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Characterization of *Perina nuda* Nucleopolyhedrovirus (PenuNPV) Polyhedrin Gene

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The *Perina nuda* nucleopolyhedrovirus (PenuNPV) polyhedrin gene was located in *Eco*RI-G (6.3 kilobase pairs; kbp) and *Pst*I-G (4.3 kbp) fragments of its genomic DNA. A portion of 1333 nucleotides (nt) containing this gene was sequenced. An open reading frame of 735 nt encoded a 245-amino-acid-long polyhedrin. A conserved TAAG motif which is associated with transcriptional start sites was identified 51 nt upstream of the translation initiation codon of PenuNPV polyhedrin gene. A putative polyadenylation signal, AATAAA, was found 116 nt downstream of the termination codon (TAA). Comparison of the amino acid sequences of PenuNPV polyhedrin with those of other NPVs showed that PenuNPV poyhedrin was most closely related to *Orgyia pseudotsugata* multiple NPV (OpMNPV) polyhedrin.

KEY WORDS: Polyhedrin gene; *Perina nuda* nucleopolyhedrovirus; baculovirus.

INTRODUCTION

Perina nuda nucleopolyhedrovirus (PenuNPV) is a member of the family Baculoviridae and belongs to the genus Nucleopolyhedrovirus (Murphy *et al.,* 1995). Baculoviruses are large, enveloped, double-stranded DNA viruses with genomes ranging in size from 90 to 160 kbp (kilobase pairs). Baculoviridae have been divided into two genus: Granulovirus (GV) and Nucleopolyhedrovirus (NPV). Both NPVs and GVs can package their virus particles (occluded virus) into occlusion bodies (polyhedra or granules) in the late phase of infection. However, baculoviruses have been isolated only from arthropods, primarily from insects of the order Lepidoptera, but also Hymenoptera, Diptera, Coleoptera, Neuroptera, Thysanura and Trichoptera as well as from the crustacean order Decapoda (shrimp) (Murphy *et al.,* 1995). *In vivo* and *in vitro* infective studies of NPVs have shown that most NPVs are effectively replicated only in a limited number of host species. Currently, over 600 baculoviruses have been reported in the literature, but fewer than 20 have been studied at the molecular level (Vlak and Rohrmann, 1985; Miller, 1988).

The baculoviruses produce polyhedrin and p10 at very high levels in the late phase of infection. Polyhedrin is the major crystalline protein matrix of the viral occlusion bodies which serve to protect the viruses from the environment outside the host insects (Vlak and Rohrmann, 1985; Rohrmann, 1986a). The molecular mass of polyhedrin is approximately 29,000 Da (daltons), and it may be produced in greater amounts than any other proteins in virus-infected insect cells (Rohrmann, 1986a). By using a strong promoter of the polyhedrin gene to drive the expression of a foreign gene, the baculovirus expression system is now widely used for large-scale production of eukaryotic proteins (Luckow and Summers, 1988).

Ficus transparent wing moth, *P. nuda* Fabricius (Lepidoptera: Lymantriidae), is a serious pest of banyan (*Ficus* spp.) and is a major defoliator of forest and shade trees in Southeastern Asia (Su *et al.,* 1983; Lo *et al.,* 1990; Wang and Tsai, 1995). An epizootic disease of nuclear polyhedrosis of *P. nuda* occurs from spring to early summer in Taiwan and mainland China. The main pathogen was found to be the baculovirus, PenuNPV. PenuNPV is a pathogen with high virulence and specificity to this moth. PenuNPV already inhibits outbreaks of *P. nuda* in nature and is therefore a good candidate for biological control of this moth (Su *et al.,* 1983). PenuNPV has been isolated from *P. nuda* larvae in Taiwan (Lo *et al.,* 1990; Wang and Tsai, 1995), and the genome size was estimated to be 121 kbp (Wang, personal communication). Recently, a highly susceptible cell line (NTU PN-HH) derived from *P. nuda* pupal ovaries was established (Wang *et al.,*

The sequence data in this paper have been submitted to the EMBL/GenBank Data Libraries under Accession No. U22824.

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1996, in press). The characteristics of PenuNPV, including its host ranges *in vitro* and *in vivo,* the protein profiles of its polyhedra and virions, the restriction enzyme profiles of its DNA, and the location of the polyhedrin gene in the genome have been studied and it is suggested to be a distinct NPV (Wang, personal communication).

In this paper we describe the isolation, cloning, and characterization of the polyhedrin gene from PenuNPV. In addition, the nucleotide sequence and transcriptional analysis of this gene are reported. Our data indicate that PenuNPV polyhedrin is very similar, at the amino acid level, to that of OpMNPV.

MATERIALS AND METHODS

Viruses, Insects, and Cells

An isolate of *P. nuda* NPV (PenuNPV) was obtained from infected larvae with nuclear polyhedrosis symptom. Infected larvae *P. nuda* specimens were collected from the campus of National Taiwan University and other sites in Northern Taiwan (Lo *et al.,* 1990; Wang and Tsai, 1995). The *P. nuda* cell line, NTU PN-HH, was established from *P. nuda* pupal ovary (Wang *et al.,* 1996, in press). The virus was multiplied in NTU PN-HH cells and fourth instar larvae of *P. nuda* were fed with polyhedra or inoculated with viral suspension for mass production of PenuNPV polyhedra (Lo *et al.,* 1990). *Autographa californica* nuclear polyhedrosis virus (AcMNPV) strain E2 (Smith and Summers, 1978) was kindly supplied by Dr. M. J. Fraser at Department of Biological Sciences (University of Notre Dame, U.S.A.) and propagated in IPLB SF-21 AE cells. NTU PN-HH and IPLB SF-21 AE cells were maintained as monolayers in plastic tissue culture flasks (Nunc and/or Cloning Co.) and incubated at 28°C in TNM-FH medium (Hink, 1970) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, 1.25 μg/ml fungizone, and 10% fetal calf serum. The polyhedra were isolated and purified as described by Lee and Miller (1978).

PCR Amplification of Viral DNA Polyhedrin Gene

In order to characterize PenuNPV polyhedrin gene, the amino acid sequences of the polyhedrin gene from several baculoviruses (Rohrmann, 1986a; Vlak and Rohrmann, 1985; Rohrmann *et al.,* 1981) were compared and appropriate highly conserved regions were chosen to design primers (primers 35 and 36) for the polymerase chain reaction (PCR, Mullis and Faloona, 1987). The primer sequences were as follows: primer 35, 5′ -AC$^{T}/_{C}$ TA$^{T}/_{C}$ GTG TAC GAC AAC AA$^{A}/_{G}$ TA$^{T}/_{C}$ TAC AAA-3′; primer 36, 5′ -GG$^{T}/_{C}$ GCG TC$^{T}/_{G}$ GG$^{T}/_{C}$ GCA AA$^{T}/_{C}$ TC$^{T}/_{C}$ TT$^{A}/_{T}$ AC$^{T}/_{C}$ TT$^{A}/_{G}$ AA-3′. Using this set of primers we were able to amplify the polyhedrin gene fragment from AcMNPV. PCR was performed in a 100-μl reaction mixture containing 2.5 units *Taq* polymerase (Promega), 100 ng of viral DNA, 0.5 μg of each primer, 200 μM of four dNTP, and 1× reaction buffer (10 mM Tris–HCl, pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100). Amplifications were performed over 30 cycles of denaturing at 94°C for 1 min, followed by annealing at 50°C for 1 min, and elongating at 72°C for 3 min. There was a final extension step of 5 min at 72°C in a MJR PTC-100 Thermocycler (Watertown, MA). PCR products were analyzed in 1.5% agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) containing 0.5 μg/ml ethidium bromide and visualized with short-wave ultraviolet light. In addition, PCR products of approximately 680 bp in length were subcloned into the *Sma*I site of plasmid pUC18 (Yanisch-Perron *et al.,* 1985) and sequenced by the dideoxy chain termination method (Sanger *et al.,* 1977) using a Sequenase kit (United States Biochemical Corp. Cleveland, OH) with either universal primers or specific internal primers. Oligonucleotides were synthesized commercially on an Applied Biosystems DNA synthesizer.

Southern Blot, Cloning, and Sequence Analysis

The genomic DNA of PenuNPV was digested with several restriction enzymes and then electrophoretically separated in 0.8% agarose gels and transferred to Hybon N nylon membrane (Amersham) by using a vacuum transfer unit (Hoefer TE-80) for 60 min. The blot was hybridized at 37°C for 16 hr to DIG-labeled probes (Sambrook *et al.,* 1989). The AcMNPV polyhedrin gene fragment was used as control. After hybridization and blocking membranes were incubated with alkaline phosphatase-conjugated anti-DIG antibody (1:10,000 dilution in buffer 2) for 1 hr and followed by two 15-min washes in washing buffer (100 mM maleic acid, 150 mM NaCl, pH 7.5, 0.3% Tween 20). The membrane was then equilibrated with buffer 3 (100 mM Tris–HCl, 100 mM NaCl, 50 mM $MgCl_2$; pH 9.5) and incubated with Lumigen-PPD solution (1:200 dilution in buffer 3) for 5 min. Membranes were exposed to Kodak XAR-5 film at 37°C for 15–60 min and then the film was developed. Cloning of positive restriction enzyme fragments of PenuNPV DNA was achieved by isolated from agarose gel and subcloning into the plasmid pUC18 (Yanisch-Perron *et al.,* 1985). DNA sequence analysis was performed using the Sequenase kit as described above.

RNA Isolation

For Northern blot hybridization and primer extension analysis, total RNA was isolated from PenuNPV-infected NTU PN-HH cells at 0, 12, 24, 48, 72, 96, 120, and 144 hr postinfection. NTU PN-HH cells were seeded in 25-cm^2 tissue culture flasks (Nunc and/or Cloning Co.) at a density of 3×10^6 cells/flask and infected with PenuNPV at a multiplicity of infection

(moi) of 1. Total cellular RNA was isolated according to BIOTECX RNAzol B RNA isolation protocol (BIOTECX Laboratories, Inc.) as briefly described below. Cells in a 25-cm^2 tissue culture flask were lysed with 1 ml of RNAzol B and then transferred to a 1.5-ml Eppendorf tube. After adding 0.1 ml of chloroform a vigorous vortex was induced for 15 sec. The tube was placed on ice for 5 min and centrifuged at 13,000*g* for 15 min. After centrifugation, the upper aqueous solution was transferred to another tube and mixed with an equal volume of isopropanol. The sample was again placed on ice for 15 min and then centrifuged at 13,000*g* for 15 min. The RNA pellet was washed with 70% ethanol and redissolved in DEPC-treated water. The RNA concentration was determined by measuring UV absorbance at 260 nm.

Northern Blot

Twenty micrograms of total RNA prepared from each of the postinfection sampling times was denatured, electrophoresed, and blotted to nylon membrane as described by Sambrook *et al.,* (1989). To identify PenuNPV polyhedrin-specific transcripts, the blot was hybridized for 16 hr at 37°C in hybridization buffer containing DIG-labeled PenuNPV polyhedrin gene probe (prepared as above). After hybridization, the blot was washed and exposed to the film as in the Southern blot procedure described above.

Primer Extension

A 30-base oligonucleotide (5′ -TTT GTA GTA TTT GTT GTC GTA CAC GTA GGT-3′) complementary to PenuNPV polyhedrin mRNA was synthesized and used to identify the transcriptional start site of the polyhedrin gene. The oligonucleotide (approximately 10 pmol) was 5′ end-labeled with [γ-^{32}P]ATP (3000 Ci/mmol, Amersham) using T4 polynucleotide kinase (8–10 units; Promega) in a forward exchange buffer (50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine) for 30 min at 37°C. The sample was heat-inactivated at 90°C for 10 min, and after adding to 90 μl DEPC-treated water, it was stored at −20°C. Two microliters of labeled primer was added to 20 μg total RNA in AMV primer extension buffer (50 mM Tris–HCl, pH 8.3, 50 mM KCl, 10 mM $MgCl_2$, 10 mM DTT, 1 mM each dNTP, 0.5 mM spermidine). The mixture was denatured at 58°C for 20 min and then annealed at 37°C for 10 min. After annealing, 5 μl of 2× primer extension buffer, 1.4 μl of 40 mM sodium pyrophosphate, 1.6 μl of DEPC-treated water, and 1 unit of AMV reverse transcriptase (Promega) were added to the mixture. The reverse transcriptase reaction proceeded for 30 min at 42°C. The reaction was stopped by the addition of 20 μl of loading buffer (98% formamide, 10 mM EDTA, pH 8.0, 0.1% xylene cyanol FF, 0.1% bromophenol blue). Five microliters of this reaction mixture was analyzed in a 6% polyacrylamide sequencing gel. After drying, the gel was subjected to autoradiography at −70°C for 24 hr.

RESULTS

Polyhedrin Gene Localization, Cloning, and Sequencing

To identify the location of the polyhedrin gene in the PenuNPV genome, Southern blot analysis was applied using AcMNPV polyhedrin gene fragment as a probe. *Eco*RI-G (6.3 kbp) and *Pst*I-G (4.3 kbp) fragments of PenuNPV DNA were found to be positive with AcMNPV polyhedrin gene probe. The *Eco*RI-G and *Pst*I-G fragments of PenuNPV DNA were isolated and subcloned into the same site on plasmid pUCl8. The resulting plasmids were analyzed and Fig. 1 shows the restriction enzyme map of both fragments. A total length of 8.4 kbp of PenuNPV DNA was found to cover these two fragments. From repetitive subcloning and Southern hybridization, we were able to localize PenuNPV polyhedrin gene to a smaller region of the *Eco*RI–*Xma*III fragment. All the 1333 nucleotides (nt) which include this *Eco*RI–*Xma*III fragment were then subjected to DNA sequence analysis and the results are shown in Fig. 2. An open reading frame (ORF) of 735 nt encoding a protein of 245 amino acid residues was found.

DNA Sequence Analysis

An amino acid comparison between PenuNPV polyhedrin and 19 other NPV polyhedrin amino acid identities is summarized in Table 1. The overall sequence identity between PenuNPV polyhedrin and that of other 19 viruses is considerably high, approximately 74–99% (GmMNPV, Gusak *et al.,* 1981; OpMNPV, Leisy *et al.,* 1986, respectively).

In the 5′-untranslated region of PenuNPV polyhedrin gene, a putative transcriptional start site was found with the core sequence TAAG (Vlak and Rohrmann, 1985; Rohrmann, 1986a) which is 51 nt upstream of the translation initiation codon of the polyhedrin gene as determined by primer extension (Fig. 3A). Other minor signals also indicated alternative start sites at positions −50 and −52.

A similar portion of lef-2 (named after the homologous AcMNPV lef-2 by Passarelli and Miller, 1993) was also found in the 5′-untranslated region of PenuNPV polyhedrin gene. The PenuNPV 3′ portion of lef-2 showed 76, 95, 70, and 67% amino acid identity with those of *Anticarsia gemmatalis* MNPV (AgMNPV; Zanotto *et al.,* 1992), BmNPV (Iatrou *et al.,* 1985), OpMNPV (Zanotto *et al.,* 1992), and AcMNPV (Possee *et al.,* 1991), respectively (paper in preparation).

The promoter regions of PenuNPV and OpMNPV upstream from the translation start codon, ATG, were

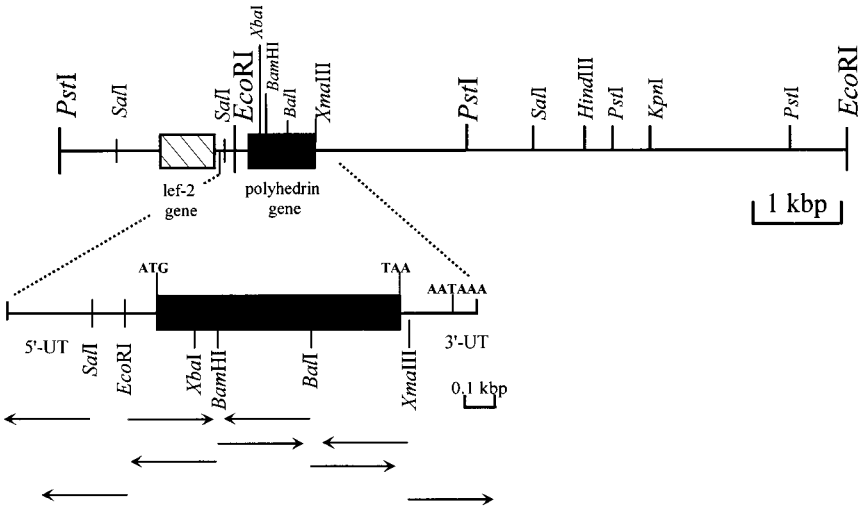

FIG. 1. Restriction map of the region containing PenuNPV polyhedrin gene. Restriction enzyme map of an 8.4-kbp PenuNPV DNA fragment containing 6.3 kbp *Eco*RI-G and 4.3 kbp *Pst*I-G fragments. The polyhedrin gene is indicated by the solid bar. The lef-2 gene is indicated by the hatched bar. The 4.3-kbp *Pst*I fragment was cloned into pUC18. The lower map is a detailed restriction map of the 1.0-kbp *Eco*RI–*Xma*III fragment. The start codons (ATG), stop codons (TAA), and the polyadenylation signal (AATAAA) of the polyhedrin gene are indicated. The flanking untranslated regions (5′-UT and 3′-UT) are also shown. The sequencing strategy is shown in the lower portion of the restriction map. Arrows show the sequencing orientation and length of the sequenced DNA.

aligned and compared in Fig. 4. They were obviously divergent from the upstream 140 nt of the PenuNPV promoter region.

In the 3′-untranslated region of the PenuNPV polyhedrin gene, the polyadenylation signal, AATAAA (Birnstiel *et al.,* 1985), was found 116 nt downstream from the translational stop codon TAA (Fig. 2).

Northern Blot Analysis

The results of Northern blot analysis are shown in Fig. 3B. Two major transcripts, 1.16 and 4.1 kb in length, were identified at 96 through 144 hr postinfection. However, whereas the 1.16-kb transcript was already strongly expressed by 120 hr postinfection, the 4.1-kb transcript was strongly expressed after 120 hr postinfection.

DISCUSSION

In this report we have determined the nucleotide sequence of PenuNPV polyhedrin gene and analyzed its transcript using primer extension and Northern blot analysis. It has been reported that polyhedrins from lepidopteran NPVs polyhedrins are closely related to one another and have 85 to 90% amino acid identity (Rohrmann, 1986b). PenuNPV polyhedrin, a protein of 245 amino acid residues, does indeed resemble other NPVs. Comparison of amino acid sequences between PenuNPV and 19 other NPV polyhedrins showed 97, 95, 91, 94, and 99% amino acid identity with those of AgMNPV, ArNPV (*Attacus ricini* NPV, Hu *et al.,* 1993), BmNPV, HcNPV (*Hyphantria cunea* NPV, Croizier *et al.,* 1994), and OpMNPV (Table 1). In addition, PenuNPV is almost identical to that of OpMNPV (Leisy *et al.,* 1986) with only 1 amino acid residue difference. This implies that PenuNPV is closely related to OpMNPV.

The examination of restriction patterns is a very convenient technique and is useful in determining whether viruses are closely related. Restriction enzymes have been used to identify and define variants of a virus (Lee and Miller, 1978, Smith and Summers, 1978) and to differentiate closely related viruses, such as those of *Rachoplusia ou* (RoMNPV), AcMNPV, *Trichoplusia ni* (TnMNPV), *Galleria mellonella* (GmMNPV) (Miller and Dawes, 1978, Smith and Summers, 1979), and the MNPVs of *Spodoptera* (Loh *et al.,* 1982). Our previous study had shown that restriction enzyme profiles of PenuNPV are distinct from other well-known NPVs (Wang, personal communication).

The *in vitro* infective studies of PenuNPV show that it can replicate in NTU PN-HH and IPLB LD-652Y cells (Wang *et al.,* 1996, in press). However, the cytopathic effects on NTU PN-HH and IPLB LD-652Y cells after inoculation with PenuNPV are different in terms of the morphological changes in the infected cells and the production of polyhedral inclusion bodies (PIBs) (Wang, personal communication). OpMNPV and LdMNPV are NPVs that are known to be able to replicate in IPLB LD-652Y cells (Bradford *et al.,* 1990; McClintock *et al.,* 1986). The IPLB LD-652Y cells and NTU PN-HH cells were derived from common *Lymantriidae* ovarian tissue (Goodwin *et al.,* 1978; Quiot, 1976; Wang *et al.,* 1996, in press). Therefore, it is not surprising that these two cell lines should be more related than others. In the PenuNPV-infected cells (NTU PH-HH or IPLB LD-652Y cells), the percentage of cells

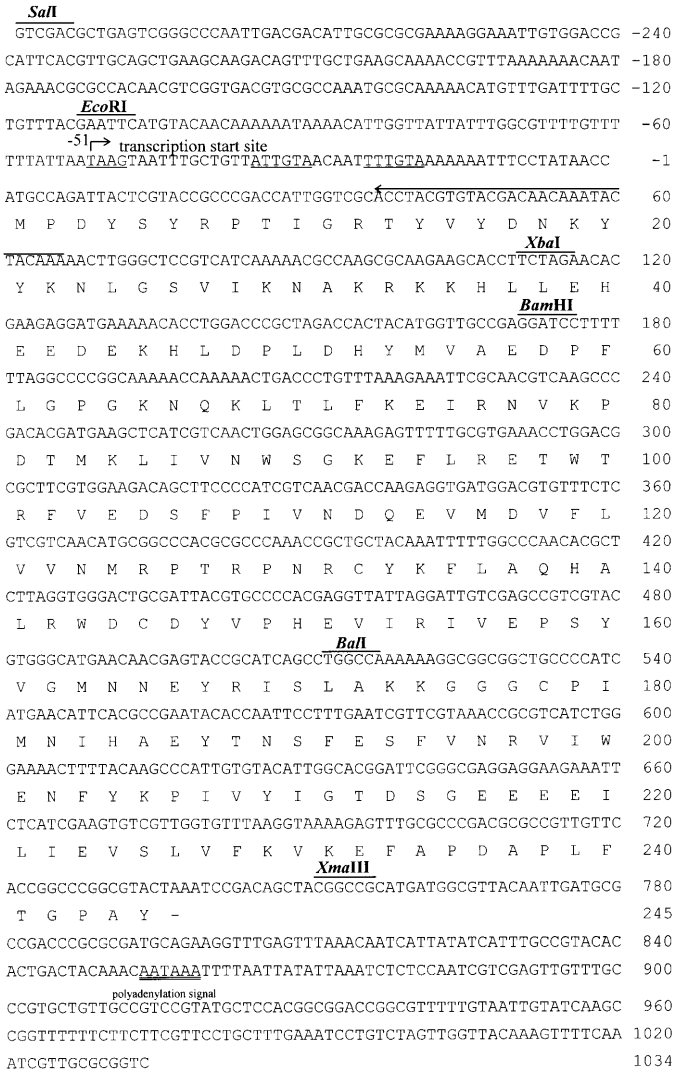

FIG. 2. Nucleotide sequence of PenuNPV polyhedrin gene and its flanking regions. The plus strand is shown. The first nucleotide of the polyhedrin gene translational start signal ATG is given number +1. The predicted amino acid sequence is indicated by one-letter code and displayed below the nucleotide sequence. The sequence at nt –51 initiates the 5′ end of the mRNA and the arrow indicates the direction of transcription. The putative transcription initiation motif (TAAG) and two 6-nt consensus sequences are underlined. The potential polyadenylation signal (AATAAA) is indicated by double underline, starting at position nt 854. Numbers of deduced amino acid residues are shown in the right. The primer used in the primer extension assay is also shown and the arrow indicates the direction of extension.

which contained polyhedra in their nuclei were observed in very late infection. Up to 98% of infected NTU PN-HH cells contained polyhedra but less than 20% of the infected IPLB LD-652Y cells contained polyhedra (Wang, personal communication).

NPVs have only been isolated from arthropods and are considered to have narrow host ranges, i.e., they replicate in only a few species of the same or similar

TABLE 1

Amino Acid Sequence Identities (%) of 20 NPVs Polyhedrins[a]

	PenuNPV	AcMNPV	AgMNPV	ArNPV	AsNPV	BmNPV	BsNPV	GmMNPV	HcNPV	HzNPV	LdMNPV	LsNPV	MbNPV	MnNPV	OpMNPV	PdNPV	PfNPV	SeMNPV	SfMNPV	SlNPV
PenuNPV	—	89	97	95	82	91	87	74	94	84	80	85	86	84	99	78	86	85	84	84
AcMNPV		—	89	88	82	86	90	79	87	86	81	88	88	86	89	79	89	85	85	84
AgMNPV			—	95	83	92	87	75	94	82	85	81	86	84	96	80	86	85	84	82
ArNPV				—	81	91	85	73	92	80	79	84	84	82	95	77	84	83	82	79
AsNPV					—	84	84	70	82	79	76	82	83	80	82	74	83	83	82	77
BmNPV						—	85	73	89	81	80	84	85	83	91	78	85	82	82	80
BsNPV							—	74	86	91	83	93	94	91	87	81	95	91	91	87
GmMNPV								—	73	70	69	71	72	70	75	69	73	71	71	72
HcNPV									—	80	80	84	85	82	93	78	85	83	82	80
HzNPV										—	81	87	87	89	84	76	88	87	86	87
LdMNPV											—	82	83	79	80	92	84	82	81	81
LsNPV												—	98	88	85	80	97	91	91	85
MbNPV													—	89	86	80	98	92	92	86
MnNPV														—	84	76	89	87	87	86
OpMNPV															—	79	86	84	84	84
PdNPV																—	81	79	79	79
PfNPV																	—	91	91	86
SeMNPV																		—	98	85
SfMNPV																			—	85
SlNPV																				—

[a] Amino acid sequences of polyhedrins are from baculoviruses as follow: AcMNPV (Hooft van Iddekinge *et al.*, 1983), AgMNPV (Zanotto *et al.*, 1992), ArNPV (Hu *et al.*, 1993), AsNPV (Kozlov *et al.*, 1992), BmNPV (Iatrou *et al.*, 1985), BsNPV (Hu *et al.*, 1993), GmNPV (Grusak *et al.*, 1981), HcNPV (Croizier *et al.*, 1994), HzNPV (Cowan *et al.*, 1994), LdMNPV (Smith *et al.*, 1988), LsNPV, MbNPV (Cameron *et al.*, 1989), MnNPV (Kozlov *et al.*, 1994), OpMNPV (Leisy *et al.*, 1986), PdNPV (Levitina *et al.*, 1981), PfNPV (Oakey *et al.*, 1989), SeMNPV (van Strien *et al.*, 1992), SfMNPV (Gonzales *et al.*, 1989), and SlNPV (Croizier *et al.*, 1994).

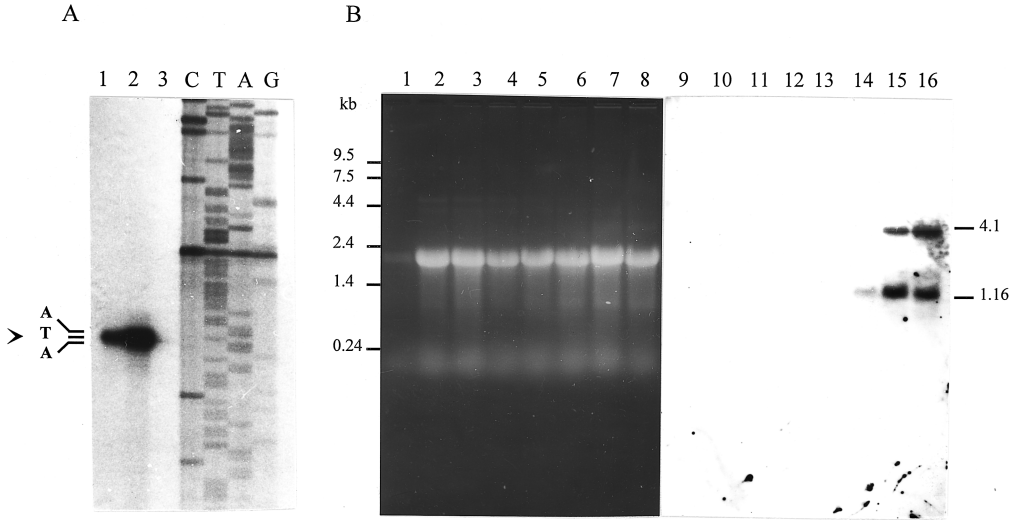

FIG. 3. (A) Primer extension analysis of the 5′ end of polyhedrin mRNA. A radiolabeled oligonucleotide (see Materials and Methods) was hybridized to total RNAs from noninfected NTU PN-HH cells (lane 3) or PenuNPV-infected NTU PN-HH cells at 48 and 120 hr pi (lanes 1 and 2), and reverse transcription was then conducted. The DNA ladder was generated from a plasmid containing PenuNPV *Pst*I G fragment using the same primer in the presence of dideoxyribonucleotides ddCTP (lane G), ddTTP (lane A), ddATP (lane T), or ddGTP (lane C). The transcriptional start site is indicated with an arrowhead in a region specified by the nucleotide sequence of the DNA strand in the same sense as polyhedrin mRNA. The size of the extension produced was determined against the sequence ladder and the sequence of the mRNA transcription start site is indicated by the letters at the side of photograph. (B) Northern blot analysis of PenuNPV polyhedrin gene transcripts. Total RNA was isolated from uninfected NTU PN-HH cells (lane 1) and PenuNPV-infected NTU PN-HH cells collected at 12, 24, 48, 72, 96, 120, and 144 hr pi (lanes 2, 3, 4, 5, 6, 7, and 8, respectively). The samples (20 μg RNA per lane) were electrophoretically separated on 1% agarose gel (containing formaldehyde), stained with ethidium bromide, and photographed under UV light. The same gels were transferred to a nylon membrane (lanes 9–16) and hybridized to a DIG-labeled *Eco*RI–*Xma*III fragment containing PenuNPV polyhedrin gene. The 1.16 and 4.1-kb transcripts are indicated. The size of the transcripts are indicated on the right. The kilobase scale on the left is derived from RNA size markers (Life Technologies, Inc.).

families. The general mechanisms of baculovirus host specificity, however, remains undetermined. The host range of OpMNPV is not clearly understood. Thus, although PenuNPV and OpMNPV are both able to replicate in IPLB LD-652Y cells, and the amino acid sequences of polyhedrin are very similar, OpMNPV and its host insect, *Orgyia pseudotsugata,* have not been recorded in Taiwan. The fact that both PenuNPV and OpMNPV can replicate in IPLB LD-652Y suggests that they might have several genes in common. Further investigation is needed.

Although we think that these two viruses are related, they are clearly not identical. First, the OpMNPV polyhedrin gene is located in *Eco*RI-B (28 kbp) and *Hin*dIII-A (21 kbp) fragments (Leisy *et al.,* 1984; Chen *et al.,* 1988), whereas the PenuNPV polyhedrin gene is located in *Eco*RI-G (6.3 kbp) and *Hin*dIII-F (6.2 kbp) fragments. Second, the restriction enzyme (*Eco*RI, *Hin*dIII, and other restriction enzymes, Wang, personal communication) profiles of PenuNPV and OpMNPV genomes are dissimilar. Third, although both NPVs can replicate in IPLB LD-652Y cells, the cytopathic effects are different (Wang, personal communication). Fourth, the viral polypeptides profile of PenuNPV (Wang, personal communication) and OpMNPV (Blissard and Rohrmann, 1989) are different.

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OpMNPV    GCGCCGGTGAACGTCGCCAAATGCGCAAAAACAATGGGCGTCGCAATACC -242
PenuNPV   ------GTG-----CGCCAAATGCGCAAAAACA---------------- -133

OpMNPV    CCGAACACTTGACAACCGTTCACAAGAGGCACCCGCTTGCAGCTGTCCTG -192
PenuNPV   ---------------------------------------------------- -132

OpMNPV    CGTTTGGTGCGACGACCAGCGCTCTTGGGACCCGCACTCCGCCAAAGGTT -142
PenuNPV   ---------------------------------------------------- -132

OpMNPV    TAATGTTTGAGCATATGTTTTGCTGCTTACGAATTTATGTACAACAAAAA -92
PenuNPV   ---TGTTTGA-------TTTTGCTGTTTACGAATTCATGTACAACAAAAA -93

OpMNPV    ATAAAACACTAGTTACTATT-GGCGTTTCGTTTTTTATTAATAAGTAATT -43
PenuNPV   ATAAAACATTGGTTATTATTTGGCGTTTTGTTTTTTATTAATAAGTAATT -43

OpMNPV    TCCTGTTATTGTAACAATTTTGTAAAAAAATTTCCTATAACC -1
PenuNPV   TGCTGTTATTGTAACAATTTTGTAAAAAAATTTCCTATAACC -1
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FIG. 4. Comparison of PenuNPV and OpMNPV 5′ -untranslated region. Alignment of the 5′ -untranslated region of PenuNPV and OpMNPV (Leisy *et al.,* 1986) is shown. To maximize the alignment, gaps are introduced and indicated as dashes. The vertical lines indicate that nucleotide sequences are the same.

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