Susceptibility of Lepidopteran Cell Lines to a *Spodoptera exigua* (Lepidoptera: Noctuidae) Nuclear Polyhedrosis Virus

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*Spodoptera exigua* nuclear polyhedrosis virus (SeNPV) was examined for replication in 20 continuous cell lines from eight lepidopteran species; *Spodoptera frugiperda*, *Spodoptera littoralis*, *Spodoptera litura*, *Spodoptera exigua*, *Pseudalecia separata*, *Mamestra brassicae*, *Plutella xylostella*, and *Bombyx mori*. Of these, only five homologous cell lines, established from *S. exigua*, were permissive for infection with the virus. The growth kinetics of SeNPV in *S. exigua* Se3FH cells showed that extracellular viruses were released from infected cells 6 h p.i. and reached a maximal titer 72 h p.i. The number of polyhedral inclusion bodies (PIBs) reached a maximum of $10^7$ PIBs/ml 96 h p.i.

**Key words:** *Spodoptera exigua*, nuclear polyhedrosis virus, *in vitro* virus replication

**INTRODUCTION**

The beet armyworm, *Spodoptera exigua*, is a serious pest of vegetables, grain and turf crops in subtropical areas and in greenhouses of temperate regions (TRUMBLE and BAKER, 1984). Because of the increased resistance to chemical insecticides, alternative biological control methods have been considered (VLAK et al., 1982) and the baculovirus *Spodoptera exigua* nuclear polyhedrosis virus (SeNPV) has been shown to be an effective agent in controlling the beet armyworm (CHAUTHANI and REHNBORG, 1971; GELERNTER et al., 1986; SMITS et al., 1987; SMITS and VLAK, 1988). The successful replication of SeNPV *in vitro* was first reported by GELERNTER and FEDERICI (1986) who used a cell line established from *S. exigua* larvae. More recently, HARA et al. (1993) established five cell lines, designated Se3FH, Se4FH, Se5FH, Se6FHA, and Se6FHB, from the larvae of *S. exigua*. In Se3FH and Se5FH, adherent spindle-shaped cells were predominant. In contrast, spherical cells, consisting of adherent and floating cells, were predominant in Se4FH, Se6FHA and Se6FHB. The population doubling time of five cell lines ranged from 19 h to 37 h. All continuous cell lines were heteroploidy and the chromosome numbers ranged from 12 to about 300. These five cell lines all supported the replication of SeNPV, Se3FH showing the highest susceptibility.

In this paper, we further investigated the host range of SeNPV in lepidopteran cell lines and the growth kinetics of SeNPV in *S. exigua* cultured cells.

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MATERIALS AND METHODS

Rearing of insect. S. exigua was kindly provided by Mr. H. Hayashi, Hiroshima Prefectural Agricultural Experiment Station, Hiroshima, Japan. The larvae were reared on an artificial diet (Okada, 1990; Hayashi, 1991) at 27°C under a photoperiod of 16L:8D.

Cell lines and culture conditions. Five Spodoptera exigua cell lines (Hara et al., 1993) and 15 other lepidopteran cell lines used in this study are listed in Table 1. The cell lines were grown in IPL-41 medium (Dougherty et al., 1981) supplemented with 10% heat-inactivated (60°C, 30 min) fetal bovine serum (FBS). Cells were seeded in 25-cm² tissue culture flasks (Falcon, 3013) at a density of 5 × 10⁵ cells/ml in 4 ml of medium, incubated at 27°C, and subcultured at 4-day intervals.

Virus preparation and inoculation. The SeNPV was propagated as previously described (Hara et al., 1993) by infection of early 4th-instar S. exigua larvae by feeding them with an artificial diet surface-contaminated with 1.0 × 10⁶ polyhedral inclusion bodies (PIBs)/larva. Four days after inoculation, infected hemolymphs, containing extracellular viruses, from several living larvae were pooled and centrifuged at 8,000 rpm for 10 min. The supernatant was stored at −80°C until use. For inoculation, infected hemolymph was diluted 1:100 in IPL-41 medium and passed through a 0.45-µm membrane filter. The dilution (0.1 ml) was incubated with cells (0.4 ml/well) in exponential phase in a multiwell tissue culture plate (Falcon, 3014) with a cell density of 6 × 10⁵/ml. Glutathion (0.6 mg/ml), for prevention of melanization, and 1% antibiotics (Gibco, penicillin 10,000 units/ml-streptomycin 10,000 µg/ml) were added. The plates were sealed and incubated at 27°C. Wells were examined for the presence or absence of PIBs by phase-contrast microscopy.

Kinetics of viral growth. S. exigua cells in exponential phase were seeded into a 50-ml conical tube (Falcon, 2070) at a density of 6 × 10⁵ cells/ml in IPL-41 medium supplemented with 10% FBS, 1% antibiotics and 0.6 mg/ml glutathion. The SeNPV was inoculated to cells at a MOI of 0.1 TCID₅₀ per cell. The virus adsorption proceeded for 1 h at 27°C with gentle stirring every 10 min, followed by two washes with the IPL-41 medium by low-speed centrifugation (600 rpm, 4 min). Cells were resuspended in fresh medium to a density of 6 × 10⁵ cells/ml and incubated in multiwell tissue culture plate (0.5 ml/well) at 27°C. At suitable intervals, the medium was removed and centrifuged at 3,000 rpm for 10 min. The supernatant was stored at −80°C until use. The virus titers were determined by end-point dilution assays. Se3FH cells in exponential phase (0.1 ml/well) were seeded in Microtestplate-II (Falcon, 3042) at a cell density of 1.0 × 10⁴ cells/ml in IPL-41 medium containing 10% FBS and 1% antibiotics. The cells were infected with 10-times serial dilutions of the virus (0.1 ml/well) in IPL-41 medium. Five wells were used for each virus dilution. After rocking for 1 min, the plates were incubated at 27°C for 10 days. The wells were scored as positive when PIBs were observed under a phase-contrast microscope. TCID₅₀ values were calculated according to the method of Reed and Muench (1938). For quantification of PIBs, infected cell cultures were harvested at adequate intervals, and centrifuged at 3,000 rpm for 10 min. The pellet was resuspended in a small volume of IPL-41 medium and sonicated to release cell-associated PIBs. The sample was repelleted at 3,000 rpm for 10 min, and three independent PIB counts were made with a hemocytometer.
RESULTS

Table 1 shows the results of in vitro host range study with SeNPV. There was no formation of PIBs and cytopathological changes in 15 cell lines derived from seven lepidopteran species, *S. frugiperda*, *S. littoralis*, *S. litura*, *P. separata*, *M. brassicae*, *B. mori*, and *P. xylostella*. Apparently, these 15 cell lines were not susceptible to the SeNPV. In contrast, five *S. exigua* cell lines were all susceptible to the *S. exigua* NPV (Fig. 1).

Figures 2 and 3 represent the growth kinetics of SeNPV in *S. exigua* cell lines. The extracellular infectivity titer of SeNPV in Se3FH cell line was the highest among five *S. exigua* cell lines. In this cell line, the extracellular virus titer began to increase at 6 h p.i. and reached the highest titer of approximately $10^6$ TCID$_{50}$ units/ml at 72 h p.i. (Fig. 2). On the other hand, the extracellular virus titer was very low in the Se4FH and Se6FHA cell lines (Fig. 3).

<table>
<thead>
<tr>
<th>Insect species</th>
<th>Cell line</th>
<th>Source</th>
<th>Susceptibility to SeNPV$^1$</th>
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</thead>
<tbody>
<tr>
<td><em>Spodoptera frugiperda</em></td>
<td>SF9</td>
<td>T. HARA$^a$</td>
<td>—</td>
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<tr>
<td></td>
<td>SF21AEII</td>
<td>J. VAUGHN$^b$</td>
<td>—</td>
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<td></td>
<td>CLS79</td>
<td>J. VAUGHN</td>
<td>—</td>
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<tr>
<td></td>
<td>TUAT-SpLi-221</td>
<td>J. MITSUHASHI$^c$</td>
<td>—</td>
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<tr>
<td></td>
<td>Se3FH</td>
<td>K. HARA$^h$</td>
<td>+</td>
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<tr>
<td></td>
<td>Se4FH</td>
<td>K. HARA</td>
<td>+</td>
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<td></td>
<td>Se5FH</td>
<td>K. HARA</td>
<td>+</td>
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<td></td>
<td>Se6FHA</td>
<td>K. HARA</td>
<td>+</td>
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<td></td>
<td>Se6FHB</td>
<td>K. HARA</td>
<td>+</td>
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<tr>
<td><em>Pseudaletia separata</em></td>
<td>NIAS-LeSe-11</td>
<td>J. MITSUHASHI</td>
<td>—</td>
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<tr>
<td></td>
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<td>J. MITSUHASHI</td>
<td>—</td>
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<td></td>
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<td>NIAS-MB-32</td>
<td>J. MITSUHASHI</td>
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<tr>
<td></td>
<td>NIAS-MaBr-92</td>
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<tr>
<td><em>Mamestra brassicae</em></td>
<td>BM-N</td>
<td>S. MAEDA$^e$</td>
<td>—</td>
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<tr>
<td></td>
<td>Bm-5</td>
<td>T. SATO$^g$</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>SES-BoMo-15A</td>
<td>H. INOUE$^f$</td>
<td>—</td>
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<tr>
<td></td>
<td>NIAS-BoMo-15AIIC</td>
<td>H. INOUE</td>
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<td></td>
<td>S.P.C.Bm36</td>
<td>J.-M. QUIOT$^g$</td>
<td>—</td>
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<tr>
<td><em>Plutella xylostella</em></td>
<td>PX1/C</td>
<td>T. SATO</td>
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</table>

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$^h$ Faculty of Agriculture, Kyushu University, Fukuoka, Japan.
$^i$ —: Polyhedral inclusion bodies were not formed. +: Polyhedral inclusion bodies were formed.
Fig. 1. Phase-contrast photographs of the *Spodoptera exigua* cell lines infected with *S. exigua* nuclear polyhedrosis virus 4 days postinoculation. Cell lines infected with the virus were as follows: (A) Se3FH, (B) Se4FH, (C) Se5FH, (D) Se6FHA, and (E) Se6FHB. Bar=50 μm.
Fig. 2. Replication of *Spodoptera exigua* nuclear polyhedrosis virus in *S. exigua* Se3FH cell line. Cells were infected with the virus at a MOI of 0.1 TCID\textsubscript{50} unit/cell. The extracellular virus (●) was monitored by TCID\textsubscript{50} assay, and the total number of polyhedral inclusion bodies produced (○) was quantified after the cells were disrupted by sonication.

Fig. 3. Replication of *Spodoptera exigua* nuclear polyhedrosis virus in *S. exigua* Se4FH, Se5FH, Se6FHA, and Se6FHB cell lines. Cells were infected with the virus at a MOI of 0.1 TCID\textsubscript{50} unit/cell. The extracellular virus was monitored by TCID\textsubscript{50} assay. Arrow shows that the titer is less than, or equal to, the value indicated.

The PIBs were first detected in *S. exigua* cells 24 h p.i. and most of them were released into the medium by lysis of infected cells at advanced stages of infection. In Se3FH cell line, the number of PIBs reached the maximum of 10\textsuperscript{7} PIBs/ml 96 h p.i. (Fig. 2). The number of PIBs per cell in the infected cells ranged from 20 to 149 with a mean value of 92 ± 40 (X ± SD).
DISCUSSION

Studies of baculovirus host specificity have shown that most NPVs possess a relatively narrow host range both in vivo and in vitro (Gröner, 1986; Granados and Hashimoto, 1989). Generally, NPVs infect only members of the genus or, in some cases, the family of the original host (Gröner, 1986; Bilimoria, 1991). In addition, transfection experiments have shown that in several NPVs the host range of virus DNA is substantially the same as that of the intact extracellular virus (Burand et al., 1980; Tsuda et al., 1990). Autographa californica NPV (AcNPV), however, has a broad host range and successfully infects over 25 insect species (Adams and McClintock, 1991). Similarly, AcNPV can replicate in many cell lines derived from six families of Lepidoptera (Granados and Hashimoto, 1989).

In the present study, the SeNPV replicated in only S. exigua cell lines, indicating high host specificity of this virus in vitro. Gelernter and Federici (1986) demonstrated that the host specificity of SeNPV was as high in vitro as it was in vivo; the infection of SeNPV did not occur in cell lines of Trichoplusia ni, Spodoptera frugiperda, S. littoralis, Estigmene acrea, Mamestra brassicae.

In general, the NPVs infect cells derived from their homologous host. However, in this study there is difference in viral susceptibility among cell lines derived from a natural host. Se3FH and Se5FH cell lines, in which spindle-shaped cells were predominant, released more extracellular viruses than the other three cell lines consisting of spherical cells. Gelernter and Federici (1986) found that the SeNPV replicated in UCR-SE-1 cell line established from minced neonate larvae consisting of epithelial-like cells and spindle-shaped cells, but only in the latter. The NPV susceptibility of cell lines may vary depending on the source of tissues. S. exigua cell lines established from minced neonate larvae probably contain many cell types of different origins. So, these cell lines may be permissive to virus with varying degrees of susceptibility.

The virus growth kinetics study with the Se3FH clearly showed the existence of three phases: the latent phase, the exponential phase, and the stationary phase. This is in good agreement with the results obtained by earlier workers (Granados, 1976), for various combinations of NPVs and cultured cells.

Knutson and Tinsley (1974) reported that, in S. frugiperda NPV infected S. frugiperda cells, the extracellular virus was first detected 12 h p.i. and the infectivity reached a maximum titer 4 days p.i. Our results showed that the SeNPV replicates more rapidly than the SfNPV; the extracellular viruses appeared as early as 6 h p.i. and the infectivity reached maximum titer 72 h p.i. This difference may be due to the difference in the virus and/or the cell line examined.

Volkman et al. (1976), in their study on the growth kinetics of AcNPV in Trichoplusia ni cultured cells, showed that the extracellular virus production would shut down with the onset of polyhedron formation, suggesting that the formation of polyhedra had an inhibitory effect on the budding of extracellular viruses. In our study with the SeNPV, the polyhedral formation occurred concomitantly with the production of extracellular virus; however, the production of extracellular viruses stopped midway in the polyhedral formation stage. Whether the accumulation of polyhedrin suppresses the virus replication will be the subject of our future work.

Attempts are currently underway to optimize the production of the virus and increase the efficiency of the assay by single-cell cloning.
SeNPV in vitro

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REFERENCES


