Susceptibility of the Cell Line Hv-AM1 from *Heliothis virescens* to Eight Selected Nucleopolyhedroviruses

Javier Gordon Ogembo1,5, Sudawan Chaeychomsiri2, Barbara Laviña Cañili1,3, Motоко Ikeda4 and Michihiro Kobayashi1*

1 Laboratory of Biodynamics and 4 Laboratory of Sericulture and Entomoresources, Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan,
2 Central Laboratory and Greenhouse Complex, Kasetsart University, Nakhon Pathom 73140, Thailand, and
3 Crop Protection Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031, Philippines

(Received April 7, 2008; Accepted May 27, 2008)

The cell line Hv-AM1 from *Heliothis virescens* was characterized for permissiveness and productivity, employing *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and seven other NPVs, including those of *Hyphantria cunea* (HycuMNPV), *Orgyia pseudotsugata* (OpMNPV), *Bombyx mori* (BmNPV), *Spodoptera exigua* (SeMNPV), *Spodoptera litura* (SpltMNPV), *Lymkantra dispar* (LdMNPV) and *Autographa californica* (AcMNPV). Based on budding virus (BV) yields, polyhedrin production, and polyhedra formation, the results showed for the first time that the cell line Hv-AM1 was permissive for HearNPV, HycuMNPV and OpMNPV, supporting moderate levels of BVs, polyhedrin and polyhedra production. Consistent with the previous results, the cell line Hv-AM1 was also shown to be highly permissive for AcMNPV infection, yielding high levels of BVs, polyhedrin and polyhedra. In the cell line Hv-AM1 infected with BmNPV, SpltMNPV and SeMNPV, few, if any, viral progeny were produced, regardless of the severity of the observed cytopathic effects and the significant production of viral DNA, suggesting its semipermissivity nature for these NPVs. In addition, the cell line Hv-AM1 was found to readily undergo apoptosis upon infection with BmNPV, SeMNPV and HycuMNPV, while it was refractive to LdMNPV. Thus, although the cell line Hv-AM1 is not very efficient for HearNPV production, the diverse observed responses and high permissiveness against NPVs indicate that the cell line Hv-AM1 provides a novel system for analyzing the possible mechanisms underlying the permissiveness and productivity of insect cells against NPVs.

Key words: Hv-AM1 cell line, *Heliothis virescens*, nucleopolyhedrovirus, NPV, baculovirus, permissiveness, productivity, apoptosis

INTRODUCTION

Insect species from the subfamily Heliothinae, such as *Helicoverpa armigera*, *H. punctigera*, *H. zea* and *Heliothis virescens*, are some of the major pests worldwide, causing severe damage to a variety of cultivated crops, including cotton, tobacco, citrus, tomato, cereals and legumes (Zalucki et al. 1986, 1994; King, 1994). Currently, the control of these insect species is extremely difficult, due to their development of resistance to major groups of existing chemical insecticides (Gunning et al., 1991). In addition, the use of chemical insecticides in the management of insect pests is a matter of great concern because of their detrimental effects on human health and the environment. Thus, insect-specific microorganisms, including bacteria, fungi and viruses, have been explored, developed and applied as alternative agents for pest management (Hunter-Fujita et al., 1998).

Nucleopolyhedroviruses (NPVs), members of the family *Baculoviridae* characterized by circular, double-stranded DNA genomes of approximately 80-180 kbp (Blissard et al., 2000), have been studied for their potential use as insecticides. These viruses are pathogenic exclusively to arthropods, with the vast majority of susceptible insects belonging to the order Lepidoptera (Adams and McClintock, 1991). The lepidopteran insects represent one major pest group affecting most agricultural crops, forest trees and ornamental plants, and a number of NPVs have been used for large-scale management of these insect pests (Hunter-Fujita et al., 1998; Moscardi, 1999).

Although a number of cell lines from diverse insect species have been established and insect cell culture technology has advanced significantly, NPVs used for biological control are still mass-produced in vivo in insect larvae, due to advantages such as feasibility and cost effectiveness over in vitro production in cell cultures (Black et al., 1997). However, in vivo insect virus production system also has some drawbacks. Aside from its being labour intensive, the most serious disadvantage is the low quality of its virus products, due to contamination by tissue debris from the infected larvae or by adventitious viruses, as well as by other pathogens such as bacteria and fungi. These disadvantages have necessitated the development of

*To whom correspondence should be addressed.
Fax: +81-52-789-4036. Tel: +81-52-789-4038.
Email: michihir@agr.nagoya-u.ac.jp

1 Present address: Division of Infectious Disease-Research, Beth Israel Deaconess Medical Center, Harvard Institute of Medicine, 4 Blackfan Circle, Boston, MA 02115, USA
an efficient in vitro system for the production of these viruses.

In an effort to enhance the application of *H. armigera* NPV (HearNPV) as a microbial insecticide, we have previously cloned NPVs from *H. armigera* larvae collected from various geographical regions in Africa and Thailand, and selected the most virulent HearNPV strain, employing the cell line Hz-AM1 derived from *Helicoverpa zea* (Ogembo et al., 2007). We have also established five cell lines from *H. armigera* and selected the cell line HaAO1 that supported the highest HearNPV productivity among the newly established cell lines and the cell line Hz-AM1 (Ogembo et al., 2008). However, the ability of the cell line HaAO1 to produce HearNPV was relatively limited, compared with those of some other NPV-cell line systems. Thus, it is still imperative to explore and/or develop cell lines that are efficient for HearNPV production and versatile for HearNPV biology and biotechnology research.

Previous studies have shown that cell lines from *H. virescens* exhibit higher permissiveness for NPVs than the cell lines previously established from other members of the subfamily Heliothinae, including *H. armigera*, *H. zeas*, *H. punctigera* and *H. subflexa*, and support a high titer of *Autographa californica* multicipsid NPV (AcMNPV) (McIntosh et al., 2005; Lynn, 2007). In this study, we examined the permissiveness and productivity of the cell line Hv-AM1 from *H. virescens*, employing HearNPV and seven other NPVs: *Hypantria cunea* MNPV (HycuMNPV), *Orgyia pseudotsugata* MNPV (OpMNPV), *Bombyx mori* NPV (BmNPV), *Spodoptera exigua* MNPV (SeMNPV), *Spodoptera litura* MNPV (SplMNPV), *Lymantria dispar* MNPV (LdMNPV) and AcMNPV.

Our results showed for the first time that the cell line Hv-AM1 is permissive for HearNPV, HycuMNPV and OpMNPV, supporting moderate levels of BVs, polyhedrin and polyhedra production. The cell line Hv-AM1 was also permissive for AcMNPV, yielding high levels of BVs, polyhedrin and polyhedra. Upon infection with BmNPV, SplMNPV and SeMNPV, the cell line Hv-AM1 exhibited its semipermissive nature, producing a significant amount of viral DNA while yielding few or no progeny virions. In addition, our results showed that Hv-AM1 cells readily underwent apoptosis upon infection with BmNPV, SeMNPV and HycuMNPV, while they were refractive to LdMNPV. These results indicate that although it does not support high HearNPV productivity, the cell line Hv-AM1 is useful in analyzing the possible mechanisms underlying the permissiveness and productivity of insect cells against NPVs, due to its diverse responses and high permissiveness for various NPVs.

**MATERIALS AND METHODS**

**Viruses and cells**

The clonal NPVs used in these experiments were: AcMNPV E2 from the fall armyworm, *A. californica* (Smith and Summers, 1978); HearNPV NNg1 from the African bollworm, *H. armigera* (Ogembo et al., 2007); HycuMNPV N9 from the fall webworm, *H. cunea* (Kamiya et al., 2003); OpMNPV from the Douglas-fir tussock moth, *O. pseudotsugata* (Leisy et al., 1986); BmNPV N9 from the silkworm, *B. mori* (Nagamine et al., 1989); SeMNPV G3 from the beet armyworm, *S. exigua* (Wu et al., 2000); SplMNPV C3 from the common cutworm, *S. litura* (Kamiya et al., 2004); and LdMNPV from the gypsy moth, *L. dispar* (Slavicek et al., 1996).

The insect cell line BCIRL-Hv-AM1 from *Heliothis virescens* (Hv-AM1; McIntosh et al., 1981) was used throughout the experiments. The cell lines BCIRL-Hz-AM1 from the American bollworm, *Helicoverpa zea* (Hz-AM1; McIntosh and Ignoffo, 1981); Sf9 from the fall armyworm, *Spodoptera frugiperda*; Se301 from the beet armyworm, *S. exigua* (Hara et al., 1995); BmN-4 from the silkworm, *B. mori* (Maeda, 1989); FRI-SpIm1229 from the mulberry tiger moth, *Spilosoma imparilis* (SpIm; Mitsuhashi and Inoue, 1988); and TUAT-SpLi-221 from the common cutworm, *S. litura* (SpLi; Mitsuhashi, 1995) were used for the plaque assays of HearNPV, AcMNPV, SeMNPV, BmNPV, HycuMNPV and SplMNPV BVs, respectively. The cell line IPLB-Ld652Y from the gypsy moth, *L. dispar* (Ld652Y; Goodwin et al., 1978) was used for the titration of OpMNPV and LdMNPV. Cell lines Hv-AM1, Hz-AM1, Sf9, BmN-4 and Ld652Y were maintained in TC100 medium (JRH Biosciences), whereas cell lines Se301 and SpLi were cultured in IPL41 medium (Gibco BRL, Grand Island, NY). Both media were supplemented with 10% fetal bovine serum (FBS). The cell line SpIm was cultured in MM medium (Mitsuhashi and Maramorosch, 1964) supplemented with 3% FBS. All cell lines were maintained at 28°C.

**Virus infection and sample preparation for budded virus titration, slot-blot hybridization and immunoblot analyses**

Hv-AM1 cells (1 × 10^6^) seeded in 12.5 cm² flasks were infected with AcMNPV, HearNPV, HycuMNPV, OpMNPV, BmNPV, SeMNPV, SplMNPV and LdMNPV at multiplicities of infection (MOIs) of 10, 5, 10, 10, 10, 1 and 1 plaque forming unit (PFU) per cell, respectively. The low input MOIs for infection with HearNPV, SplMNPV and LdMNPV were due to the low titers of the inocula obtained. After a 60-min period of virus adsorption at room temperature, the infected cells were washed twice with the medium and cultured in 2 ml of
media. The samples for budded virus (BV) titration and analyses by slot-blot hybridization and immunoblotting were prepared from a single flask at 0, 24, 48, 72 and 96 h postinfection (pi) as described previously (Laviña et al., 2001; Katou et al., 2006; Ogembo et al., 2007, 2008). In brief, 500-μl aliquots of the culture medium were harvested at 24-h intervals and stored at −80°C for BV titration. The infected cells were scraped into the culture media using a rubber policeman, and 100-μl aliquots of the cell suspensions were transferred into microcentrifuge tubes for viral DNA replication analysis by slot-blot hybridization. The remaining cell suspensions were pelleted and lysed in 100 μl of sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% glycerol (v/v)) for SDS-polyacrylamide gel electrophoresis, and subjected to polyhedrin and GP64 analyses by immunoblotting.

**Viral DNA isolation and slot-blot hybridization analysis**

Analyses of viral DNA replication by slot-blot hybridization were carried out as described previously (Ikeda and Kobayashi, 1999; Laviña et al., 2001). The aliquots of virus-infected Hv-AM1 cells were pelleted at 3000 rpm for 5 min and suspended in 100 μl of distilled water. The suspended cells were treated with 81 μl of heat-saturated sodium iodide and boiled for 10 min before being chilled on ice for 5 min. The chilled cells were blotted onto the Hybond-N+ nylon membranes (Amersham Biosciences) and hybridized with respective NPV probes for viral DNA detection.

The probes were prepared from a portion of the viral DNA polymerase gene for HearNPV, SeMNPV and SpltMNPV, the gp64 gene for AcMNPV, the iap3 gene for OpMNPV, the p35 gene for BmNPV, the ep32 gene for HycuMNPV, and the hrf-1 gene for LdMNPV. These probes were amplified by PCR using specific primers and genomic DNA of the respective NPVs as templates. The specific paired primers used for PCR amplification were:

- 5′-CACCGTCGAGGCG-3′ for LdMNPV.
- 5′-CGCCTCGACGGTGTGGAA-3′ and 5′-TTCCA GCCAGCC-3′ for HycuMNPV.
- 5′-GACCGTGACGGTGTGGAA-3′ for BmNPV.
- 5′-CTTGGTTAGCAACGCTTACATGCGCACCGCT-3′ for OpMNPV.
- 5′-GGTAGCTTACATGCGCACCGCT-3′ for BmNPV.
- 5′-TTGCCGAGCGAAGACTG-3′ for AcMNPV.
- 5′-GACCGTGACGGTGTGGAA-3′ for HycuMNPV.
- 5′-TACGATACGATTTGAGTCCGG-3′ and 5′-CGCATA GCCAGCC-3′ for SeMNPV.
- 5′-TGCAACGAGAGATGTCGTCGTCGTCTG-3′ and 5′-GATTACATTTGGGACACGATTTGAGTCCGG-3′ for SpltMNPV.
- 5′-ACGATACGATTTGAGTCCGG-3′ and 5′-GATTACATTTGGGACACGATTTGAGTCCGG-3′ for SeMNPV.
- 5′-TTGCCGAGCGAAGACTG-3′ for AcMNPV.

**Immunoblot analysis**

Polypeptides from NPV-infected Hv-AM1 cells were resolved on 12% SDS-polyacrylamide gels and transferred onto immobilon (Millipore, Bedford, MA) or nitrocellulose (Advantec Toyo, Tokyo, Japan) membranes for immunoblot analysis as described previously (Shirata et al., 1999; Laviña et al., 2001). The polyhedrin protein was probed with anti-BmNPV polyhedrin and goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (HRP) (Zymed Laboratories, San Francisco, CA) as the primary and the secondary antibodies, respectively. For GP64 protein detection, the monoclonal antibody against AcMNPV GP64 protein (Clontech) and HRP-conjugated anti-mouse IgG antibody (Zymed Laboratories) were used as the primary and the secondary antibodies, respectively. Positive signals for the polyhedrin protein were visualized by Konica Immunostain HRP-1000 (Konica, Osaka, Japan), whereas positive signals for the GP64 protein were visualized by ECL Western blotting detection reagents (Amersham Biosciences). The antibody against BmNPV polyhedrin was raised in rabbits using purified BmNPV polyhedrin (Shirata et al., 1999). Benchmark pre-stained protein ladder (Invitrogen) was used as the molecular size marker.

**Budded virus (BV) titration**

Culture media were harvested from the infected Hv-AM1 cell cultures at 24-h intervals until 96 h pi, and BVs were titrated by plaque assay with appropriate cell lines as described previously (Shirata et al., 1999; Laviña et al., 2001).

**DNA fragmentation assay**

Apoptotic fragmented DNA was isolated from the infected cells by NP-40 extraction procedure as described previously (Ishikawa et al., 2003). The infected cells were washed twice with PBS (8 mM Na2HPO4, 1.5 mM KH2PO4, 137 mM NaCl, 2.7 mM KCl) and lysed in 100 μl of lysis buffer (1% NP-40, 50 mM Tris-HCl, pH 7.5, 20 mM EDTA). The cell lysates thus obtained were centrifuged at 4500 rpm for 5 min at 4°C in a microcentrifuge, and the supernatant containing apoptotic fragmented DNA was removed. The supernatants were mixed well with 20 μl of 10% SDS and RNase A (10 mg/ml) and incubated at 56°C for 2 h. The mixtures were then supplemented with 5 μl of proteinase K (15.6 mg/ml) and further incubated at 37°C for 4 h. The DNA in the mixture was ethanol-precipitated at −20°C overnight, dissolved in TE-8.
(10 mM Tris-HCl, pH 8, 1 mM EDTA) and analysed on a 1.5% agarose gel.

**Caspase activity assay**

The caspase activity assay was performed using a caspase-3 fluorescent assay kit ApoProbe-3 (Biodynamics Laboratory) as described previously (Ishikawa et al., 2003). Monolayer cultures of the Hv-AM1 cells were infected with SeMNPV, HycuMNPV and BmNPV at an MOI of 10 PFU/cell. At 24 h-intervals until 96 h pi, these cells were scraped into the culture medium with a rubber policeman and collected using microcentrifuge at 3000 rpm for 5 min at 4°C. The cells were suspended in the cell lysis buffer included in the kit and incubated on ice for 10 min, and the cell lysates were then centrifuged at 12,000 rpm for 3 min at 4°C. The resultant supernatant was analysed for caspase-3-like protease activity using vAc-DEVD-AMC as the substrate. The accumulation of fluorescent product was monitored using a spectrofluorometer (Fluoroskan Ascent, Thermo Labsystems) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm.

**RESULTS**

### Cytopathology and polyhedra production in infected Hv-AM1 cells

Hv-AM1 cells were infected with each of eight different NPVs (AcMNPV, HearNPV, HycuMNPV, OpMNPV, BmNPV, SeMNPV, SpltMNPV and LdMNPV) and examined for cytopathology and polyhedra production under a microscope. The infected Hv-AM1 cells exhibited cytopathic effects (CPE) following infection with seven NPVs, with the exception of LdMNPV, of which four NPVs (AcMNPV, HearNPV, HycuMNPV and OpMNPV) produced polyhedra (Fig. 1, Table 1). In AcMNPV-infected cells, CPE and polyhedra were first observed at 12 and 24 h pi, respectively, and cells with polyhedra increased rapidly until 36 h pi, at which time pi about 95% of total cells had polyhedra. In the HycuMNPV-infected cells, CPE was first observed at 24 h pi, followed by polyhedra formation at 48 h pi. When Hv-AM1 cells were challenged with HearNPV, CPE and polyhedra formation were observed simultaneously at 24 h pi, and cells with polyhedra increased at 36 h pi, remaining at this level until 96 h pi. At 96 h pi, about 95, 56, 26 and 5% of total infected Hv-AM1 cells produced polyhedra when infected with AcMNPV, HycuMNPV, HearNPV and OpMNPV, respectively. The number of polyhedra produced on a per cell basis was also highest in the AcMNPV-infected cells, followed by the cells infected with HycuMNPV, HearNPV and OpMNPV. The OpMNPV-infected cells contained only a few polyhedra per cell. Only AcMNPV-infected cells were ruptured by 96 h pi, releasing polyhedra into the culture medium.

When Hv-AM1 cells were infected with BmNPV, SeMNPV and SpltMNPV, a hallmark CPE was observed from 24 h pi and became more severe with time until 96 h pi. However, no polyhedra were detected in the infected cells, suggesting the abortive infection of these three NPVs in the cell line Hv-AM1. The CPE was most severe in BmNPV-infected cells, followed in succession by the cells infected with HycuMNPV, HearNPV and OpMNPV. The LdMNPV-infected cells contained only a few polyhedra per cell. The LdMNPV-infected cells continued to proliferate.

![Fig. 1. Cytopathology and polyhedra formation in Hv-AM1 cells infected with eight different NPVs. Monolayer cultures of 1 × 10⁶ Hv-AM1 cells in 12.5-cm² flasks were infected with AcMNPV, HearNPV, HycuMNPV, OpMNPV, BmNPV, SeMNPV, SpltMNPV and LdMNPV at MOIs of 10, 5, 10, 10, 10, 10, 1 and 1 PFU per cell, respectively. The infected cells were washed twice with TC100 medium after a 60-min adsorption period at room temperature and cultured in 2 ml of fresh TC100 medium at 28°C. Cytopathic effects and polyhedra formation were examined at 12-h intervals and photographed at 24-h intervals. Mock-infected Hv-AM1 cells were also presented for comparison. The bar indicates 100 μm.](image-url)
and reached confluence without any appreciable CPE.

**Budded virus yield in infected Hv-AM1 cells**

Four NPVs (AcMNPV, HearNPV, HycuMNPV and OpMNPV) produced a significant amount of BVs in the culture medium of the infected Hv-AM1 cells. In the cells infected with these NPVs, BV titer reached a maximum at 48 or 72 h pi and formed a plateau thereafter until 96 h pi (Fig. 2). At 96 h pi, BV titers accounted for $1.94 \times 10^{10}$, $1.94 \times 10^7$, $2.15 \times 10^6$ and $5.24 \times 10^5$ PFU per ml of culture medium for AcMNPV, HycuMNPV, HearNPV and OpMNPV, respectively. In the SpltMNPV-infected cells, BV titer increased slightly, if any, only during the first 24 h of infection. No significant BV yields were detected in the Hv-AM1 cells infected with BmNPV and SeMNPV, regardless of the presence of severe CPE resulting in apoptosis. LdMNPV yielded no detectable BV in the Hv-AM1 cells, consistent with lack of appreciable CPE upon infection.

**Viral DNA production in infected Hv-AM1 cells**

Viral DNA production in Hv-AM1 cells was observed...
for all NPVs examined, with the exception of LdMNPV (Fig. 3). Viral DNA was first detected at 24 h pi in all of the NPVs producing viral DNA in the Hv-AM1 cells. At 24 h pi, AcMNPV, BmNPV and SeMNPV produced relatively high amounts of DNA, compared with HearNPV, HycuMNPV, OpMNPV, and SpltMNPV. The amount of viral DNA increased with time for all of the NPV-infected cells, with the exception of HycuMNPV, in which viral DNA decreased drastically at 96 h pi.

**GP64 production in infected Hv-AM1 cells**

To determine if viral structural proteins were synthesized in the Hv-AM1 cells following infection with the respective NPVs, the production of the envelope fusion protein GP64 was examined by immunoblot analysis. Examination of GP64 protein production in the infected Hv-AM1 cells was performed only for AcMNPV, BmNPV, HycuMNPV and OpMNPV, since GP64 was encoded exclusively by the genome of group I NPVs. The results showed that AcMNPV and HycuMNPV produced high levels of GP64 protein, as compared with those for BmNPV and OpMNPV (Fig. 4). In both AcMNPV and HycuMNPV, GP64 protein was detected clearly in the infected cells at 24 h pi, increased at 48 h pi and then formed a plateau until 96 h pi. A unique pattern was observed in GP64 protein production in BmNPV-infected Hv-AM1 cells, in which the GP64 protein were detected as a faint band at 24 h pi, increased dramatically at 48 h pi, and then decreased drastically to a negligible level at 72 and 96 h pi. In the OpMNPV-infected Hv-AM1 cells, the GP64 protein exhibited faint bands throughout the experiments, which appeared at 24 h pi, increased to 72 h pi, and then declined at 96 h pi (Fig. 4).

**Polyhedrin production in infected Hv-AM1 cells**

To determine whether polyhedron formation observed in the infected cells was correlated with polyhedrin production, we conducted immunoblot analysis using antiserum against BmNPV polyhedrin. In agreement with the observed polyhedra production, AcMNPV, HearNPV, HycuMNPV, OpMNPV and SpltMNPV produced polyhedrin in Hv-AM1 cells (Fig. 5). Polyhedrin production was detected at 24 h pi for AcMNPV, HearNPV and HycuMNPV, while Hv-AM1 cells infected with OpMNPV and SpltMNPV showed polyhedrin production at 48 h pi. There was no detectable polyhedrin in the cells infected with BmNPV, SeMNPV and LdMNPV, consistent with the observed absence of polyhedron formation.

**Cellular DNA fragmentation and caspase-3-like activity in infected Hv-AM1 cells**

Examination under a microscope showed that the Hv-
AM1 cells exhibited hallmarks of apoptosis upon infection with HycuMNPV, BmNPV and SeMNPV (Fig. 1; Table 1). To determine whether the morphological changes observed in the infected cells were due to apoptosis, the cellular DNA isolated from the Hv-AM1 cells infected with these three NPVs were analyzed on an agarose gel. An oligomeric DNA ladder was clearly observed as early as 24 h pi in the cells infected with BmNPV and SeMNPV, whereas HycuMNPV-infected cells exhibited the oligomeric ladder at 48 h pi (Fig. 6A), consistent with the time course for apoptosis in the infected cells observed under a microscope. No oligomeric DNA ladder was detected in the mock-infected cells.

Consistent with the apoptotic morphological changes and the DNA fragmentation observed in the infected cells, caspase-3-like protease activity increased clearly in the Hv-AM1 cells following infection with HycuMNPV, BmNPV and SeMNPV (Fig. 6B).

**DISCUSSION**

In an effort to develop an effective biopesticide for *H. armigera*, we previously cloned a HearNPV strain with high insecticidal activity (Ogembo et al., 2007) and screened homologous and heterologous cell lines that supported high levels of HearNPV production (Ogembo et al., 2008). In this study, expecting to obtain an additional versatile and feasible cell line that would be useful for mass production and to further study the biology and biotechnology of HearNPV, we have evaluated the cell line Hv-AM1, derived from *H. virescens*, with respect to its permissiveness and productivity for HearNPV. The cell line Hv-AM1 has previously been found to be permissive for AcMNPV, HzSNPV and *Anticarsia gemmatalis* MNPV (AgMNPV) (McIntosh et al., 1981; Grasela and McIntosh, 1998), but has not been examined for its permissiveness for HearNPV. Our results showed for the first time that the cell line Hv-AM1 supported the productive infection of HearNPV, although the production of HearNPV BVs, polyhedra and polyhedrin observed was lower than those of previously characterized cell lines Hz-AM1 from *H. zeas* and HaAO1 from *H. armigera* (Ogembo et al., 2008). These results show that the potential of the cell line Hv-AM1 for the production of HearNPV and as a research material for HearNPV are limited.

Our results for eight different NPVs showed that the cell line Hv-AM1 exhibited variable types of responses against NPV infections, resulting in abortive infection and apoptosis as well as productive infection. Among the NPVs examined in this study, AcMNPV, HearNPV, HycuMNPV and OpMNPV productively infected the cell line Hv-AM1, yielding moderate to high levels of BVs, polyhedrin and polyhedra. These results, together with results that showed the productive infection of HzSNPV and AgMNPV in the cell line Hv-AM1 (McIntosh et al., 1981), indicate that the cell line Hv-AM1 is permissive for a minimum of six NPV species. The high permissiveness of cell line Hv-AM1 for such a broad range of NPV species is consistent with that of most other cell lines established from *H. virescens*, while the permissiveness of the cell lines for other members of the subfamily Heliothinae, including *H. zeas*, *H. punctigera* and *H. armigera*, is restricted commonly to one or a few numbers of NPV species (Lynn, 2007; Ogembo et al., 2008). It is unknown why cell lines from insect species within the same subfamily exhibit such diverse responses to NPV infections.

In addition to AcMNPV, HearNPV, HycuMNPV and OpMNPV, SpltMNPV yielded limited but significant amounts of BVs and polyhedrin in the cell line Hv-AM1, manifesting CPE from 24 h pi onward, although no clear polyhedron formation was observed even as late as 96 h pi. These results indicate that the cell line Hv-AM1 is semipermissive for SpltMNPV infection. Comparative studies between SpltMNPV and those NPVs which represented the productive infection of the cell line Hv-AM1, such as AcMNPV, HearNPV and HycuMNPV, will provide an excellent opportunity to determine the possible viral factors responsible for sufficient progeny production.

Our results showed that the cell line Hv-AM1 was...
highly susceptible to AcMNPV. In the AcMNPV-infected Hv-AM1 cells, the BV yield in the culture medium increased rapidly during the first 24 h of infection, reaching a maximum level as high as $1.94 \times 10^{10}$ PFU/ml, and the proportion of cells with polyhedra increased with time pi to reach more than 95% of the total infected cells at 24 h pi, indicating that the productivity of AcMNPV in the cell line Hv-AM1 was superior or comparable to those in cell lines used conventionally for AcMNPV infection, such as Sf21 and Sf9 from Spodoptera frugiperda, and Tn368 and High Five from Trichoplusia ni (McIntosh and Ignoffo, 1981; Goodman et al., 2001; McIntosh et al., 2005). Our findings in this study are supported by previous results indicating that the Hv-AM1 is a better cell line for AcMNPV production than other lepidopteran cell lines (McIntosh and Ignoffo, 1989; McIntosh et al., 2005). The results obtained in this study thus indicate that the cell line Hv-AM1 will be invaluable in optimizing the mass production of AcMNPV and may also be useful in the production of foreign proteins using expression vectors and bacmids generated from AcMNPV.

The abortive infection of NPVs has not been found in cell lines from heliothine species. In this study, we showed that the Hv-AM1 cells infected with BmNPV and SeMNPV manifested severe CPE and synthesized substantial amounts of viral DNA, without yielding any detectable BVs or polyhedra. Immunoblot analyses of BmNPV-infected Hv-AM1 cells showed that the envelope fusion protein GP64 was detected substantially only at 48 h pi and remained at a negligible level at 24, 72 and
96 h pi. This result implies that a unique interaction occurs between BmNPV and the cell line Hv-AM1, yet further experiments are required to explore the mechanisms involved in this interaction. As the abortive infection of SeMNPV, it is not clear in this study if SeMNPV infection is restricted at a step either prior or subsequent to the expression of viral structural proteins.

The cell line Hv-AM1 underwent apoptosis upon infection with BmNPV, HycuMNPV and SeMNPV, as indicated by the presence of apoptotic cellular DNA fragmentation and increased caspase-3-like protease activity in the infected cells. Among these NPVs, HycuMNPV yielded substantial amounts of BVs, polyhedra and polyhedrin, while BmNPV and SeMNPV produced no detectable BVs or polyhedra, despite their considerable synthesis of viral DNA. This difference may reflect variations in viral strategies against apoptosis induction. In the HycuMNPV-infected Hv-AM1 cells, it is worthy of further study to identify the viral product(s) that may be involved in the suppression of apoptosis until the yield of considerable viral progenies, or that is responsible for the induction of apoptosis after the achievement of sufficient viral yields. It would also be interesting to determine if the apoptosis after the yield of progeny viruses, as observed in the HycuMNPV-infected Hv-AM1 cells, occurs in vivo in H. virescens larvae following infection with HycuMNPV, and if such apoptosis is of any benefit to HycuMNPV in promoting the quick spread of the viral infection within an insect.

Our results thus showed that although its potential to serve in the mass production of HearNPV was limited, the cell line Hv-AM1 could provide an excellent opportunity to analyze the molecular mechanisms underlying permissiveness and productivity for NPVs, due to its characteristic properties of diverse responses and high permissiveness against NPVs.

ACKNOWLEDGMENTS

We thank Dr. T. Yaginuma and Dr. T. Niimi, the Laboratory of Sericulture and Entomoresources, Nagoya University, Japan, for their helpful discussion during this study. J.G.O. was a recipient of a scholarship from the Ministry of Education, Science, Sports and Culture of Japan. B.L.C. was supported by a Postdoctoral Fellowship for Foreign Researchers from the Japan Society for the Promotion of Science (JSPS). This work was supported by the JSPS-NRCT Joint Research Program between Japan and Thailand and, in part, by grants-in-aid (16380040, 19208006) from the JSPS.

REFERENCES


