Comparative in Vitro Replication and Serial Passaging of BMNPV in the DZNU-BM-12 and Other Cell Lines

C.G. Deshmukh*, R.S. Bahekar**

* Department of Zoology, A.C.S. College, Koradi (MS) India-.441111

Abstract- The newly established ovarian cell line of Bombyx mori, DZNU-Bm-12 was tested for its susceptibility to homologous nucleopolyhedrovirus, BmNPV. The BmNPV was serially passaged in the cell line for five times along with the other four cell lines Bm-1, Bm-16 and Bm-17 which is established in our laboratory and Bm-5 is a widely used cell line. All the cell lines are susceptible to BmNPV. 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 9-13 and only 7-8 in Bm-17.

Index Terms- Bombyx mori, Ovarian cell line, DZNU-Bm-12, BmNPV, Baculovirus expression system.

I. INTRODUCTION

Insect cell cultures are widely used in viral diagnosis and biotechnology, for the production of recombinant proteins, viral pesticides and vaccines. In recent years, there is renewed interest in developing new lepidopteran cell lines due to their potential application in biotechnology for the production of recombinant proteins by the use of the Baculovirus expression vector (Granados and McKenna, 1995; Smith et al., 1983; Vaughn, 1981; Summers, M.D., 1989). *Bombyx mori* nucleopolohedrovirus (BmNPV) is one such baculovirus, which is being used in BEV system for expression of recombinant proteins in susceptible cell lines (Meada, 1987, 1989; Maeda et al., 1991 Raghow, et al., 1974).

Several cell lines have been established from silkworm embryos (Inoue and Mitsuhashi 1984; Chen et al. 1988; Pandharipande 1994; Pan et al. 2007) and larval and pupal ovaries (Sudeep et al 2002; Khurad et al. 2006) of this economical important insect however, only a few of these cell lines are susceptible to *B. mori* nucleopolohedrovirus (BmNPV) and support its replication efficiently *in vitro*.

In the present study the indigenously developed larval ovarian cell line DZNU-Bm-12 (Khurad *et al.*, 2009) was tested for susceptibility to homologous virus (BmNPV). The other ovarian cell lines of *B. mori* Bm-1 (Khurad *et al.*, 2006), Bm-16 and Bm-17 and a widely used, Bm-5 cell lines were also tested for susceptibility of BmNPV and its replication to compare with Bm-12 cell line.

II. MATERIALS AND METHODS

BmNPV was obtained from diseased fifth instar larvae of the silkworm, *B. mori*. Turbid haemolymph was collected through an incision on proleg. After centrifugation (3000 rpm, 10 min) the supernatant was diluted with equal volume of 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 3×10^5 cells /ml to higher densities ($1\text{-}1.8 \times 10^6$ 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.

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.

III. OBSERVATION AND DISCUSSION

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	273	9.67	8.70	85.75	7.46	9	0.73
2	8	281	10.35	9.91	87.16	11.25	9	0.78
3	10	289	10.07	9.06	88.89	8.05	10	0.79

4	8	295	9.57	8.61	84.49	7.27	10	0.76
5	9	300	10.42	9.37	79.43	7.44	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	294	3	2.7	87.29	2.35	15	0.55
2	6	297	3.1	2.79	88.78	2.47	15	0.59
3	5	301	3.3	2.97	90.36	2.68	16	0.61
4	5	311	4.02	3.61	90.06	3.25	16	0.64
5	9	316	4	3.6	86.28	3.10	16	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 (X10^7)[d]
1	5	8	6.75	6.07	91.78	5.57	18	1.17
2	5	12	6.51	5.85	93.06	5.45	18	1.20
3	4	17	5.82	5.23	93.57	4.89	18	1.17
4	4	26	5.02	4.51	90.28	4.07	17	0.95
5	4	31	4.68	4.21	90.64	3.81	17	0.85

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 (X10^7)[d]
1	5	34	6.81	6.12	93.19	5.70	18	1.07
2	4	37	6.4	5.76	90.60	5.21	19	1.08
3	5	41	18.32	16.48	91.85	15.14	16	1.11
4	4	52	7.7	6.93	86.69	6	18	1.14
5	5	57	10.5	9.45	83.61	7.90	17	NR

Table 5: 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 (X10^7)[d]
1	8	74	10.02	9.01	81.90	7.37	7	0.58
2	8	79	9.2	8.28	78.94	6.53	8	0.56
3	10	82	10.23	9.20	78.05	7.18	8	0.61
4	8	92	9.5	8.5	81.20	6.94	8	0.56
5	10	97	14.1	12.69	77.43	9.82	7	0.62

[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.

In the present study, the BmNPV was serially passaged in the the newly established cell line DZNU-Bm-12 as well as in four other *B. mori* cell lines for five times. All the cell lines were susceptible to BmNPV infection.

At early stage of infection, the cytopathic effect such as hypertrophy of nuclei, heavy clumping and adherence of the cells to the substratum of the culture flask in TNM-FH medium were prominent in all the five cell lines. Numerous large clumps of cells were observed 16-20 h post inoculation (h pi) in Bm-1, Bm-16 and Bm-12 and about 40-48 h pi small refractive OBs were appeared in the nuclei of cells. By 72 h pi OBs were prominently seen in the nuclei of aggregated and dislodged cells. The replication of BmNPV between 16 and 18 h pi and formation of OBs by 40-48 h pi in *B. mori* cells have also been reported earlier (Raghow and grace 1974, Khurad *et al.*, 2006). Some of the cell aggregates that exhibited infected cells were removed from the infected cultures and examined under microscope. The cells were loaded with OBs in the nuclei depending on the cell size in each cell line. In DZNU-Bm-1, Bm-5 and Bm-16 the lysis of cells loaded with OBs was a common feature 96 h pi , 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.p.i. This may be the characteristic feature of these cell lines.

IV. Serial Passaging

The BmNPV was serially passaged in all the five cell lines for 5 times. The infection rate was highest (93.19%) in Bm-16 followed by Bm-5 (91.78%), Bm-1 (87.26%), Bm-12 (85.75%) and Bm-17 (81.29%). However, the yield of OBs was 1.17×10^7/ml in Bm-5 followed by 1.07×10^7/ml in Bm-16, 0.73×10^7/ml in Bm-12, 0.58×10^7/ml in Bm-17 and 0.55×10^7/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 9-13 and only 7-8 in 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.

V. CONCLUSION

Thus the data obtained from the present study revealed that all the four cell lines all four indigenously developed cell lines DZNU-Bm1, Bm-12, Bm-16, and Bm-17 are highly susceptible to baculovirus BNPV and can be comparable to widely used Bm-5 cell line of Japanese origin. Among indigenously developed cell lines, Bm-1, Bm-12 and Bm-16 are highly productive cell lines and they can be utilized to express recombinant proteins using BmNPV derived expression vectors/bacmids, however further studies using an appropriate BmNPV expression system and comparative BmNPV replication assays are essential to confirm the utility of these cell lines.

References

[1] Blissard, G.W., 1996. Baculovirus-insect cell interactions. Cytotechnology 20, 73- 93.

[2] Chen Q., Li L., Yu Z., Peng J. Establishment of cell lines from embryos of the silkworm, Bombyx mori In: Kuroda Y; Kurstak E; Maramorosch K. (eds) invertebrate and fish tissue culture Springer, New York, pp 259-261; 1988.

[3] Grace, T.D.C., 1967. Establishment of a cell line from the silkworm, *Bombyx mori*. Nature (London). 216, 613.

[4] Granados, R.R., McKenna, K.A., 1995. Insect Cell Culture Methods and Their Use in Virus Research: In: Schuler, M.L., Wood, H.A., Granados, R.R., Hammer, D.A., (Eds.), Baculovirus Expression Systems and Biopesticides. Wiley-Liss. New York, pp. 13-39.

[5] Inoue H., Mitsuhashi J. ABombyx mori cell line susceptible to a nuclear polyhedrosis virus. J. Seric. Sci. Jpn. 53; 108-113; 1984

[6] Khurad, A.M., Mahulikar, A., Rathod, M.K., Rai, M.M., Kanginakudru, S., Nagaraju, J., 2004. Vertical transmission of nuclear polyhedrosis virus in the silkworm, *Bombyx mori* L. J. Invertebr. Pathol. 87, 8-15.

[7] Khurad, A.M., Kanginakudru,S., Qureshi, S.O., Rathod, M.K., Rai, M.M., Nagraju, J., 2006. A new *Bombyx mori* larval ovarian cell line highly susceptible to nucleopolyhedrovirus J. Invertebr. Pathol. 92, 59-65.

[8] Khurad, A.M., Min-Juan Zhang., Deshmukh, C.G.2009. A new continuous cell line from larval ovaries of silkworm, *Bombyx mori*.

[9] Meda S.,1987. Epression of humaninterferon α in silkworm with a baculovirus Vector. In: Maramorosch K.(ed) Biotechnology in invertebrate pathology and cell culture. Academic, New York, pp 221-233;

[10] Meda S.,1989. Increased insecticidal effect by a recombinant baculovirus carrying a synthetic diuretic hormone gene. Biochem. Biophys. Res. Commum.165: 1177-1183.

[11] Meda S.,Volrath, S.L., Hanzlink, T.N., Harper, S.A., Majima, K., Maddox, D.W., Hammock, B.D., Fowler, E., 1991. Increased insecticidal effect of an insect specific neurotoxin expressed by a recombinant baculovirus. Virology 184, 777-780.

[12] Pan M.H., Xiao S.Q., Chen M; Hong X.J; Lu C. Establishment and characterization of two embryonic cell lines of Bombyx mori. IN vitro cell dev. Biol. Anim, 43; 101-104; 2007.

[13] Pandhripande T.N. Two new continuously growing cell lines from Bombyx mori (Lepidoptera:Bombycidae). App. Entomol Zool 29;604-607; 1994.

[14] Raghow, R., Grace, T.D.C., 1974. Studies on a nuclear polyhedrosis virus in *Bombyx mori* cell *in vitro*. I. Multiplication kinetics and ultrastructural studies. J. Ultrastruct. Res. 47, 384-399.

[15] Smith, G.E., Fraser, M.J., Summers, M.D., 1983. Molecular engineering of the *Autographa californica* nuclear polyhedrosis virus genome-deletion mutations within the polyhedrin gene. J. Virol. 46, 584-593.

[16] Summers, M.D., 1989. Recombinant proteins expressed by baculovirus vectors. In: Notkins, M.B.A. and Oldstone, M.B.A. (Eds.), Concepts in Viral Pathogenesis III. Springer-Verlag, New York, pp. 77-86.

[17] Sudeep A.B., Mishra A.C.,Shouche Y.S.; Pant U; Mourya D.T. Establishment of two new cell lines from Bombyx mori (L.) (Lepidoptera; Bombycidae) and their susceptibility to baculoviruses. Indian J. Med. Res. 115; 189-193; 2002

Authors

First Author – C. G. Deshmukh, Department of Zoology, A.C.S. College Koradi, chanchaldeshmukh@gmail.com
Second Author – R.S. Bahekar, Department of Zoology, A.C.S. College Koradi., rbahekar@yahoo.com