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Molecular Cloning and Sequencing of the Ecdysteroid UDP-Glucosyltransferase Gene, *EGT*, from *Bombyx mori* Nuclear Polyhedrosis Virus K1

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The ecdysteroid UDP-glucosyltransferase (*egt*) gene isolated from *Bombyx mori* nuclear polyhedrosis virus (BmNPV) K1 strain was compared to its homologue from *Autographa californica* NPV (AcNPV) and Bm NPV T3. The *egt* gene of BmNPV-K1 encoded 506 amino acid open reading frame, and was 99.6% identical at the amino acid level and 99.2% identical at the nucleotide level to BmNPV T3. The BmNPV-K1 *egt* gene showed highly identity to AcNPV and BmNPV T3 strain. The BmNPV-K1 *egt* gene was different from amino acid sequence at 2 positions, 19 and 72, in BmNPV T3. The genomic location of *egt* gene in the BmNPV-K1 was confirmed by Southern blot analysis and its expression patterns at the transcriptional level in the infected cells were confirmed by Northern hybridization analysis. Transcripts of the *egt* of Bm NPV-K1 peaked around 12 hrs postinfection (p.i.) and reduced at 24 hrs p.i.

Key words : Baculovirus, *Bombyx mori* nuclear polyhedrosis virus, Ecdysteroid UDP-glucosyltransferase gene (*egt*)

Introduction

In insects, the moulting hormone ecdysone initiates and regulates a cascade fashion of gene expressed during moulting. Baculoviruses are naturally occurring pathogens which are generally insect specific. Baculoviruses have evolved mechanisms to delay normal larval moulting in lepidopteran insects. It has been reported that, during virus replication, infected insects fail to moult (O'Reilly and Miller, 1989; Burand and Park, 1992). This effect has been attributed to the activity of a virus-encoded enzyme, an ecdysteroid UDP-glucosyltransferase (EGT) (O'Reilly and Miller, 1989). The egt genes from baculovirus have been identified in *Autographa californica* nuclear polyhedrosis virus (AcNPV), *Bombyx mori* NPV (BmNPV), *Choristoneura fumiferana* NPV (CfNPV), *Lymantria dispar* NPV (LdNPV), *Spdoptera exigua* NPV (SeNPV) (Barrett *et al.*, 1995; O'Reilly and Miller, 1989; Riegel *et al.*, 1994; Gomi *et al.*, 1999).

The baculovirus egt gene encodes the enzyme EGT which catalyses the transfer of a sugar moiety from UDP-sugars to ecdysteroids (O'Reilly and Miller, 1989). The EGT catalyses the conjugation of ecdysone and UDP-glucose, and results in the clearing of ecdysone from the hemolymph. The direct result is a delay in moulting so that virus-infected larvae feed longer and cause greater foliar damage. Since the discovery of the *egt* gene in the baculovirus, a significant amount of information has been obtained on the gene structure and function of EGT (Barrett *et al.*, 1995; O'Reilly and Miller, 1989,1990; O'Reilly *et al.*, 1991, 1992). Insects infected with the genetic engineered baculovirus egt virus fed less and died more rapidly than those infected with unaltered baculovirus (Kang *et al.*, 1998; O'Reilly and Miller, 1991). These results demonstrated that alteration in the expression of *egt* in baculoviruses, either through deletion or inactivation, might also provide a more effective biological control insecticide.

AcNPV and BmNPV are widely studied members of baculovirus. These NPVs have been utilized in studies of the virus genetic structure, gene expression, development of baculoviruses as expression vectors of foreign genes, and genetically modified virus insecticides (Ayres *et al.*, 1994; Gomi *et al.*, 1999; King and Possee, 1992; Maeda *et al.*, 1985; O'Reilly *et al.*, 1992; Smith *et al.*, 1983). In

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BmNPVs, one of well-known strains is a BmNPV T3, which was originally isolated by Maeda (1984). The BmNPV T3 strain has been studied extensively (Gomi *et al.*, 1999; 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), *ie1* (Park *et al.*, 2001), *vlf-1* (Park *et al.*, 2000), *p35* (Lee *et al.*, 2001) genes from BmNPV-K1 were identified and developed polyhedrin gene- and *p10* gene-based expression vectors (Kang *et al.*, 1997; Woo *et al.*, 1995).

In this study, we have cloned and characterized the *egt* gene from BmNPV-K1. The sequence of BmNPV-K1 *egt* gene presented here was aligned to that of AcNPV and BmNPV T3.

Materials and Methods

Cells and virus

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

Viral genome isolation and polymerase chain reaction (PCR)

Polyhedra and viral DNA of BmNPV-K1 were obtained from Bm5 cells by standard methods (OReilly *et al.*, 1992). Viral DNAs were used as templates. The *egt* gene was amplified from viral DNAs using the primers 5-CAGTACAGTTATTCGGGTTGAAGC-3 and 5-GAGTGGTTGGTCTCGTCAGTA-3, annealing to the translation start region and translation termination region respectively (Gomi *et al.*, 1999; O'Reilly and Miller, 1989). 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

The PCR product was purified with PCR purification kit (QIAGEN) following manufacturers instruction and then cloned into pGem-T vector (Promega). The deletion mutants of egt gene were constructed using an Exo Mung Bean Deletion Kit (Stratagene). DNA sequencing was performed using an automatic sequencer (model 310 Genetic Analyzer; Perkin-Elmer Applied Biosystems, Foster City, CA). Sequence alignment was performed using IBI MacVector (ver. 6.5).

Southern blot analysis

Viral DNAs digested with *Pst*I and *Eco*RV were electrophoresed through a 1.0% agarose gel as described previously (OReilly *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, 50% formamide, 0.1% (W/V) *N*-lauroylsarcosine, 0.02% sodium dodecyl sulphate (SDS) and 2% blocking agent (Boehringer Mannheim, Mannheim, Germany). The probe used to detect DNA fragment containing *egt* was about 1.5 kb BmNPV-K1 *egt* amplified by PCR in this study. The probe was radiolabeled with [α-^{32}P] dCTP (Amersham, Arlington Heights, IL). 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.

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

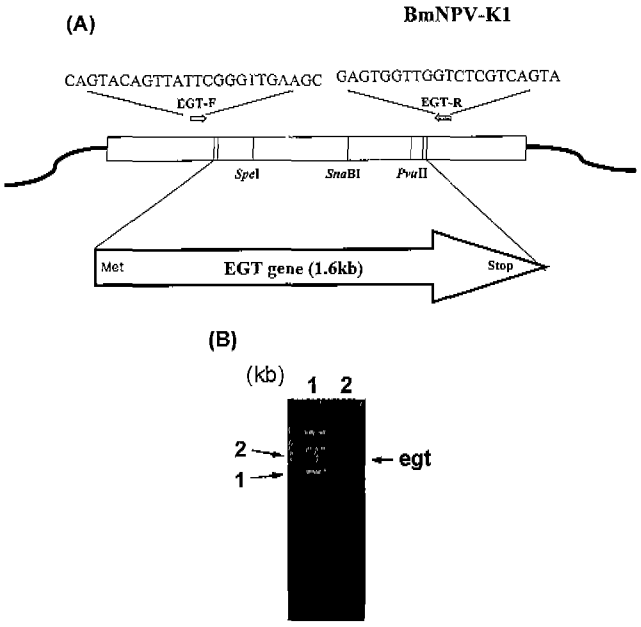

Fig. 1. PCR of *egt* gene from BmNPV-K1. The PCR primers for identification of BmNPV-K1 *egt* were based on the previously identified *egt* genes of AcNPV and BmNPV T3 (A). The amplified PCR product was analyzed by 1% agarose gel electrophoresis (B). Lane 1, molecular size marker; lane 2, Bm NPV-K1.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

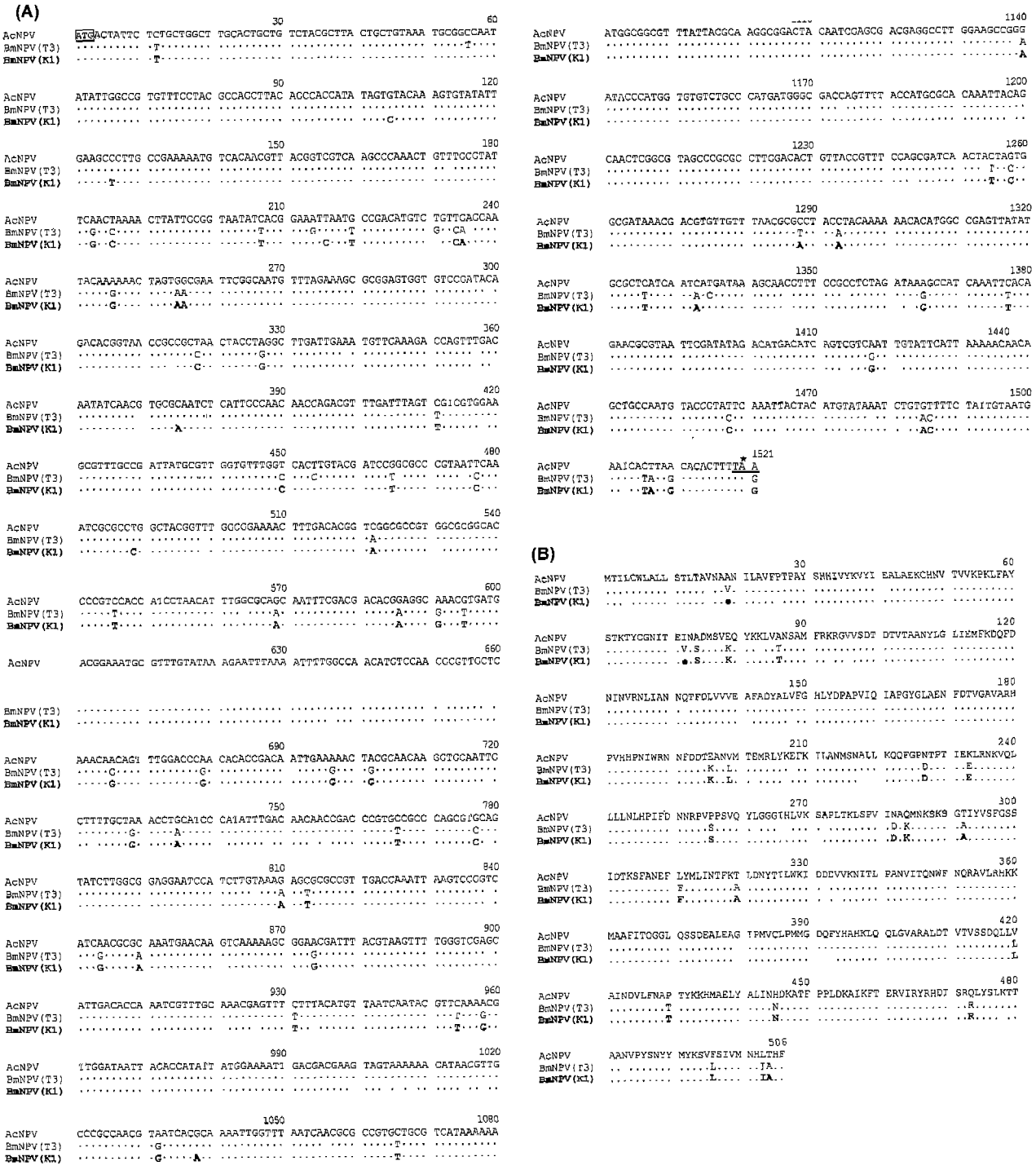

Fig. 2. Nucleotide (A) and deduced amino acid (B) sequences of BmNPV-K1 *egt*. The sequences of BmNPV-K1 were compared with those of AcNPV and BmNPV T3. The translation initiation codon (open box) and translation termination codon (asterisk) of *egt* gene are indicated. The differences among BmNPV-K1, AcNPV and BmNPV T3 sequences are indicated in boldface at nucleotide and amino acid sequence positions. Identical sequences are indicated by dots below the AcNPV sequence. The sequence of BmNPV-K1 has been deposited in GenBank (Accession number AY048771).

at 4, 8, 12, 16, 20, and 24 hrs postinfection (p.i.). Total cellular RNA was isolated using Total RNA extraction kit (Promega). Total cellular RNA (10 μg per lane) from 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 buffer containing 2 × PIPES, 50% formamide, 1% SDS and blocking agent (Boehringer Mannheim). The probe used to detect *egt* transcripts was about 1.5 kb BmNPV-K1 *egt* amplified by PCR in this study. The probe was radiolabeled with [α-^{32}P] dCTP (Amersham). The other procedures for washing the membrane filter and exposing *X*-ray film were performed as in the Southern blot analysis described above.

Results and Discussion

The genome of AcNPV and BmNPV T3 was sequenced and analyzed (Ayres *et al.*, 1994; Gomi *et al.*, 1999). 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 at nucleotide level and about 93% at amino acid level. To identify *egt* gene in BmNPV-K1, therefore, we have employed PCR based on the conserved region of the *egt* of AcNPV and BmNPV T3 so far known (Fig. 1A). The amplified PCR product, as expected, was observed in BmNPV-K1 (Fig. 1B). As the result of agarose gel electrophoresis analysis, the molecular size (about 1.5 kb) of the amplified product in BmNPV-K1 was identical to that expected. The PCR product was cloned and then sequenced.

The nucleotide sequence of PCR product was analyzed and its amino acid was deduced. As the result of the complete nucleotide sequence (GenBank accession number; AY048771) in Fig. 2, the 1,518 bp sequence of this region revealed an open reading frame that could encode a 57 kDa protein of 506 amino acids. The nucleotide and deduced amino acid sequences of BmNPV-K1 *egt* gene were compared with those of AcNPV and BmNPV T3. When the BmNPV-K1 *egt* and BmNPV T3 *egt* are aligned, nucleotide and amino acids sequence homologies of 99.2% and 99.6% are respectively observed (Table 1 and 2). The nucleotide sequences of BmNPV-K1 *egt* were different from 12 positions in BmNPV T3. In addition, BmNPV-K1 *egt* was different from amino acid sequence at 2 positions, 19 and 72, in BmNPV T3. The sequences of the nucleotide and amino acid of BmNPV-K1 *egt* gene are 95.9% and 96% identical to those of AcNPV. The sequences of BmNPV-K1 *egt* gene showed highly identity to AcNPV and Bm NPV T3 strain (Gomi *et al.*, 1999; O'Reilly and Miller, 1989). High identity among baculovirus *egt* genes was indicated in phylogenetic relationships that one group was composed of AcNPV and BmNPV and the other group included SeNPV and LdNPV (Barrett *et al.*, 1995).

The genomic localization of *egt* gene in the BmNPV-K1 was confirmed by using Southern blot analysis. BmNPV-K1 genome was extracted, digested with *Pst*I and *Eco*RV, and probed with amplified *egt* gene (Fig. 3). The Southern hybridization result showed that the entire BmNPV-K1 *egt* encoding region was located on the 3.6 kb *Pst*I fragment and 12 kb *Eco*RV fragment in the genome.

To verify whether the *egt* transcripts were correlated with virus replication, we examined Northern blot analysis with egt probe (Fig. 4). Total cellular RNA purified from Bm5 cells 4, 8, 12, 18, 24, 36 and 48 hrs p.i. with wild-type BmNPV-K1 was hybridized with an excess of

Table 1. Alignment of the nucleotide sequence of the *egt* coding region from BmNPV-K1

	1	2	3
AcNPV	-	4.01	4.07
BmNPV T3	61	-	0.79
BmNPV-K1	62	12	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

Table 2. Alignment of the amino acid sequence of the *egt* coding region from BmNPV-K1

	1	2	3
AcNPV	-	4.34	3.95
BmNPV T3	22	-	0.39
BmNPV-K1	20	2	-

Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.

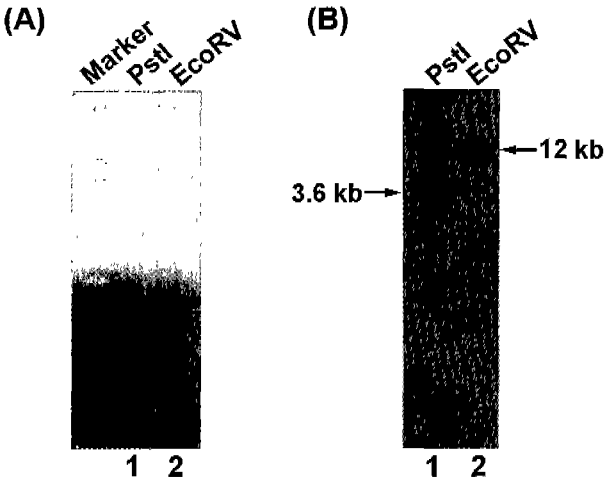

Fig. 3. Southern blot analysis of BmNPV-K1 genome. Viral DNAs digested with *Pst*I (lane 1) and *Eco*RV (lane 2) were electrophoresed through a 1.0% agarose gel (A) and hybridized at 42°C with a labeled probe (B). The probe used to detect DNA fragment containing *egt* was about 1.5 kbp BmNPV-K1 *egt* amplified by PCR in this study. Hybridized bands are indicated by arrow with molecular size.

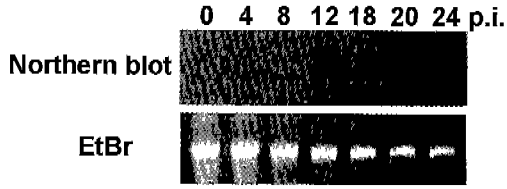

Fig. 4. Northern blot analysis of *egt* transcripts from BmNPV-K1-infected cells. Total RNA was collected from Bm5 cells at various times p.i. as indicated at the top of each lane. The probe used to detect *egt* transcripts was about 1.5 kbp BmNPV-K1 *egt* amplified by PCR in this study.

probe. As shown in Fig. 4, a single transcript of *egt* gene was observed for Bm5 cells infected with BmNPV-K1. Time course analysis indicated that for the BmNPV-K1-infected cells, an *egt* transcript was clearly observed at 8 hrs p.i., peaked around 12 hrs p.i., and then decreased. Thus, this result was consistent with previous result for time course analysis of the *egt* transcript in CfNPV-infected cells (Barrett *et al.*, 1995).

In conclusion, we have cloned a novel *egt* gene from the BmNPV-K1. Knowledge of the *egt* gene in this study will provide the information for establishing BmNPV-K1 strain.

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