

A New Continuous Cell Line of *Spodoptera exigua* and Its Susceptibility to *Autographa californica* Multicapsid Nucleopolyhedrovirus

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Abstract—A continuous cell line, designated KU-SEN1-1 (SEN1), has been established from minced neonate larvae of *Spodoptera exigua* (Lepidoptera: Noctuidae) treated with collagenase. The primary culture was maintained in TC100 medium supplemented with 10% Fetal Bovine Serum (FBS), 3% *Helicoverpa armigera* hemolymph and incubated at 27 °C. This continuous cell line was cultured in TC100 medium supplemented with 10% FBS and subcultured at 5-day intervals. The cell line consisted of a mixture of two cell types, epithelial-like cells and spindle-shaped cells, both of which grown as attached monolayers. The population doubling time of this new cell line during the logarithmic phase of growth was 45h. RAPD and DAF analyses confirmed that the origination of the SEN1 cell line was *S. exigua*. The susceptibility of this cell line to the *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) was high and by 3 days postinfection (pi) greater than 90% of the cells contained Occlusion Bodies (OBs) or were greatly hypertrophied, indicating they were infected. This cell line was highly effective for Budded Viruses (BV) titration of the AcMNPV. Therefore, the SEN1 cell line will be a valuable new tool for biological characterization of AcMNPV in cell culture and also for protein expression using the baculovirus-insect cell expression vector system.

Index Terms—*Spodoptera exigua*, *Autographa californica*, nucleopolyhedrovirus, insect cell culture, plaque assay

I. INTRODUCTION

Insect cell lines are valuable in a wide range of biological research, including physiology, pathology and are useful for the production of recombinant proteins [1]. Given the wide applicability of insect cell lines, the establishment of more insect cell lines would be beneficial [2]. In particular, cell lines from insect pests of economical importance were established for the primary purpose of studying and optimizing baculovirus production for the pest control [3]-[6]. Many cell lines derived from lepidopteran pests such as the cell lines from *Spodoptera frugiperda*, *S. exigua*, and *Trichoplusia ni* are susceptible to *Autographa californica* multicapsid

nucleopolyhedrovirus (AcMNPV) which is frequently used for recombinant protein expression [7].

NPVs represent a group of insect virus in the Family Baculoviridae [8] whose virions are embedded into polyhedron-shaped Occlusion Bodies (OBs) or polyhedra in the nuclei of host cells. The baculovirus AcMNPV is one of the most popular, commonly used and well-developed of eukaryotic protein expression vectors. This is due in large part to the so-called hyper expression of genes inserted under the control of the polyhedrin promoter of this virus. While very late genes of AcMNPV are expressed at extremely high levels, the level of expression achieved from heterologous proteins may vary considerably in different insect cell lines. The most widely used lepidopteran cells for AcMNPV replication studies, plaque assays and recombinant protein expression by the Baculovirus Expression Vector System (BEVS) are the SF9 and SF21 cell lines isolated from ovarian tissue of *S. frugiperda* [9]-[11] and BTI-Tn-5B1-4 cell line, commercially known as High Five, originally established from the *T. ni* eggs [12]. While *S. frugiperda* cell lines, either SF9 or SF21 and High Five cell line likely represent the most highly productive cell line currently in widespread use, there remains enormous potential for development of new cell lines from different insect species, from different tissue sources, and by engineering existing cell lines for specific improvements [13].

In the present study, the establishment and characterization of a new continuous cell line from the neonate larvae of *S. exigua* were described. In order to evaluate the potential of this new cell line for the production of AcMNPV, the virus was propagated in cell monolayers and the kinetics of virus replication were determined.

II. MATERIALS AND METHODS

A. Primary Culture and Subculture

A laboratory colony of *S. exigua* was reared on an artificial diet [14] and allowed to grow to the pupal stage. At adult emergence, 10 females and an equal number of

males were selected for mating in single pairs, transferred to plastic boxes that were lined internally with wax paper to facilitate egg laying and which contained 10% honey-soaked cotton balls in the cup for adult feeding. The eggs were surface-sterilized by being soaked for 10 min in 10% formaldehyde, rinsed twice with sterile distilled water and then dried under the laminar flow cabinet in a container lined with sterile filter paper. The explants were obtained by crushing two hundred neonate larvae which hatched within a 24-h period in a sterile 15-ml centrifuge tube containing a solution of 0.3% (w/v) collagenase dissolved in Hank's balanced salt solution [15] and incubated 30 min at 37 °C. Following the incubation period, the collagenase was removed by centrifugation 5 min at 550g, and the supernatant was discarded. The primary cultures were maintained in 5ml of TC100 medium [16] supplemented with 10% Fetal Bovine Serum (FBS) and 3% hemolymph of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in a 25-cm² tissue culture flask and incubated at 27°C.

The first subculture was made when cells in the culture flask became confluent. Subculture was carried out by detaching the cells from the surface of the flask, and transferred these cells into a new flask. Once the cells had been cultured for 3 months, the concentration of hemolymph was gradually reduced and deleted. Finally, the cells were cultured in TC100 medium supplemented with 10% FBS and were subcultured at 5-day intervals.

B. Growth Kinetics

The kinetics of cell growth were determined at passages 50 by recording cell densities for 7 days. *S. exigua* cells (2×10^5 cells/ml) were seeded into 25-cm² culture flasks with a culture volume of 5 ml of TC100 medium supplemented with 10% FBS. The cell density and viability were determined at 24 h intervals by using a hemocytometer and trypan blue staining. A cell growth curve was generated from the average of three independent experiments. An exponential regression was used to calculate the population doubling time during the logarithmic phase of growth [17].

C. DNA Extraction

Insect cells grown in the logarithmic phase were harvested from the culture flask and pellet was washed twice in Phosphate-Buffered Saline (PBS). Total genomic DNA was extracted using PureLink[®] Genomic DNA Mini Kit (Invitrogen), according to the manufacturer's instructions. For comparison, DNA was also extracted from *S. exigua* neonate larvae and other insect cell lines maintained in the laboratory. These cell lines were as follows: SE301 (*S. exigua*) [18], SF9 (a clonal isolate of IPLBSF21-AE (SF21) [9], [19], HZ-AM1 (*H. zea*) [20], one cell line established from pupal ovaries of *S. litura*, SLPO1, and three cell lines initiated from pupal ovaries of *H. armigera*, HAPO2, HAPO2 B-5 and HAPO2 D-4. The SLPO1, HAPO2 and its clonal cell lines (B-5 and D-4) were developed in this laboratory.

D. Cell Line Characterization

In order to identify *S. exigua* cell line established in this study, two Polymerase Chain Reaction (PCR)-based methods including Random Amplified Polymorphic DNA (RAPD) and DNA amplification fingerprinting (DAF) were performed. Two 10-mer random primers No. 212 (5'-TTG CTG TCC A-3') and No. 273 (5'-GCT GAC CTG T-3') were used for RAPD-PCR as described by [21]. PCR amplification reaction volume was 50µl which contained 1X PCR buffer (Invitrogen), 1.5mM MgCl₂, 0.2mM dNTPs, 1 U Taq DNA polymerase (Invitrogen), 40 pmol of primer No. 212 or primer No. 273 and 200 ng of cellular DNA. The RAPD-PCR thermal profile consisted of an initial incubation at 94 °C for 5 min, followed by 40 cycles of 20 sec at 94 °C, 20 sec at 45 °C and 30 sec at 72 °C, then a final extension at 72 °C for 6 min using a GeneAmp PCR System 9700, PE Applied Biosystems DNA thermal cycler.

DAF-PCR was performed with primers aldolase 1 (5'-CCG GAG CAG AAG AAG GAG CT-3') and aldolase 2 (5'-CAC ATA CTG GCA GCG CTT CA-3') as described by [22]. Each PCR consisted of 50µl mixture which contained 1X PCR buffer (Invitrogen), 1.5mM MgCl₂, 0.2mM dNTPs, 1 U Taq DNA polymerase (Invitrogen), 20 pmol of primer aldolase 1, 20 pmol of primer aldolase 2 and 200 ng of cellular DNA. The PCR was performed under the following conditions: initial incubation at 94 °C for 5 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 56 °C and 1 min at 72 °C, then a final extension at 72 °C for 5 min. The PCR products were subjected to agarose gel electrophoresis using 1X Tris-Borate-EDTA (TBE) buffer to determine the size, and DNA banding patterns.

E. Virus Susceptibility

Fourth instar *S. exigua* larvae were fed on formalin-free artificial diet [14] that had been contaminated with AcMNPV (a kind gift from M. Ikeda, Nagoya University, Japan). Five days after inoculation, hemolymph containing Budded Viruses (BV) was collected by bleeding the infected larvae from the first proleg. The hemolymph was transferred immediately into ice-cold, sterile microcentrifuge tubes containing L-cysteine to prevent melanization [23], pooled and diluted 1:1 in complete culture medium. The diluted infectious hemolymph was passed through a 0.45 µm membrane filter and used as an inoculum for infection of the *S. exigua* cells to generate the BV inoculum. The BV from the culture medium of *S. exigua* cells 4 days postinfection (pi) was titrated three times by plaque assay [24] prior to use as inoculum for the next experiment. Further analyses of the virus were performed to study the kinetics of virus replication. Cell monolayers were infected at a Multiplicity of Infection (MOI) of 1 Plaque Forming Unit (PFU)/cell, the virus inoculum was allowed to adsorb for 1h at room temperature and then replaced with fresh medium. Time zero was defined as the time when the inoculum was replaced with fresh medium. The cultures were observed daily for evidence of Cytopathic Effects

(CPE) and culture media were collected and then subjected to virus titration by plaque assay [24].

F. Plaque Assay

The plaque assay was performed as described by [24]. Briefly, a confluent monolayer of *S. exigua* cells grown in 35-mm culture dishes was infected with the serial 10-fold dilutions of AcMNPV. Three replicates of each dilution were performed. The plaque assay used a SeaPlaque GTG agarose (Lonza) overlay and an incubation period of 6 days. Plaques were counted after staining with crystal violet.

G. Electron Microscopy

The mock-infected cells and AcMNPV-infected cells were harvested by using a rubber policeman, pelleted at 1000g and washed twice in PBS. Pellets were resuspended and fixed with 3% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, for 2h at 4 °C, postfixed in 1% osmium tetroxide for 1 h and washed twice with PBS. Samples were then seeded onto stubs and were allowed to air dry. Then stubs were coated with gold and observed under scanning electron microscope.

III. RESULTS AND DISCUSSION

A. Primary Culture and Subculture

The explant tissues began to adhere to the culture flask and cell migration occurred within 1 day of initiation of the primary culture (Fig. 1A). The migrated cells were either epithelial-like cells and spindle-shaped cells. The number of cells started to increase slowly during the first month. They initially dispersed themselves around tissue explants, gradually distributed to the surrounding areas, and finally formed adherent monolayers in the culture flask after 3 months of culturing (Fig. 1B). The first successful subculture was achieved after 6 months of culturing when cells in the culture flask became confluent. The culture flask was gently tapped to dislodge the adherent cells which were subsequently transferred into a new 25-cm² flask. At this stage, the active proliferation of cells was observed (Fig. 1C).

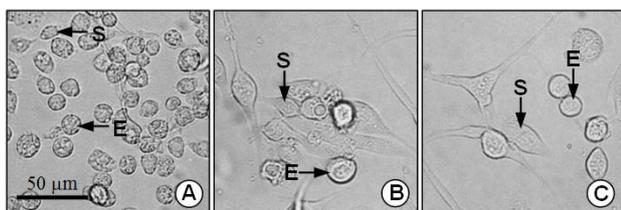


Figure 1. Micrographs of the primary culture derived from neonate larval tissues of *S. exigua*. (A) Primary culture observed at 1 day after culture initiation. (B) Cells in primary culture after 3 months of culturing. (C) The morphology of cells at passage 1. E, epithelial-like cells; S, spindle-shaped cells.

In the present study, attempts to obtain the first subcultures as early as 3 months were unsuccessful. Cultures ceased growing and gradually deteriorated. The required period from an established primary culture to the first successful subculture are diverse. It can take a month

[25], a few months [26]-[28], or even more than 6 months [29], relying on insect species and tissue origins.

After the 10th passage, the cells exhibited stable growth and proliferation. The resulting cell line was considered a new continuous cell line after the 50th passage and designated as KU-SENL-1 (SENL1). The morphological characteristics of SENL1 cells as revealed by light and scanning electron microscopy were similar. Two dominant shapes can be distinguished in the SENL1 cell line, epithelial-like cells and spindle-shaped cells (Fig. 2A, 3A). Cells of both types grown in monolayers attached to the culture flask. As cells grown beyond confluency, the morphology of spindle-shaped cells changed slightly, becoming more rounded (Fig. 2B-C, 3B-C). Some cell aggregates were observed in SENL1 cell culture as cell density approached confluency (Fig. 2C). The observed clumping may result from a low level of contact inhibition, and that may contribute to the observation of high cell density in adherent insect cell cultures. Contact inhibition shown by various mammalian cell lines [30] is low or absent for insect cells, and they tend to aggregate in suspension or in adherent cultures. Therefore, overgrown cells pile up, or detach from the culture vessel and float in the medium [29], [31].

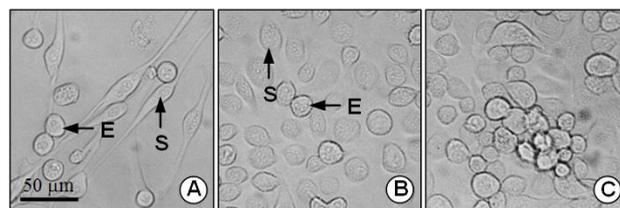


Figure 2. Micrographs of the cell characteristics of the SENL1 cell line. (A) Subconfluent monolayers. (B) Confluent monolayers. (C) Multicellular aggregations as cell density approached confluency. E, epithelial-like cells; S, spindle-shaped cells.

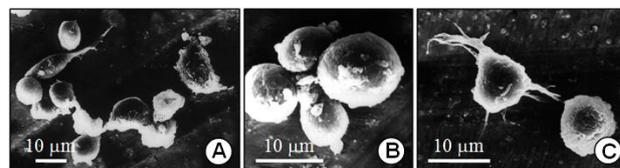


Figure 3. Scanning electron micrographs of the cultured SENL1 cells. (A) Cells in the logarithmic phase of growth. (B) The morphology of spindle-shaped cells became more rounded as cell densities were confluent. (C) Cells in the stationary phase of growth.

B. Growth Kinetics

Growth pattern of SENL1 cells in monolayer culture was characterized (Fig. 4). Cell density increased by 1 day after seeding, indicating that the seeded cells were able to adapt to the culture environment and preparing for fast growth. By 2-6 days of growth, cells increased exponentially and reached a maximum cell density of 8×10^5 cells/ml on day 6, then entered the stationary phase. At the stationary phase, high density culture and the absence of nutrients caused a change in cell shape (Fig. 2B-C, 3B-C) and cells ceased growing (Fig. 4). The population doubling time of this new cell line during the logarithmic phase of growth was 45h. The SENL1 cells showed a faster growth rate than the other *S. exigua* cells

from the growth curves [32]-[34]. Thus, this newly established cell line from *S. exigua* is a fast growing cell line and a promising candidate for future insect-cell based applications.

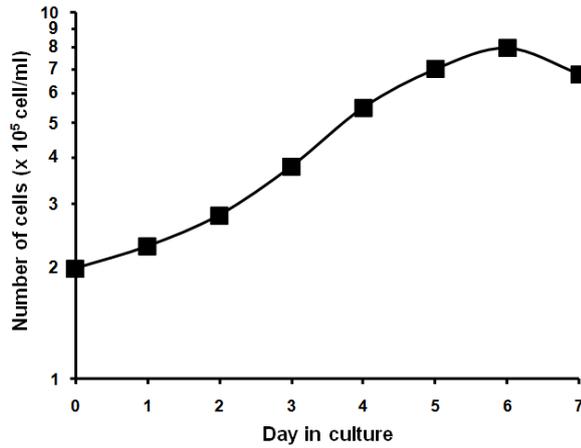


Figure 4. Growth curve of SENL1 cell line.

C. Cell Line Characterization

Characterization of the cell lines was carried out using RAPD-PCR and DAF-PCR. The RAPD analysis showed similar DNA profiles for the SENL1 cell line and the neonate larval tissues of *S. exigua* (Fig. 5). These results indicated that SENL1 cell line was derived from the *S. exigua*. In addition, comparing patterns obtained with other continuous cell lines maintained in this laboratory showed that the SENL1 cell line is unique, verifying it is a new cell line. Similar DNA banding profiles were obtained for the SENL1 and SE301 cell lines, using primer No. 212 (Fig. 5A). However, there were minor differences in band intensity. The results of RAPD analysis using primer No. 273 revealed that this primer was able to distinguish the SENL1 cell line from the SE301 cell line (Fig. 5B). As shown in Fig. 5, the RAPD analysis showed identical DNA profiles for the clonal cell lines (B-5 and D-4) and the parental line HAPO2. In contrast, the DNA profiles mentioned above were quite distinct from those of other cell lines including the cell lines from *H. zea*, *S. exigua*, *S. frugiperda* and *S. litura*.

The electrophoresis band patterns obtained after DAF analysis on the DNA extracted from SENL1 cell line, *S. exigua* neonate larvae and seven additional insect cell lines were compared (Fig. 6). The major amplified band at approximately 400bp was shared by SENL1 and SE301 cell lines and their host, while the other cell lines showed a different profile. This major band was also observed in cell lines from the larval fat bodies of *S. exigua* [22]. The profile of SE301 cell line was distinctive from that of SENL1 cell line by the presence of a major band at approximately 660bp. Analysis of clones derived from the parental line HAPO2 produced patterns that were all identical. This confirms the capability of the PCR procedure to distinguish between related cell lines.

The need to identify specific insect cell lines quickly and reliably, using the PCR-based methods such as RAPD and DAF is a crucial element of good quality

control both in the laboratory and in industry. These methods are widely used for the identification of insect cell lines as well as for the detection of a cross-contaminated cultures [21], [22], [28], [35], [36].

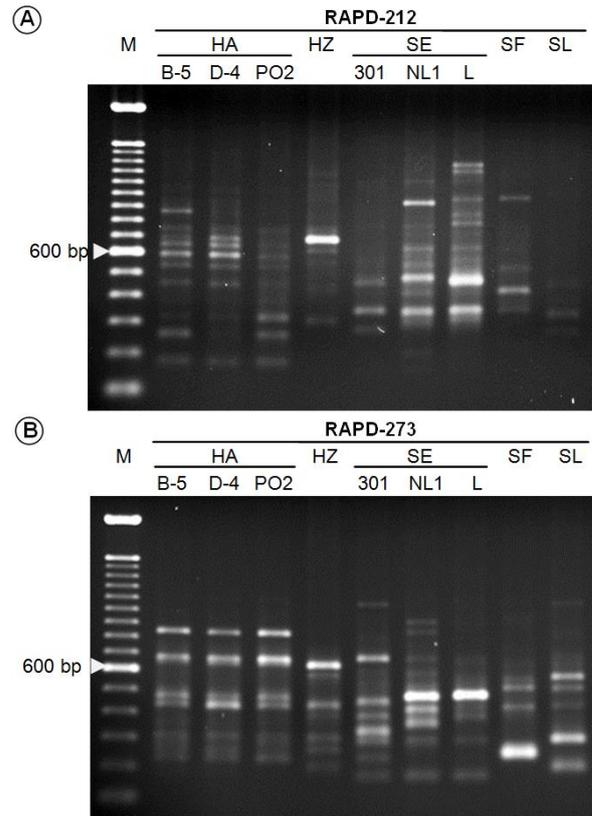


Figure 5. RAPD-PCR profiles of insect cell lines obtained with primers No. 212 and 273. (A) Primer No. 212. (B) Primer No. 273. The PCR products were electrophoresed on a 1% agarose gel along with 100-bp DNA ladder (M) with an arrowhead indicating band of 600 bp. HA, *H. armigera*; HZ, *H. zea*; SE, *S. exigua*; SF, *S. frugiperda*; SL, *S. litura*; L, neonate larvae; PO, pupal ovaries.

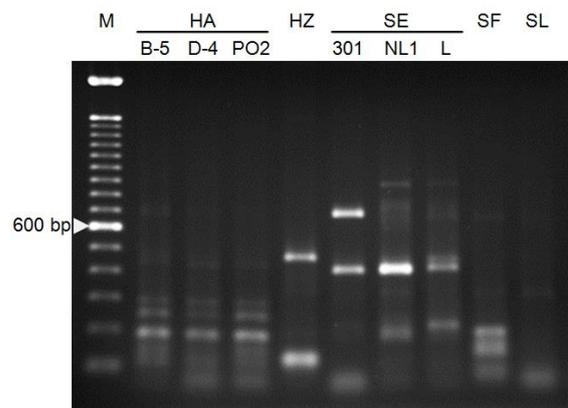


Figure 6. DAF-PCR profiles of insect cell lines obtained with aldolase primer. The PCR products were electrophoresed on a 1% agarose gel along with 100-bp DNA ladder (M) with an arrowhead indicating band of 600 bp. HA, *H. armigera*; HZ, *H. zea*; SE, *S. exigua*; SF, *S. frugiperda*; SL, *S. litura*; L, neonate larvae; PO, pupal ovaries.

The PCR-based methods used in the present study were able to distinguish inter- and intraspecies cell lines. They can be also applied to distinguish the two *S. exigua* cell lines originated from different laboratories. It is

important to note that SENL1 and SE301 cell lines originated from the neonate larval tissues of *S. exigua* revealed little similarity in DNA banding profiles. This was the case in the pairwise comparisons of the DNA profiles between SENL1 and SE301 cell lines using primer No. 273 (Fig. 5B) and aldolase primer (Fig. 6). This pattern could be reproduced. Availability of this base to laboratories involved in cell culture would allow easy identification of individual cell lines by comparison to reference profiles as well as comparison of similar lines from different sources.

D. Virus Susceptibility

For determining the susceptibility of the SENL1 cell line to AcMNPV, the infectious medium containing BV was used as an inoculum. Typical CPE like granulated and rounded cells, nuclear hypertrophy and impairment in cell proliferation were observed within 1 day pi. The CPE developed rapidly with subsequent OBs formation. The AcMNPV replicated in both epithelial-like cells and spindle-shaped cells and OBs were clearly seen within 2 days pi (Fig. 7B). These cells became loosely attached to the substrate. The susceptibility of this cell line to the AcMNPV was high and by 3 days pi greater than 90% of the cells contained OBs and some of the infected cells were dislodged from the culture flask (Fig. 7C). At the late stage of infection (4 days pi), numerous OBs were formed in the nuclei and some infected cells ruptured, releasing a large number of OBs into the medium (Fig. 7D).

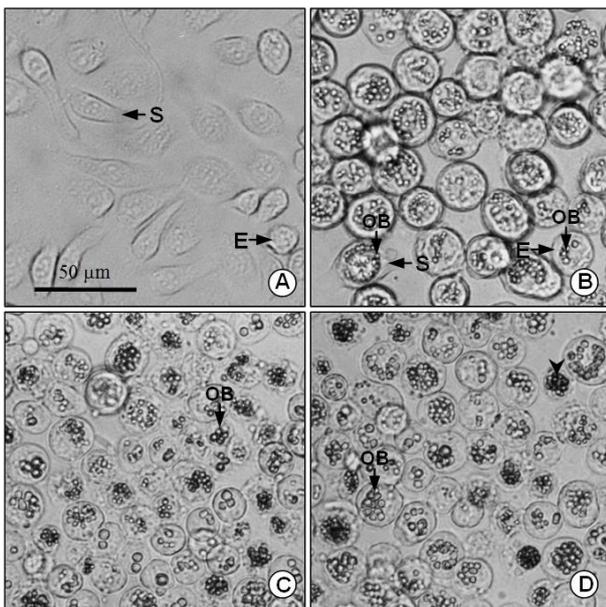


Figure 7. Cytopathology and occlusion bodies (OBs) formation in the AcMNPV-infected SENL1 cells. (A) Mock-infected cells. (B) At 2 days pi, the AcMNPV replicated in both epithelial-like cells and spindle-shaped cells. (C) At 3 days pi, the numerous OBs were formed in the hypertrophied nuclei of infected cells. (D) At 4 days pi, the infected cells were filled with OBs and some cells ruptured (arrowhead).

Scanning electron microscope observation revealed an increase in cell size by 2 days pi (Fig. 8B). The hypertrophy of infected cells observed by electron microscope could result from accumulation of OBs as

observed under light microscope (Fig. 7B). OBs were formed and packed in the nuclei which caused the cellular hypertrophy. These OBs were released upon cell lysis. The immature OBs showing pitted surfaces were found by 3 days pi (Fig. 8C), while the mature OBs possessing smooth surface and sharp edges were found by 4 days pi (Fig. 8D). Mature OBs are surrounded by an electron-dense structure called the polyhedron envelope (PE) or polyhedron calyx [37]. A protein called the PE protein (PEP) appears to be an integral component of the polyhedron envelope [38]-[40].

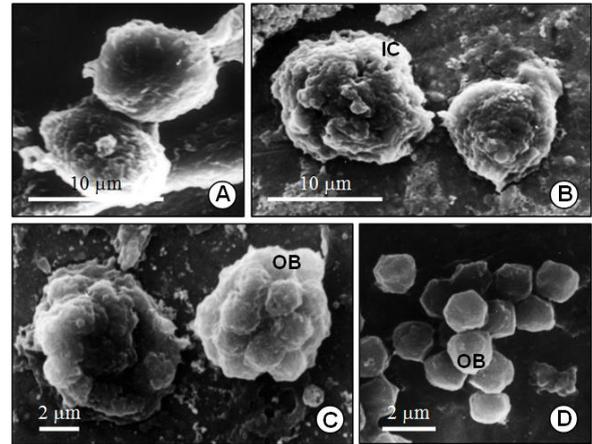


Figure 8. Scanning electron micrographs of the occlusion bodies (OBs) of AcMNPV in the infected SENL1 cells. (A) Mock-infected cells. (B) At 2 days pi, infected cells (IC) appeared larger. (C) At 3 days pi, immature OBs showing pitted surfaces were formed. (D) At 4 days pi, mature OBs possessing smooth surface and sharp edges were released from infected cells.

The PE has been shown to play a major role in sealing the periphery of the OBs of *Orgyia pseudotsugata* NPV [37] and *H. armigera* single nucleopolyhedrovirus (HaSNPV) [41], thereby ensuring retention of nucleocapsids that may otherwise become dislodged and lost. The PE may also stabilize OBs and protect them from mechanical damage. Another possible function of a properly assembled PE could be to prevent the fusion or aggregation of OBs [37]. Examination of the AcMNPV-infected SENL1 cells showed OBs with relatively smooth surface and sharp edges by 4 days pi (Fig. 8D), whereas most OBs produced by AcMNPV by 3 days pi (Fig. 8C) had pitted surfaces, indistinct margins and aggregated into clumps. Therefore, these results indicated that mature OBs surrounded by PE were produced in the infected SENL1 cells by 4 days pi. All these features could contribute to the ability of the virus to transmit a maximal number of intact virions in individual stable OBs and the stability of virus in the environment.

The study of AcMNPV was greatly facilitated by the establishment of *T. ni* [42] and *S. frugiperda* [9] cells in continuous culture, as well as by the subsequent development of a plaque assay using these cells for titration of infectious AcMNPV [43]. In the present study, the established SENL1 cells were tested with regard to the capability to serve as indicator cell in the plaque assay for AcMNPV. SENL1 cells were infected with AcMNPV at a MOI of 1 PFU/cell, and then BV was harvested daily

for 5 days from the medium of infected cells. BV titer was determined by plaque assay employing SENL1 cell. Cell monolayers in 35-mm culture dishes were infected with AcMNPV and foci of infection were clearly seen within 1 day pi. AcMNPV was able to induce plaque formation within 2 days, plaques comprised areas of inhibited cell division and the cells with hypertrophied nuclei. The increase in plaque size as well as severe loss of cell viability and attachability was clearly observed under inverted microscope after 5 days of incubation (Fig. 9). Plaques could be seen microscopically without staining but were easier to count after the monolayers were stained with crystal violet. Most plaques composed of infected cells containing OBs and appeared as round opalescent areas which remained colorless after crystal violet staining. Easily countable plaques of 0.5 to 2mm diameter were detected after 6 days of incubation (Fig. 10A). This plaque assay method was highly effective for BV titration of the AcMNPV during a 5 days time course (Fig. 10B).

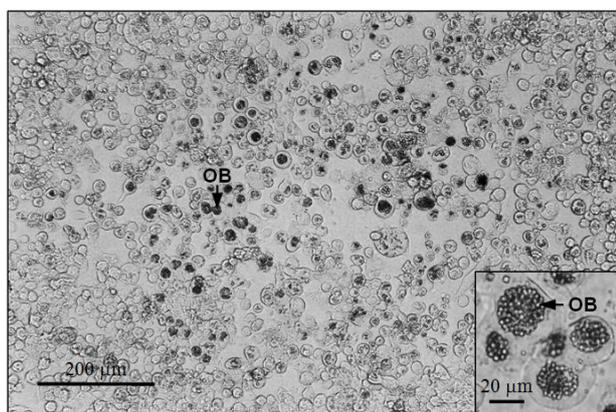


Figure 9. Plaque formation of AcMNPV in SENL1 cells. Plaque consisting of numerous cells containing AcMNPV occlusion bodies (OBs) at 5 days pi. Inset: numerous OBs in the infected cells.

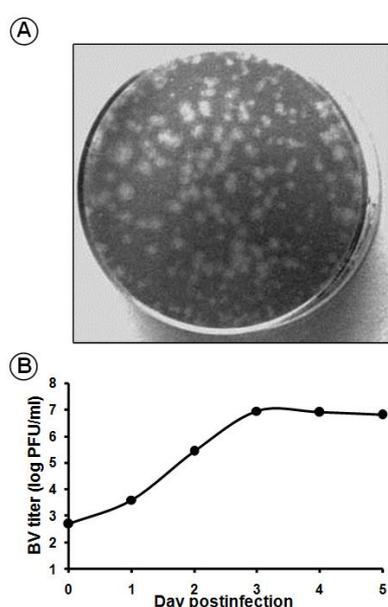


Figure 10. Titration of AcMNPV by plaque assay in SENL1 cells. (A) Clear areas of plaques in a stained background after staining with crystal violet. (B) BV yields in culture medium from the AcMNPV-infected SENL1 cells.

SENL1 cells were infected at a MOI of 1 PFU/cell and at various times pi cell culture media were completely removed and then subjected to plaque assays as described in materials and methods. The onset of BV release occurred within 1 day pi, rapidly reached peak levels at 3 days pi and remained constant through 5 days pi. The maximum rate of BV production occurred between 1 day and 2 days pi. The rate of BV production declined or plateaued when OBs appeared in the infected cells 2 days after infection (Fig. 7, 10B). The titer of AcMNPV BV determined on SENL1 peaked at 8.9×10^6 PFU/ml (Fig. 10B), while that of BV produced by the infected SF21 cells was 3.0×10^7 PFU/ml [44]. These results demonstrated that SENL1 cells were sensitive to infection with AcMNPV and produced similar yields of BV compared to AcMNPV-infected SF21 cells. Therefore, this new insect cell line can be especially useful for titration and plaque purification of AcMNPV.

The plaque assay technique was first applied to baculoviruses by [43], who utilized a methyl cellulose overlay procedure. Subsequent techniques utilizing SeaKem agarose, SeaPlaque agarose or agarose overlay were developed by [44]. This overlay has the advantage of simplicity of quantitation and cloning of the virus. The method reported here is a modification of those reported by [24], [44]. The infected monolayers were covered with 3 ml of 0.75% SeaPlaque GTG agarose (Lonza) overlay prepared in TC100 medium supplemented with 10% FBS. This overlay was chosen for routine plaque assay in the laboratory since the techniques pioneered with AcMNPV are being applied to a variety of other baculoviruses.

Several insect cell lines were compared for their ability to act as target cells for plaque assay of AcMNPV [44]. Both SF21 and *Manduca sexta* cells could be utilized for assay of AcMNPV, but *M. sexta* cells were less sensitive than SF21 cells. The satisfactory results were obtained when AcMNPV was titered by plaque assay on SENL1 cells and SF9 cells, which is known to be highly permissive to AcMNPV, were used as a positive control. AcMNPV was able to form clear and distinct plaques in both SENL1 and SF9 cell monolayers. The assays in which SENL1 and SF9 cells were used as indicators were comparable for both sensitivity to infection with AcMNPV and plaque morphology (data not shown).

Interestingly, the continuous cell lines from a variety of tissues of *S. exigua* have been established and reported [32], [33], [45], [46]. These cell lines do support replication of AcMNPV. Thus, the *S. exigua* cell lines represent an attractive alternative to the baculovirus expression system, particularly for the production of recombinant proteins that can be used as research reagents and leads for new pharmaceuticals and insecticides.

In this study, a cell line was established from minced neonate larvae, and susceptible to the AcMNPV. The SENL1 cell line was confirmed to have originated from *S. exigua* by both RAPD and DAF analyses as shown in Fig. 5, 6. The PCR-based methods used in the present study were able to distinguish inter- and intraspecies cell lines, and also applied to distinguish the two *S. exigua* cell lines originated from different laboratories. Infection of

AcMNPV in SENL1 cells was successfully completed as in vitro. The results and observations strongly indicate that AcMNPV can multiply in SENL1 cells. The ability of the SENL1 cells to support efficient replication of AcMNPV has allowed to study biological characterization of AcMNPV in cell culture system. Further cloning of the cell line is underway to obtain a homogeneous clonal cell line for use in both fundamental and applied studies including production of viral insecticides and recombinant proteins.

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