



Studies on cytopathic effects of BmNPV infection in three lepidopteran insect cell lines in MGM-448 medium

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ABSTRACT

Three insect cell lines, namely, DZNU-Bm-1, DZNU-Bm-12, and NIAS-MaBr-92, were tested for susceptibility to *Bombyx mori* nucleopolyhedrovirus (BmNPV). The cytopathic effects of infection on cultured cells were observed. BmNPV was then serially passaged in these cell lines to assess their long-term ability to support BmNPV replication. The cell lines were cultured in MGM-448 medium supplemented with 10% fetal bovine serum. Virus inoculum was prepared from *B. mori* larvae infected with BmNPV. The early signs of infection started appearing within 16–36 hours post inoculation (hr pi). DZNU-Bm-1 and DZNU-Bm-12 cells showed heavy clumping. NIAS-MaBr-92 cells lost their motility and tended to form loose aggregations. Cytopathic effects such as hypertrophy of nuclei and increase in cell size were prominent in all three cell lines. Beginning of occlusion body (OB) formation in DZNU-Bm-1 was first observed at about 36–42 hr pi, whereas, in DZNU-Bm-12, they were observed only after 42 hr pi. NIAS-MaBr-92 cells exhibited OB formation only after 84 hr pi. The large mature OBs were visible in DZNU-Bm-1 cells by 66 hr pi, whereas, in DZNU-Bm-12 cells by 72 hr pi. Mature OBs were observed in NIAS-MaBr-92 cells only after 120 hr pi. A number of OBs per cell varied from 12 to 82 in DZNU-Bm-1, 3 to 23 in DZNU-Bm-12, and 3 to 14 in NIAS-MaBr-92 cells. In this study, DZNU-Bm-1 and DZNU-Bm-12 cell lines were found to be better than NIAS-MaBr-92 in terms of percentage infection. The yield of OBs per infected cell is also the highest in DZNU-Bm-1, whereas DZNU-Bm-12 and NIAS-MaBr-92 have comparable yields of OBs per cell.

1. INTRODUCTION

Insect cell lines have served a wide range of purposes in areas, such as genetics, cell biology, agriculture, endocrinology, and virology. In recent years, lepidopteran cell lines have been used for the biomanufacturing of proteins of human interest, biopesticides, and vaccines [1–4]. Baculoviruses are DNA viruses that infect arthropods. Many of these baculoviruses have been shown to possess the potential to be used as biopesticides. Two properties of nucleopolyhedroviruses have made them attractive for use as bioinsecticides. First, they are safe for mass spraying because they do not infect vertebrates and other non-target animals [5]. The second desirable property of these baculoviruses is their high pathogenicity [6]. Advances in recombinant DNA technology have now armed researchers with the ability to engineer novel

nucleopolyhedroviruses with high insecticidal potential [7–9]. Baculoviruses are also being employed as gene vectors forming baculovirus expression vector systems (BEVS) [10]. Insect cell lines that have a high susceptibility to a baculovirus can be used to develop efficient insect cell-baculovirus expression vector system (IC-BEVS) [11]. An efficient IC-BEVS production platform can be used for foreign gene expression, production of therapeutic compounds, and vaccines. *Bombyx mori* nucleopolyhedrovirus (BmNPV) has been successfully utilized in BEVS for the expression of recombinant proteins in susceptible cell lines [12,13]. BmNPV has another important economic implication as it causes a devastating disease of silkworms called grasserie. Studying the cytopathic effects and progression of virus replication in permissive cell lines is the first step toward understanding the replication cycle of BmNPV. The studies carried out on the abilities of different baculoviruses to infect heterologous cell lines have given varying results. *Helicoverpa armigera* nuclear polyhedrosis virus (HaNPV) and BmNPV have been reported to replicate only in respective cell lines and appeared to be highly species specific,

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whereas AcNPV could infect seven cell lines from different insects [14]. However, BmNPV has been shown to replicate in cell line from *Mamestra brassicae* [15]. In the present study, three insect cell lines [16–18], namely, DZNU-Bm-1, DZNU-Bm-12, and NIAS-MaBr-92, were tested for susceptibility to BmNPV. DZNU-Bm-1 and DZNU-Bm-12 have been previously used for growth rates and karyotype studies [19]. Cytopathic effects on cultured cells were observed after successful infection with the virus. The BmNPV was then serially passaged in these cell lines to assess their long-term ability to support BmNPV replication.

2. MATERIALS AND METHODS

2.1. Cell Lines

Three cell lines such as DZNU-Bm-1, DZNU-Bm-12, and NIAS-MaBr-92 were cultured in MGM-448 medium supplemented with 10% fetal bovine serum (FBS). No antibiotics were added to the medium. The cell lines were grown in glass tissue culture flasks, incubated at $25 \pm 1^\circ\text{C}$, and passaged regularly.

2.2. Preparation of Virus Inoculum

The virus inoculum was prepared from the diseased larvae of silkworm *B. mori*. Fresh mulberry leaves were cut into pieces of 2 cm² size. Each piece was then smeared with a 10- μl suspension of occlusion bodies (OBs) of BmNPV obtained from the Centre for Sericulture and Biological Pest Management, Ambavihar, R.T.M. Nagpur University, Nagpur. Each smeared piece of leaf was fed to a healthy larva of *B. mori* for inoculation. The larvae were further reared till the fifth instar stage by feeding them with fresh mulberry leaves. At the fifth instar stage, an incision was made on proleg of the inoculated larva to collect hemolymph. After centrifugation (1,000 g for 10 minutes), the supernatant was diluted with an equal volume of MGM-448 medium supplemented with 10% FBS medium and sterilized by passing through 0.45- μm pore size membrane filter. This served as BmNPV inoculum for infecting the cell lines.

2.3. Inoculation

BmNPV inoculum was taken in a sterile pipette, and 3–4 drops of it were added to each culture flask containing growing cells. The infected cultures were incubated at $25 \pm 1^\circ\text{C}$ and examined for the cytopathic effects and formation of OBs. The contents of the inoculated cultures were collected after 10–12 days post inoculation and centrifuged at 3,000 rpm for 15 minutes. The supernatant containing BmNPV-budded virus was collected in a sterile centrifuge tube. The supernatant was stored in the refrigerator at 4°C to be used as a virus inoculum for subsequent passaging of the virus. Serial passaging of virus was carried out by inoculating healthy cells with undiluted supernatant from the previous passage of the virus. Harvesting of OBs was done by resuspending the precipitate containing the infected cells in sterile distilled water, washed with 0.5% (w/v) of sodium lauryl sulfate, and rinsed thrice in distilled water.

2.4. Phase-Contrast Microscopy

The cultures were examined regularly for the presence of infected cells with a Magnus INVI-inverted phase-contrast microscope. The criterion of BmNPV infection was the presence of polyhedral inclusion bodies in a cell. In cultures that showed heavy clumping of cells, a small number of cells were removed from the inoculated cultures and transferred to microscopic glass slides. The cells were then spread out by placing a cover glass over the slide. From each flask, about 200–300 cells were used for making differential counts of healthy and infected cells. The percentage infection and number of OBs per cell were determined for each cell line.

3. RESULTS AND DISCUSSION

The observation of inoculated cultures under phase-contrast microscope indicated that the early signs of infection started appearing within 16–36 hours post inoculation (hr pi). By 24 hr pi, all infected cultures of DZNU-Bm-1 and DZNU-Bm-12 cells showed heavy clumping. Cells from NIAS-MaBr-92 did not show heavy clumping but tended to form loose aggregations. The infected cells of NIAS-MaBr-92 lost their motility. All three cell lines exhibited loose adherence to the bottom of the culture flask. The cytopathic effects such as hypertrophy of nuclei and increase in cell size were prominent in all three cell lines.

The beginning of OB formation in DZNU-Bm-1 was first evidenced from the appearance of refractive bodies in the nuclei of infected cells at about 36–42 hr pi, whereas, in DZNU-Bm-12, they were observed only after 42 hr pi. The nuclei of NIAS-MaBr-92 cells exhibited OB formation only after 84 hr pi. The large mature OBs were visible in the nuclei of aggregated cells of DZNU-Bm-1 by 66 hr pi, whereas in DZNU-Bm-12 cells by 72 hr pi. Mature OBs were observed in NIAS-MaBr-92 cells only after 120 hr pi. OB formation by 40–48 hr pi in *B. mori* cells has been reported in the previous studies [20]. Small OBs have been observed in MB-19 cell line by 52 hr pi, whereas mature OBs have been reported by 76 hr pi [15]. Some of the cell aggregates were taken out from the infected cultures and examined under a microscope for the determination of the percentage of infected cells. The percentage infection in DZNU-Bm-1 cells remained between 89% and 93% (Table 1) for five serial passages of BmNPV. DZNU-Bm-12 cell line showed a consistent percentage infection that ranged from 82% to 88% (Table 2). These results agree with other studies that have reported no decrease in the yield of OBs even after 10 passages [21,22]. During passage I of virus, about 62% of NIAS-MaBr-92 cells were infected. The percentage infection gradually increased to 88% by passage III. It declined thereafter to a level of about 77% by passage V (Table 3). This agrees with the other reports of a decrease in percentage infection and yield of OBs following serial passaging of NPV in permissive cell lines [23–25]. A number of OBs per cell varied from 12 to 82 in DZNU-Bm-1, whereas, in DZNU-Bm-12, it ranged from 3 to 29. The number of OBs per cell depended on the cell size. NIAS-MaBr-92 exhibited about 3 to 19 OBs per infected cell. Infected cells started to lyse by 72 hr pi in DZNU-Bm-1 cells. This is consistent with the previous observations on DZNU-Bm-1 cells [26]. DZNU-Bm-12 cells did not

Table 1. Five serial passages of BmNPV in DZNU-Bm-1 cells.

Virus passage	Days p.i.	Cells per ml ($\times 10^5$)	Percentage infection	Infected cells per ml ($\times 10^5$) ^b	OBS per infected cell ^c
I	4	5.22	89.86	4.69	20 \pm 2
II	4	4.89	92.45	4.52	24 \pm 2
III	4	4.92	90.21	4.44	20 \pm 3
IV	4	5.52	92.52	5.11	20 \pm 3
V	4	5.68	93.21	5.29	21 \pm 3

Table 2. Five serial passages of BmNPV in DZNU-Bm-12 cells.

Virus passage	Days p.i.	Cells per ml ($\times 10^5$)	Percentage infection ^a	Infected cells per ml ($\times 10^5$) ^b	OBS per infected cell ^c
I	5	4.45	82.64	3.68	10 \pm 2
II	6	4.96	86.35	4.28	09 \pm 2
III	6	5.06	87.16	4.41	12 \pm 3
IV	6	5.15	88.98	4.58	12 \pm 2
V	6	5.10	85.57	4.31	11 \pm 2

Table 3. Five serial passages of BmNPV in NIAS-MaBr-92 cells.

Virus passage	Days p.i.	Cells per ml ($\times 10^5$)	Percentage infection ^a	Infected cells per ml ($\times 10^5$) ^b	OBS per infected cell ^c
I	9	5.51	62.23	3.43	08 \pm 2
II	7	5.20	78.87	4.10	09 \pm 2
III	7	5.24	88.44	4.63	13 \pm 3
IV	8	5.98	87.16	5.21	14 \pm 2
V	8	5.34	77.65	4.15	11 \pm 3

^aThe presence of OBS in a cell was the criterion of its infection with BmNPV.

^bCalculated by multiplying the cell number by percentage infection.

^cMean \pm standard error (SE).

show much cell lysis even after 96 hr pi. The lysis of NIAS-MaBr-92 cells started at 172 hr pi. In the present study, DZNU-Bm-1 showed an average of 21 OBS/cell, whereas DZNU-Bm-12 gave an average yield of about 10 OBS/cell over five passages. All three cell lines have a higher susceptibility to BmNPV than BmN cell line that has been reported to give 64%–73% of infection [27].

4. CONCLUSION

The results point that DZNU-Bm-1 and DZNU-Bm-12 cell lines are better than NIAS-MaBr-92 in terms of percentage infection in MGM-448 culture medium. The yield of OBS per infected cell is the highest in DZNU-Bm-1, whereas DZNU-Bm-12 and NIAS-MaBr-92 have comparable yields of OBS per cell. These cell lines should be adapted to low-cost serum-free media and checked for percentage infection and OB yield. Furthermore, the cell lines should also be screened for susceptibility to other baculoviruses such as HaNPV to ascertain their efficiency for the large-scale production of such biopesticides.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest.

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