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# Comparative study of *Bombyx mori* nucleopolyhedrovirus replication in larval cell lines of *Bombyx mori*

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## ABSTRACT

A four new cell lines viz., Bm-1, Bm-12 Bm-16, Bm-17 and Bm -5 is a worldwide used cell line are tested for BmNPV (*Bombyx mori* nuclepolyhdedrovirus) susceptibility and its replication The BmNPV was serially passaged in the Bm-12, Bm-1, Bm-16, Bm-17 and Bm-5 cell lines for five times. All the cell lines were found susceptible to BmNPV infection when inoculated with the haemolymph of BmNPV infected silkworm larvae. The infection rate was Bm-5 (93.79%), Bm-16 (91.30%), Bm-1 (90.30%), Bm-12 (87.20%) and Bm-17 (80.43%).

**Keywords:** *Bombyx mori*, Ovarian cell lines, BmNPV infection

## INTRODUCTION

Insect cell culture has found many applications in the field of physiology, biochemistry, genetics, developmental biology and insect pathology. This is true with lepidopteran cells. They are commonly used to study insect viruses (Blissard, 1996 and T.V Sathe et al, 2014) and have been considered for production of certain pathogenic viruses and recombinant proteins by the use of the Baculovirus expression vector (Granados and McKenna, 1995; Smith et al., 1983; Vaughn, 1981; Summers, M.D., 1989). BmNPV has a narrow host range can infect larval ovary of B. mori and also transmits to next generation (Khurad et al., 2004). Bm-12 cell line shows susceptibility of BmNPV (C.G.Deshmukh et al 20013; K. Srivastava and V.B. Upadhyay. 2013) in the present study larval ovarian cell line Bm-12, Bm-1, Bm-16 and Bm-17 and a widely used, Bm-5 cell lines were also tested for susceptibility of BmNPV for studying the rate of infection at different passage number.

## MATERIALS AND METHODS

### Preparation of Virus Inoculum:

BmNPV was obtained from diseased larvae of the silkworm, *B. mori*. Turbid haemolymph of fifth instar larvae infected with BmNPV was collected. After centrifugation (3000 rpm, 10 min) the supernatant was diluted with equal volume of TNM-FH medium and passed through 0.45 µm membrane filter and used as an inoculum. It was stored in refrigerator at 4°C.

### Inoculation of Cultures:

The cells of each cell line at log phase were harvested, counted and transferred to three 30 mm Falcon plastic Petri-plates at about 3 × 105 cells /ml to higher densities (1-1.8 × 106 cell/ml). The cultures were inoculated by adding 2-3 drops of the inoculum with a Pasteur pipette. The infected cultures were maintained at 25°C and examined every day for cytopathic effect and occurrence of occlusion bodies (OBs) in the nuclei (Figure-1).

**Figure-1. Cytopathic effect of NPV infected OBs (occlusion bodies) in the nuclei against Bm-12, Bm-1, Bm-16, Bm-17 and Bm-5 cell lines**

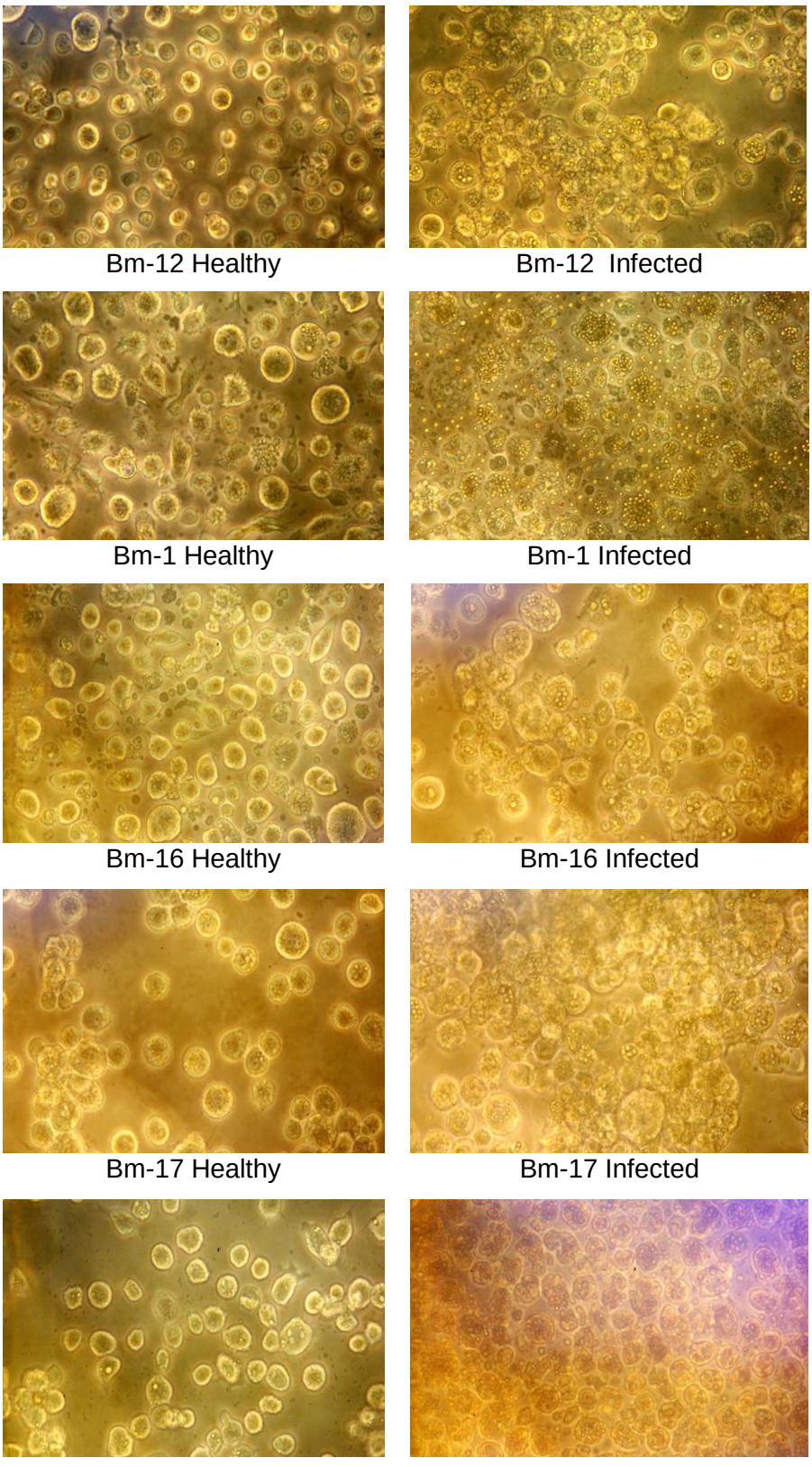


  

**Table-1. Serial passage of *B. mori* nucleopolyhedrovirus (BmNPV) in DZNU-Bm-12 cell line**

| S.P No. | Days (PI) | Cell passage No. | Cells/ml (X $10^5$) | Viable cells (X $10^5$) | % Infection[a] | Infected Cells/ml (X $10^5$) [b] | OBs/ cell[c] | OBs/ml (X $10^7$)[d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 275 | 9.67 | 8.70 | 83.71 | 7.28 | 10 | 0.74 |
| 2 | 8 | 283 | 9.57 | 9.07 | 85.66 | 7.30 | 10 | 0.79 |
| 3 | 9 | 291 | 10.07 | 9.06 | 87.20 | 7.9 | 9 | 0.78 |
| 4 | 8 | 297 | 9.57 | 8.61 | 83.30 | 7.17 | 10 | 0.79 |
| 5 | 9 | 302 | 10.42 | 9.37 | 80.60 | 7.55 | 10 | 0.76 |

**Table-2. Serial passage of *B. mori* nucleopolyhedrovirus (BmNPV) in DZNU-Bm-1 cell line**

| S.P No. | Days (PI) | Cell Passage No. | Cells /ml (X $10^5$) | Viable cells (X $10^5$) | % Infection[a] | Infected Cells/ml (X $10^5$) [b] | OBs/ cell[c] | OBs/ml (X $10^7$)[d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 6 | 296 | 3 | 2.7 | 88.21 | 2.38 | 16 | 0.56 |
| 2 | 6 | 299 | 3.1 | 2.79 | 89.60 | 2.5 | 16 | 0.59 |
| 3 | 5 | 303 | 3.3 | 2.97 | 90.30 | 2.68 | 17 | 0.63 |
| 4 | 5 | 313 | 4.02 | 3.61 | 89.37 | 3.23 | 17 | 0.63 |
| 5 | 9 | 318 | 4 | 3.6 | 87.36 | 3.14 | 17 | 0.62 |

**Table-3. Serial Passage of *B. mori* nucleopolyhedrovirus (BmNPV) in DZNU-Bm-5 cell line**

| S.P No. | Days (PI) | Cell passage No. | Cells /ml (X $10^5$) | Viable cells (X $10^5$) | % Infection[a] | Infected Cells/ml (X $10^5$) [b] | OBs/ cell[c] | OBs/ml (X$10^7$) [d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 10 | 6.75 | 6.07 | 90.20 | 5.48 | 17 | 1.20 |
| 2 | 5 | 14 | 6.51 | 5.85 | 91.46 | 5.35 | 17 | 1.29 |
| 3 | 4 | 19 | 5.82 | 5.23 | 93.79 | 4.91 | 18 | 1.10 |
| 4 | 4 | 26 | 5.02 | 4.51 | 91.32 | 4.12 | 18 | 0.93 |
| 5 | 4 | 31 | 4.68 | 4.21 | 90.25 | 3.8 | 17 | 0.87 |

**Table-4. Serial passage of *B. mori* nucleopolyhedrovirus (BmNPV) in DZNU-Bm-16 cell line**

| S.P No. | Days (PI) | Cell passage No. | Cells /ml (X $10^5$) | Viable cells (X $10^5$) | % Infection[a] | Cells/ml (X $10^5$) [b] | OBs/ cell[c] | OBs/ml (X$10^7$) [d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 36 | 6.81 | 6.12 | 91.30 | 5.59 | 17 | 1.15 |
| 2 | 4 | 39 | 6.4 | 5.76 | 91.18 | 5.25 | 19 | 1.17 |
| 3 | 5 | 43 | 18.32 | 16.48 | 90.26 | 14.87 | 17 | 1.16 |
| 4 | 4 | 55 | 7.7 | 6.93 | 87.66 | 6.07 | 17 | 1.15 |
| 5 | 5 | 59 | 10.5 | 9.45 | 84.73 | 8.01 | 17 | NR |

**Table-2. Serial passage of *B. mori* nucleopolyhedrovirus (BmNPV) in DZNU-Bm-17 cell line**

| S.P No. | Days (PI) | Cell passage No. | Cells /ml (X $10^5$) | Viable cells (X $10^5$) | % Infection[a] | Infected Cells/ml (X $10^5$) [b] | OBs/ cell [c] | OBs/ml (X$10^7$) [d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 8 | 76 | 10.02 | 9.01 | 80.26 | 7.23 | 7 | 0.53 |
| 2 | 8 | 81 | 9.2 | 8.28 | 79.35 | 6.57 | 7 | 0.55 |
| 3 | 10 | 84 | 10.23 | 9.20 | 79.63 | 7.33 | 8 | 0.60 |
| 4 | 8 | 95 | 9.5 | 8.5 | 80.43 | 6.84 | 7 | 0.58 |
| 5 | 10 | 99 | 14.1 | 12.69 | 78.20 | 9.92 | 7 | 0.61 |

[a] The presence of OBs in cell was the criterion of its infection with BmNPV. Each value is the mean of sample of three hundred cells each from three plates.
[b] Calculated by multiplying the cell number by percentage infection.
[c] Mean number of OBs in infected cells counted randomly under microscope.
[d] Mean value based on samples of two plates.

**Serial Passaging of BmNPV:**
After 10-12 days post inoculation, the content of the inoculated cultures were centrifuged at 3000 rpm for 15 min and the supernatant was collected separately of each infected cell line in a sterile centrifuge tube. This served as inoculum for the next passage of the virus. OBs were harvested from the cell pellet by resuspending in sterile distilled water, washing with 0.5% (w/v) sodium lauryl sulphate and rinsing thrice in distilled water. The harvested OBs were counted in the haemocytometer and recorded. A comparative data of initial number of cells, viable cells, infected cells, OBs /cell and total production of OBs in each cell line were determined and presented in tables.

## OBSERVATIONS

**Phase Contrast Microscopy:**
At early stages of infection, the cytopathic effects such as hypertrophy of nuclei, heavy clumping of cells in MGM-448 medium and adherence of the cells to the substratum of the culture plate in TNM-FH medium were prominent. Numerous clumps of cells observed 16-20 h post inoculation (h pi) in Bm-1, Bm-12 and Bm-16 cells, whereas cells of Bm-5 and Bm-17 were remain adhered to the substratum. At about 40-48 h pi small refractive OBs were appeared in the nuclei of cells of all the five cell lines. By 72 h pi OBs were prominently seen in the nuclei of aggregated and dislodged cells. Some of the cell aggregates that exhibited about 85-90% infected cells were removed from the infected culture and examined under microscope. The Bm-12 cells were loaded with 3-17 OBs/cells in the nuclei depending on the cell size, whereas the number of OBs /cell was ranging from-13-29, 16-34, 17-35, and 3-16 in Bm-1, Bm-5, Bm-16 and Bm-17, respectively. The OBs loaded Bm-12 and Bm-17 cells remained in the infected cultures for a long time and only a few cells exhibited lysis and release of OBs by 96 h pi.

**Serial Passaging:**
The BmNPV was serially passaged in all the five cell lines for 5 times. The infection rate was highest (93.79%) in Bm-5 followed by Bm-16 (91.30%), Bm-1 (90.30%), Bm-12 (87.20%) and Bm-17 (80.43%). However, the yield of OBs was 1.17 × 107/ml in Bm-16 followed by 1.29 × 107/ml in Bm-5, 0.79 × 107/ml in Bm-12, 0.61 × 107/ml in Bm-17 and 0.63 × 107/ml in Bm-1 (Tables 1, 2, 3, 4, and 5). The overall range of BmNPV infection during serial passaging was more than 90% was observed in Bm-1, Bm-5 and Bm-16, whereas in Bm-12 and Bm-17 it was between 77 and 89%. The average number of OBs/infected cell was 16-19 in Bm-1, Bm-5 and Bm-16 but in Bm-12 it was 9-10 and only 7-8 in Bm-17. Comparative study on the infection and production of OBs in five cell lines indicated that Bm-12 was not much more productive than the Bm-5 and Bm-16 but slightly more and comparable with Bm-1 and Bm-17. The results obtained further indicate that Bm-12 cell line has one advantage that it is a fast growing cell line as compared to those of the remaining four cell lines and the susceptibility and production of BmNPV are also comparable with the other indigenously developed cell lines.

## CONCLUSION

Results of the present study indicate that all the five cell lines support Bm-NPV replication in Vitro. Infection with this virus not only appeared at the first instance in the first passage but also continued in the serial passaging. Thus, it can be concluded that the all the cell lines of B. mori can be used for in vitro production of wild-type BmNPV using suitable medium as well as recombinant proteins by infecting with BmNPV expression vector having a gene of interest.

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