATGAAATTATC
41   S  E  V  E  K  L  R  R  F  K  I  F  Q  H  N  L  N  E  I  I

181 AATAAAAACCAAAACGATTCGGCCAAATATGAAATAAACAAATTCTCGGATTTGTCCAAA
61   N  K  N  Q  N  D  S  A  K  Y  E  I  N  K  F  S  D  L  S  K

241 GACGAAACTATCGCAAAATACACAGGTCTGTCTTTGCCTACTCAGACTCAAAATTTTTGC
81   D  E  T  I  A  K  Y  T  G  L  S  L  P  T  Q  T  Q  N  F  C

301 AAGGTCATACTCTTAGACCAGCCGCCGGGTAAAGGGCCCCTTGAATTTGACTGGCGTCGT
101  K  V  I  L  L  D  Q  P  P  G  K  G  P  L  E  F  D  W  R  R

361 CTCAACAAAGTCACTAGCGTAAAAAATCAGGGCATGTGTGGCGCCTGCTGGGCGTTTGCC
121  L  N  K  V  T  S  V  K  N  Q  G  M  C  G  A  C  W  A  F  A

421 ACTCTGGCTAGTTTGGAAAGTCAATTTGCAATCAAACATAACCAGTTGATTAATCTGTCG
141  T  L  A  S  L  E  S  Q  F  A  I  K  H  N  Q  L  I  N  L  S

481 GAGCAGCAAATGATCGATTGTGATTTTGTCGACGCTGGCTGTAACGGCGGCTTGTTGCAC
161  E  Q  Q  M  I  D  C  D  F  V  D  A  G  C  N  G  G  L  L  H

541 ACAGCGTTCGAAGCCATCATTAAAATGGGCGGCGTACAGCTGGAAAGCGACTATCCATAC
181  T  A  F  E  A  I  I  K  M  G  G  V  Q  L  E  S  D  Y  P  Y

601 GAAGCAGACAATAACAATTGCCGTATGAACTCCAATAAGTTTCTAGTTCAAGTAAAAGAT
201  E  A  D  N  N  N  C  R  M  N  S  N  K  F  L  V  Q  V  K  D

661 TGTTATAGATACATTACCGTGTACGAGGAAAAACTTAAAGATTTGTTACGCCTTGTCGGC
221  C  Y  R  Y  I  T  V  Y  E  E  K  L  K  D  L  L  R  L  V  G

721 CCTATTCCTATGGCCATAGACGCTGCCGACATTGTTAACTATAAACAGGGTATTATAAAA
241  P  I  P  M  A  I  D  A  A  D  I  V  N  Y  K  Q  G  I  I  K

781 TATTGTTTCGACAGCGGTCTAAACCATGCGGTTCTTTTAGTGGGTTATGGTGTTGAAAAC
261  Y  C  F  D  S  G  L  N  H  A  V  L  L  V  G  Y  G  V  E  N

841 AACATTCCATATTGGACCTTTAAAAACACTTGGGGCACGGATTGGGGAGAGGACGGATTT
281  N  I  P  Y  W  T  F  K  N  T  W  G  T  D  W  G  E  D  G  F

901 TTCAGGGTACAACAAAACATAAACGCCTGTGGTATGAGAAACGAACTTGCGTCTACTGCA
301  F  R  V  Q  Q  N  I  N  A  C  G  M  R  N  E  L  A  S  T  A

961 GTCATTTATTAA
321  V  I  Y  *
```

Fig. 2. The nucleotide and deduced amino acid sequences of the BmNPV-K1 *v-cath* gene. The start codon of ATG is boxed and the termination codon is shown by asterisk. The BmNPV-K1 *v-cath* sequence has been deposited in GenBank under accession number AY817140.

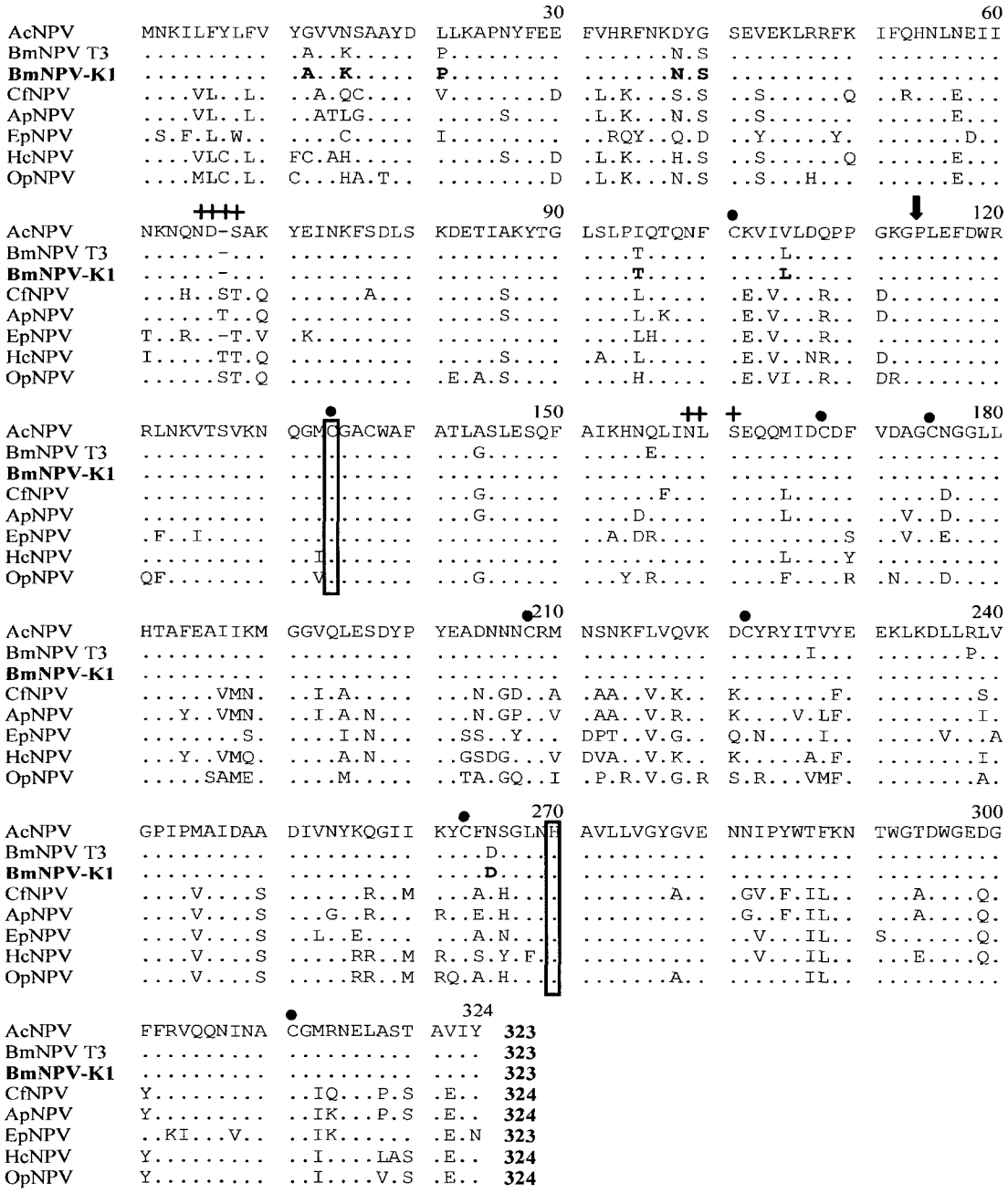

Fig. 3. Alignment of the amino acid sequence of BmNPV-K1 *v-cath* with known baculovirus *v-cath*. Residues are numbered according to the aligned baculovirus *v-cath* sequences, and invariant residues are dotted. Gap (-) is introduced to improve alignment. The proline (P) residue at the predicted preproenzyme cleavage site is indicated by an arrow and the two conserved residues (C and H) which are included in the catalytic site of other members of the papain family are boxed. The eight conserved cysteine residues are circled. Two potential N-liked glycosylation sites are crossed. The abbreviation and GenBank accession number for the *v-cath* sequences aligned are: BmNPV-K1, *Bombyx mori* NPV K1 strain (AY817140; this study); BmNPV T3, *B. mori* NPV T3 strain (L33180); AcNPV, *Autographa californica* NPV (M67451); CfMPV, *Choristoneura fumiferana* NPV (M97906); ApNPV, *Anthetaea pernyi* NPV (AB072731); EpNPV, *Epiphyas postvittana* NPV (AY043265); HcNPV, *Hyphantria cunea* NPV (AF120926); OpNPV, *Orgyia pseudotsugata* NPV (U75930).

stitute the catalytic sites of other members of the papain family are Cys and His, and these are also conserved in the baculovirus *v-cath* amino acid sequences (Hill *et al.*, 1995). In addition, eight cysteine residues and two poten-

Percent similarity

Species	GenBank No.	1	2	3	4	5	6	7	8
1. **BmNPV-K1**	AY817140		98	97	89	88	87	89	87
2. BmNPV T3	L33180	89		96	89	88	86	88	87
3. AcNPV	M67451	97	96		90	89	88	89	88
4. CfNPV	M97906	78	78	79		95	87	91	91
5. ApNPV	AB072731	78	78	79	90		89	92	88
6. EpNPV	AY043265	78	77	79	74	75		87	87
7. HcNPV	AF120926	77	76	77	85	85	74		89
8. OpNPV	U75930	75	75	76	82	80	72	81	

Percent identity

Fig. 4. Pairwise identities and similarities of the deduced amino acid sequence of BmNPV-K1 *v-cath* among baculovirus *v-cath* sequences. The abbreviation and GenBank accession number for the *v-cath* sequences aligned are described in Fig. 3 legend.

tial N-linked glycosylation sites are well conserved in the baculovirus *v-cath* aligned. One of these cysteine residues was involved in the active site cysteine residue (Hill *et al.*, 1995; Sivaraman *et al.*, 2000).

When the BmNPV-K1 *v-cath* and BmNPV T3 *v-cath* are aligned, the deduced amino acids sequence showed 98% identity to each other (Fig. 4). The deduced amino acid residues of BmNPV-K1 *v-cath* differed at 4 positions (144, 156, 227 and 238) from BmNPV T3. In addition, the deduced amino acid sequence of the BmNPV-K1 *v-cath* showed 97% protein sequence identity to that of AcNPV, demonstrating a high identity among them (Rawlings *et al.*, 1992; Ohkawa *et al.*, 1994; Slack *et al.*, 1995). BmNPV-K1 *v-cath* differed at 8 amino acid positions from AcNPV. While the deduced amino acid sequence of BmNPV-K1 *v-cath* showed 75%-78% protein sequence identity to known baculovirus *v-cath* genes excluding BmNPV T3 and AcNPV.

Fig. 5 shows the BmNPV-K1 *v-cath* model, which consists of a ββαβαααββαββββββ structural motif. The active site Cys and His are present in the substrate binding site. The structure of catenpsin was intensively studied in human cathepsins and has been solved as a globular protein (Sivaraman *et al.*, 2000). The overall fold of the BmNPV-K1 *v-cath* is similar to that of other proteases of the papain family.

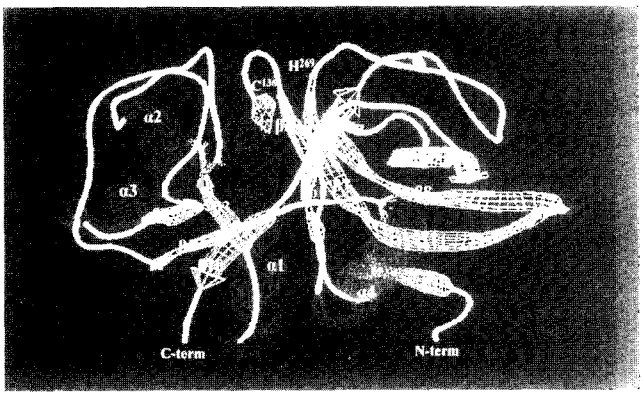

Fig. 5. Predicted three-dimensional structure of BmNPV-K1 *v-cath*. The active site Cys136 and His269 are colored yellow. The four α-helices and ten β-strands are indicated. N-term, N-terminus; C-term, C-terminus.

Localization of *v-cath* gene in BmNPV-K1 genome

The location of *v-cath* gene in the BmNPV-K1 genome was confirmed by Southern blot analysis. BmNPV-K1 genome was digested with EcoRV and XhoI, and probed with the PCR-amplified *v-cath* (Fig. 6). The *v-cath* in

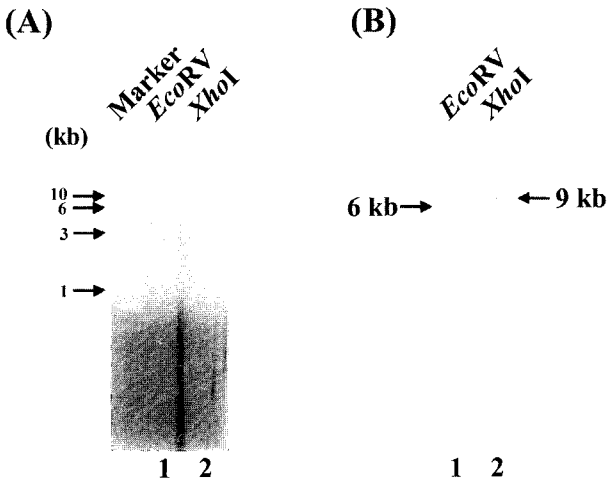

Fig. 6. Southern blot analysis of BmNPV-K1 genome. Viral DNAs digested with *Eco*RV (lane 1) and *Xho*I (lane 2) were electrophoresed through a 1.0% agarose gel (A) and hybridized at 42°C with a labeled probe (B). The probe used to detect DNA fragment containing *v-cath* was a 1.0 kb BmNPV-K1 *v-cath* amplified by PCR in this study. Hybridized bands are indicated by arrow with molecular size.

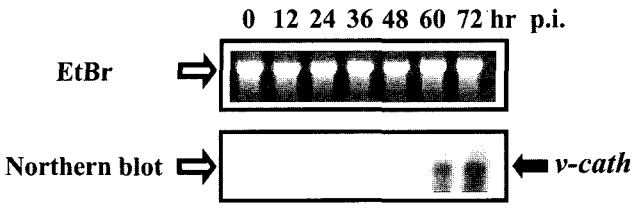

Fig. 7. Northern blot analysis of *v-cath* transcripts from BmNPV-K1-infected cells. Total RNA was collected from Bm5 cells at various times p.i. as indicated at the top of each lane. Total RNA was separated by 1.0% formaldehyde agarose gel electrophoresis (upper panel), transferred on to a nylon membrane, and hybridized with the appropriate radiolabelled 1.0 kb BmNPV-K1 *v-cath* probe (lower panel). Transcripts are indicated by arrow on the right side of the panel.

BmNPV-K1 genome was located on the 6 kb EcoRV fragment and 9 kb XhoI fragment.

Expression of *v-cath* gene in BmNPV-K1-infected *B. mori* cells

To verify whether the *v-cath* transcripts were correlated with virus replication, we examined the virus-infected cells by Northern blot analysis with *v-cath* probe (Fig. 7). Total cellular RNA purified from Bm5 cells 12, 24, 36, 48, 60 and 72 hrs p.i. with wild-type BmNPV-K1 was hybridized with an excess of probe. As shown in Fig. 6, *v-cath* transcripts were clearly detected at 48 hrs p.i., and maximally observed from 60 hrs p.i. This result is similar to the previous result that the AcNPV *v-cath* was first detected in lysates of infected cells at 22 hrs p.i. and its expression level was significantly increased from 48 hrs p.i. (Hom *et al.*, 2002). In C. fumiferana MNPV, a single transcript for *v-cath*, initiating at 26 nucleotides was detected from 2 – 7 days p.i. (Hill *et al.*, 1995).

In conclusion, we reported a novel *v-cath* gene from the BmNPV-K1. Knowledge of the *v-cath* in this study will provide the genetic information for establishing BmNPV-K1 strain.

Acknowledgements

This work was supported by a grant from BioGreen21 Program, Rural Development Administration, Republic of Korea.

References

Ahrens, C. H., R. L. Russell, C. J. Funk, J. T. Evans, S. H. Harwood and G. F. Rohrmann (1997) The sequence of the *Orgyia pseudotsugata* multinucleocapsid nuclear polyhedrosis virus genome. *Virology* **229**, 381-399.

Ayres, M. D., S. C. Howard, J. Kuzio, M. Lopez-Ferber and R. D. Possee (1994) The complete DNA sequence of the *Autographa californica* nuclear polyhedrosis virus. *Virology* **202**, 586-605.

Gal, S. and M. M. Gottesman (1988) Isolation and sequence of a cDNA for human pro-(cathepsin L). *Biochem. J.* **253**, 303-306.

Gomi, S., K. Majima and S. Maeda (1999) Sequence analysis of the genome of *Bombyx mori* nucleopolyhedrovirus. *J. Gen. Virol.* **80**, 1323-1337.

Grace, T. C. D. (1962) Establishment of four strains of cells from insect tissue grown in vitro. *Nature* **195**, 788-789.

Hawtin, R. E., T. Zarkowska, K. Arnold, C. J. Thomas, G. W. Gooday, L. A. King, J. A. Kuzio and R. D. Possee (1997) Liquefaction of *Autographa californica* nucleopolyhedrovirus-infected insect is dependent on the integrity of virus-encoded chitinase and cathepsin genes. *Virology* **238**, 243-253.

Hill, J. E., J. Kuzio and P. Faulkner (1995) Identification and characterization of the *v-cath* gene of the baculovirus, CfMNPV. *Biochim. Biophys. Acta* **1264**, 275-278.

Hom, L. G., T. Ohkawa, D. Trudeau and L. E. Volkman (2002) *Autographa californica* M nucleopolyhedrovirus Pro*V-CATH* is activated during infected cell death. *Virology* **296**, 212-218.

Hom, L. G. and L. E. Volkman (2000) *Autographa californica* M nucleopolyhedrovirus chiA is required for processing of *V-CATH*. *Virology* **277**, 178-183.

Kang, S. W., B. R. Jin, E. Y. Yun, S. H. Kim, K. Y. Kim and S. K. Kang (1997) Construction of the novel baculovirus transfer vector using the p10 gene of BmNPV. *Korean J. Seric. Sci.* **39**, 180-185.

King, L. A. and R. D. Possee (1992) The baculovirus expression system. A laboratory giude. Chapman & Hall. London.

Lanier, L. M., J. M. Slack and L. E. Volkman (1996) Actin binding and proteolysis by the baculovirus AcMNPV: The role of virion-associated *V-CATH*. *Virology* **216**, 380-388.

Lee, K. S., H. J. Park, Y. H. Je, H. D. Sohn and B. R. Jin (2001) Molecular cloning of the antiapoptotic gene, *p35*, from *Bombyx mori* nuclear polyhedrosis virus K1. *Int. J. Indust. Entomol.* **3**, 25-29.

Maeda, S. (1984) A plaque assay and cloning of *Bombyx mori* nuclear polyhedrosis virus. *J. Seric. Sci. Jpn* **53**, 547-548.

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

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.

Ohkawa, T., K. Majima and S. Maeda (1994) A cysteine proteinase encoded by the baculovirus *Bombyx mori* nuclear

polyhedrosis virus. *J. Virol.* **68**, 6619-6625.

O'Reilly, D. R., L. K. Miller and V. A. Luckow (1992) Baculovirus expression vectors: a laboratory manual. W. H. Freeman & Co., New York.

Park, H. J., K. S. Lee, E. S. Cho, E. Y. Yun, S. W. Kang, K. Y. Kim, H. D. Sohn and B. R. Jin (2000) Molecular cloning and characterization of very late expression factor 1 gene, *vlf-1* from *Bombyx mori* nuclear polyhedrosis virus K1. *Int. J. Indust. Entomol.* **1**, 29-33.

Park, H. J., K. S. Lee, Y. H. Je, H. D. Sohn and B. R. Jin (2001a) Molecular cloning and sequence analysis of the immediate early I gene, *IE1*, from *Bombyx mori* nuclear polyhedrosis virus K1. *Int. J. Indust. Entomol.* **3**, 43-49.

Park, H. J., E. H. Chung, K. S. Lee, J. H. Han, S. J. Lee, H. D. Sohn and B. R. Jin (2001b) Molecular cloning and sequencing of the ecdysteroid UDP-glucosyltransferase, *EGT*, from *Bombyx mori* nuclear polyhedrosis virus K1. *Int. J. Indust. Entomol.* **3**, 37-41.

Rawlings, N. D., L. H. Pearl and D. J. Buttle (1992) The baculovirus *Autographa californica* nuclear polyhedrosis virus genome includes a papain-like sequence. *Biol. Chem. Hoppe-Seyler* **373**, 1211-1215.

Schwede, T., J. Kopp, N. Guex and M. C. Peitsch (2003) SWISS-MODEL: an automated protein homology-modeling server. *Nucl. Acids Res.* **31**, 3381-3385.

Sivaraman, J., D. K. Nagler, R. Zhang, R. Menard and M. Cygler (2000) Crystal structure of human procathepsin X: A cysteine protease with the proregion covalently linked to the active site cysteine. *J. Mol. Biol.* **295**, 939-951.

Slack, J. M., J. Kuzio and P. Faulkner (1995) Characterization of *v-cath*, a cathepsin L-like proteinase expressed by the baculovirus *Autographa californica* multiple nuclear polyhedrosis virus. *J. Gen. Virol.* **76**, 1091-1098.

Vernett, T., D. C. Tessier, C. Richardson, F. Laliberte, H. E. Khouri, A. W. Bell, A. C. Storer and D. Y. Thomas (1990) Secretion of functional papain precursor from insect cells. Requirement for N-glycosylation of the pro-region. *J. Biol. Chem.* **265**, 16661-16670.

Woo, S. D., W. J. Kim, B. R. Jin and S. K. Kang (1995) Construction of new transfer vector of nuclear polyhedrosis virus of the silkworm, *Bombyx mori*. *Korean J. Seric. Sci.* **37**, 46-51.