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Comparative study of susceptibilities of two cell lines to *Bombyx mori* nucleopolyhedrovirus (BmNPV)

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Abstract

Insect cell lines have emerged as a prime candidate for study of baculovirus replication and production of recombinant viruses leading to foreign gene expression. Study of variations in cell line susceptibilities to nucleopolyhedroviruses may shed light on in vitro replication of these viruses. The present study compares susceptibilities of two cell lines viz. DZNU-Bm-1 and NIAS-MaBr-92 to *Bombyx mori* nucleopolyhedrovirus (BmNPV). The cell lines were grown at 25 ± 1°C in MGM-448 medium supplemented with 10% FBS. At early stages of infection, hypertrophy of nuclei, cell aggregation and adherence of cells to the bottom of the culture flask were observed in DZNU-Bm-1. NIAS-MaBr-92 cells showed hypertrophy of nuclei and loss of motility. The criterion of BmNPV infection was presence of polyhedral inclusion bodies (PIBs) in a cell. In DZNU-Bm-1, mature OBs were seen in the nuclei of infected cells by 66 h.p.i. while in NIAS-MaBr-92 mature OBs could be seen only after 120 h.p.i. At passage I of BmNPV, the infection rate was higher in DZNU-Bm-1 cells (92.50%) than that in NIAS-MaBr-92 cells (64.33%). DZNU-Bm-1 cells exhibited an average yield of 23 OBs/cell while NIAS-MaBr-92 cells showed an average of 10 OBs/cell. Thus, DZNU-Bm-1 cell line exhibits better figures of percentage infection and yield of OBs/cell. The results indicate that DZNU-Bm-1 cell line can be used to produce recombinant BmNPV and foreign gene expression through BmNPV/*B. mori* system.

Hundreds of baculovirus species occur in nature that are almost exclusively parasitic on arthropods. These viruses have the potential to be used as bioinsecticides in pest control[1]. However, large-scale production of baculoviruses in insect larvae suffers from the drawbacks of being labour-intensive and not entirely contaminant-free. Insect cell lines have emerged as an attractive alternative for production of baculoviruses which are highly virulent against some agricultural and forest pests. *Mamestra brassicae* NPV has been developed as a commercial biological insecticide in China. In addition, baculoviruses have also been genetically engineered to produce recombinant proteins through the use

of baculovirus expression vector (BEV) derived from *Autographa californica* nucleopolyhedrovirus (AcNPV)[14,21]. Other baculoviruses like *Bombyx mori* nucleopolyhedrovirus (BmNPV) can also serve as vector for cloning of heterologous genes[3]. BmNPV has been used for expression of recombinant proteins in susceptible cell lines through BEV system[8,12].

Susceptibility to infection can vary between cell lines[11]. Study of variations in cell line susceptibilities to nucleopolyhedroviruses is crucial in understanding replication of these viruses. Comparative susceptibilities of insect cell lines to infection by baculoviruses have been previously reported[11,24]. Permissiveness of cell lines to baculovirus infection may be affected by at higher passage levels[25]. In India, a larval ovarian cell line from *B. mori* DZNU-Bm-1 has been reported to show high susceptibility to BmNPV[7,19]. For comparative analysis, NIAS-MaBr-92, a cell line[12] derived from larval hemocytes of *Mamestra brassicae* was also tested for susceptibility to BmNPV. Morphological, growth rate studies and karyotype analyses of DZNU-DZNU-Bm-1 have been reported previously.[1] A cell line having higher susceptibility to BmNPV can produce large number of recombinant BmNPV and can also be used in BEV system for production of desired proteins. This paper compares the susceptibilities of DZNU-Bm-1 and NIAS-MaBr-92 cell lines to BmNPV and occlusion body yield in the two cell lines.

Cell lines :

The cell lines, DZNU-Bm-1 and NIAS-MaBr-92 were grown in MGM-448 medium[15] supplemented with 10% Foetal Bovine Serum (FBS). No antibiotics were added to the medium. The cell lines were grown in glass tissue culture flasks and incubated at 25± 1^0C. The cell lines were then passaged regularly.

BmNPV inoculum :

The virus inoculum was prepared from diseased larvae of silkworm *B. mori*. Each larva was fed on 2 cm^2 piece of mulberry leaf coated with 10 µl suspension of Occlusion Bodies (OBs) of BmNPV. The OB suspension was provided by Centre for Sericulture and Biological Pest Management Research (CSBR), Ambavihar, R.T.M. Nagpur University, Nagpur. These inoculated larvae were reared till fifth instar stage by feeding fresh mulberry leaves. At fifth instar stage an incision was made on proleg of the inoculated larva to collect turbid haemolymph containing BmNPV. After centrifugation (1000g for10 min), the supernatant was diluted with equal volume of MGM-448 medium supplemented with 10% FBS medium, passed through 0.45 µ pore size membrane filter and used as BmNPV inoculum. This BmNPV inoculum was then used to infect both the cell lines.

Virus inoculation :

The cell lines were inoculated by adding 3-4 drops of the BmNPV inoculum with a Pasteur pipette to a glass culture flask containing growing cells. The infected cultures were maintained at 25± 1^oC and examined every day for cytopathic effects and manifestation of occlusion bodies (OBs) in the nuclei. After 10 days post inoculation, the contents of the inoculated cultures were centrifuged at 3000

rpm for 15 min. The supernatant was collected in a sterile centrifuge tube. The supernatant containing BmNPV budded virus (BV) was stored in the refrigerator at 4°C for further use as virus inoculum. Serial passaging of virus was carried out by inoculating healthy cells with undiluted infected medium from the previous passage of the virus. Occlusion bodies (OBs) were harvested by resuspending the precipitate containing the infected cells in sterile distilled water, washed with 0.5% (w/v) sodium lauryl sulphate and rinsed thrice in distilled water.

Light microscopy :

The cultures were routinely examined for presence of healthy and infected cells with a Magnus INVI inverted phase-contrast microscope. The criterion of BmNPV infection was presence of polyhedral inclusion bodies (PIBs) in a cell. Since the infected cells were heavily clumped, it was difficult to make differential counts of healthy and infected cells in a flask. To overcome this problem, a small number of cells was 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. Differential counts of healthy and infected cells were made. 200 to 300 cells were counted from each flask. Percentage infection and number of OBs per cell were determined for each cell line.

Cells from both DZNU-Bm-1 as well as NIAS-MaBr-92 cell lines were successfully infected with BmNPV. Cytopathic effects such as hypertrophy of nuclei and clumping of cells were observed in DZNU-Bm-1 after BmNPV inoculum was added to culture flasks. In NIAS-MaBr-92, at early stages of infection, cell enlargement was observed due to hypertrophy of nuclei. The NIAS-MaBr-92 cells infected with BmNPV also showed loss of motilility. Cytopathogenic effects like uniform rounding and distinctly enlarged nuclei have also been reported in BmN-SWU1 cell line[24].

Numerous small and large clumps of cells were observed 16-18 hours post inoculation (h.p.i.) whereas small refractive OBs first made their appearance in the nuclei of infected cells at about 36-42 h.p.i. in DZNU-Bm-1. Small OBs were observed in nuclei of NIAS-MaBr-92 cell only after 84 h.p.i. Mature OBs could be prominently seen in the nuclei of aggregated cells of DZNU-Bm-1 by 66 h.p.i. Mature OBs were observed in NIAS-MaBr-92 cells only after 120 h.p.i. OB formation by 40-48 h.p.i. in *B. mori* cells has been reported in previous studies[20]. Some of the cell aggregates from DZNU-Bm-1 were removed from the infected cultures and examined under microscope for determination of percentage infection. The percentage infection in DZNU-Bm-1 cells remained between 89-94% for five serial passages of BmNPV (Table-1). Number of OBs/cell varied from 15 to 96 depending on the cell size. Lysis of infected cells commenced by 72 h.p.i. releasing OBs in to the medium in DZNU-Bm-1. This is consistent with previous observations on DZNU-Bm-1 cells[19]. Lysis of NIAS-MaBr-92 cells started at 172 h.p.i. During passage I of virus, about 64% NIAS-MaBr-92 cells were found to be infected. The infection level rose up to 91% by third passage and declined thereafter to a level of about 75% by fifth passage (Table-2). These results agree with others studies that have not reported any decrease in the yield of

OBs even after 10 passages[6,22]. However, decrease in yield of OBs following serial passaging of NPV in permissive cell lines has also been reported[2,9,10].

In the present study, DZNU-Bm-1 showed an average of 23 OBs/cell while NIAS-MaBr-92 gave an average yield of 10 OBs/cell. Thus DZNU-Bm-1 has better yield of OBs/cell. Another cell line from *Bombyx mori*, DZNU-Bm-12 has been reported to yield 9-10 OBs/cell[5]. Both DZNU-Bm-1 and NIAS-MaBr-92 cell lines have higher susceptibility to BmNPV than BmN cell line that has been reported to show OB formation in 64-73% cells[4]. The cell lines BM-1296 and BM-197 have been reported to show 90 and 70% infection of BmNPV[18,23]. Some other indigenous *B. mori* cell lines susceptible to BmNPV have been reported but there is no data on percentage of OB forming cells in these cell lines[17].

Table-1.
Serial passage of BmNPV in DZNU-Bm-1cells

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	5.86	92.50	5.42	24±3
II	4	4.89	93.26	4.56	25±3
III	4	5.22	89.47	4.67	19±2
IV	4	5.34	94.65	5.05	21±4
V	4	6.22	92.21	5.73	24±3

Table-2.
Serial passage of BmNPV in NIAS-MaBr-92 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	9	5.81	64.33	3.73	6±2
II	7	5.32	81.54	4.33	7±3
III	8	6.24	91.44	5.70	16±4
IV	9	4.80	88.15	4.23	11±2
V	9	4.43	75.47	3.34	9±2

[a] The presence of OBs in a cell was the criterion of its infection with BmNPV.

[b] Calculated by multiplying the cell number by percentage infection.

[c] Mean ± standard error (SE).

Since the percentage infection and yield of OBs/cell is better in DZNU-Bm-1 cell line than in NIAS-MaBr-92 cell line, the former should be used for production expression of foreign genes of human interest through BmNPV/*B. mori* system.

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