Growth of a clonal cell line of *Helicoverpa zea* (Lepidoptera: Noctuidae) in suspension culture and replication of its homologous baculovirus HzSNPV

Arthur H. McIntosh, James J. Grasela, Cynthia L. Goodman and Carlo M. Ignoffo

USDA, Agricultural Research Service, Biological Control of Insects Research Laboratory, Columbia, Missouri 65203, U.S.A.

(Received 26 January 2001; Accepted 11 April 2001)

**Abstract**

A clonal cell line (BCIRL-HZ-AM1-11) of *Helicoverpa zea* was grown in stationary and suspension cultures in Ex-Cell 401\textsuperscript{TM} medium containing 10% fetal bovine serum at 28°C. The cell population doubling time was 22 h in stationary culture as compared with 27 h in suspension culture. A lag time of approximately 24 h was observed during the first 24 h of the suspension culture following initiation but no lag time was observed in the stationary culture. Maximum viral titers were achieved in stationary and suspension cultures at 120 h (1.80×10\textsuperscript{6} TCID\textsubscript{50}/ml) and 168 h (1.48×10\textsuperscript{7} TCID\textsubscript{50}/ml), respectively, following inoculation with the *Helicoverpa zea* baculovirus (HzSNPV/Br-CL2). Infected cells harvested at 168 h from the 50 ml suspension culture produced a total number of occlusion bodies of 3×10\textsuperscript{9}.

**Key words:** *Helicoverpa zea*, suspension culture, HzSNPV

**INTRODUCTION**

Insect cell cultures have found many applications in virology including the growth and replication of baculoviruses as well as the study of the molecular biology and genetics of these viruses. More recently, an important use of cell culture has been as baculovirus expression vector (BEV) systems for the expression of foreign genes (Smith et al., 1983; Maeda, 1994; Jarvis, 1997). Other applications include large scale production of insect cells for the production of biologicals of medical importance by recombinant baculoviruses (Lakey et al., 1996; Trenor et al., 1996) as well as the production of viral insecticides (Vaughn, 1976; Weiss and Vaughn, 1986; Maiorella et al., 1988; Agathos, 1994; Goodman and McIntosh, 1994). Although none of the baculovirus insecticides presently on the market have been produced in large scale cell culture, the technology for achieving this goal is for the most part presently available. In order to achieve large scale production many processes had to be developed and tested, including the generation of cell lines and the evaluation of their susceptibility to candidate viruses. In addition, media which could support the growth of cell lines had to be developed and evaluated. The identification of serum substitutes reduced media cost, as well as facilitating recovery of recombinant proteins from reactor vessels (Maiorella et al., 1988; Vaughn and Fan, 1989; Hink, 1991).

*Helicoverpa zea* is a major pest of field crops and there have been a number of cell lines derived from this insect that have been used in the study of the homologous baculovirus HzSNPV (Granados et al., 1981; McIntosh and Ignoffo, 1981; Goodwin et al., 1982; Gettig et al., 1987; Goodman et al., unpublished). The present report utilizes a clone (BCIRL-HZ-AM1-A11) from the parental cell line BCIRL-HZ-AM1 which was shown to be one of the better producers of HzSNPV in stationary cultures (Lenz et al., 1991) as well as the best *H. zea* clonal cell line for the plaque assay of HzSNPV (McIntosh et al., 1997). It was therefore of interest to determine whether this cell line could grow in suspension culture with concomitant production of high levels of its homologous baculovirus that would be useful for laboratory scale production of HzSNPV.

**MATERIALS AND METHODS**

**Insect cell line.** The cell line employed in this study was BCIRL-HZ-AM1-A11 (Lenz et al., 1991), a clone from the parental cell line BCIRL-HZ-AM1 derived from pupal ovarian tissue (McIntosh and Ignoffo, 1981). This cell line which will be referred to as HZ-A11 in this report has been
subcultured for over 100 passages (at split ratios of 1:10 and 1:40) in ExCell™ 401 medium (JRH Biosciences, Lenexa, KS) supplemented with 10% inactivated (56°C/30 min), fetal bovine serum (FBS, Summit Biotechnology, Fort Collins, Colorado), penicillin (50 units/ml) and streptomycin (50 μg/ml). This cell line is routinely maintained as a stationary culture in T-25 cm² flasks at 28°C but was passed serially three times in suspension culture before being employed in the present study.

**Cell growth studies.** For stationary attached cultures, HZ-A11 cells were counted and seeded at 2×10⁵ cells/ml in 5 ml of growth medium in T-25 cm² flasks. For each time interval, cells from each of three flasks were harvested by pipetting back and forth and triplicate counts were performed with a hemocytometer. Suspension cultures (in triplicate) were initiated at the same cell concentration (2×10⁵ cells/ml), in 50 ml of growth medium in 250 ml Erlenmeyer flasks and placed on a rotary shaker at 120 RPM. At each time interval 0.5 ml was removed from each of the three flasks and triplicate cell counts were performed on each sample. Cell population doubling times were calculated as previously reported (McIntosh and Ignoffo, 1983).

**Inoculation of cells and quantitation of virus.** Cells were inoculated at a multiplicity of infection (MOI) of 2, with a clone from the wild-type virus HzSNPV/Br designated as HzSNPV/Br-CL2 at passage 5 (McIntosh et al., 1997). For stationary attached cultures, adsorption was allowed to take place for 2 h on a rocker platform (Bellco, Vineland, NJ) at a setting of 3. At the end of this period, the inoculum was removed and the cells in each flask were washed twice with a total of 10 ml of Hanks’ balanced salt solution (HBSS). Five milliliters of growth medium was then added to each flask and placed in an incubator at 28°C. For the suspension aspect of this study, cultures were inoculated at the same MOI of 2 by first allowing the cells to attach to the surfaces of T-75 cm² flasks and then introducing the virus inoculum and treating them in the same manner as the stationary cultures. After adsorption and washing, the inoculated cells were then seeded into 250 ml Erlenmeyer flasks (50 ml/flask) and placed on the rotary shaker at 28°C. Extracellular virus (ECV) was quantitated by the TCID₅₀ assay (Grasela and McIntosh, 1998) from both stationary and suspension cultures at specified time intervals as shown in Fig. 2. The growth medium from inoculated stationary cultures was removed from each of 3 flasks and centrifuged at 1,400×g for 10 min. The supernatant fluids were recovered and assayed for ECV in HZ-A11 cells. For suspension cultures, 0.5 ml was removed from each of 3 Erlenmeyer flasks at specified intervals and transferred into sterile 1.5 ml microfuge tubes and spun in a table-top microcentrifuge (Hermle, Woodbridge, NJ) for several seconds to pellet the cells. The supernatant fluids were removed and titered by TCID₅₀ as described.

**Cell line and virus identification.** The HZ-A11 cell line was identified by the DAF-PCR procedure (McIntosh et al., 1996) and HzSNPV/Br-CL2 by REN analysis (McIntosh and Ignoffo, 1986).

**RESULTS AND DISCUSSION**

The results of HZ-A11 grown in stationary attached culture and in suspension culture are presented in Fig. 1. There was a lag time in the increase of cell numbers following initiation of the suspension culture over the first 24 h but no lag time was observed for the stationary culture. The maximum density attained for the stationary culture was 6.4×10⁶ cells/ml at 168 h post-initiation, as compared with 13.8×10⁶ cells/ml for the suspension culture at 192 h. The cell population doubling times for the stationary and suspension cultures were 22 h and 27 h, respectively, and no obvious morphological differences could be observed between the cells from these two types of cultures.
Cell population doubling times have not been reported previously for HZ-A11, but the doubling time for the parental cell line HZ-AM1 in ExCell™ 401 serum-free medium in stationary culture was 27 h (McIntosh et al., 1995). It was also demonstrated that HZ-A11 can also grow in the serum-free medium ExCell™ 401 in suspension culture reaching a cell density of $3 \times 10^6$ cells/ml over a one week period.

The DAF-PCR cell line identification technique showed no differences in the DNA banding patterns in cells grown in either stationary or suspension cultures, and confirmed the identity of the HZ-A11 cell line as originating from *H. zea* (data not shown).

The replication of HzSNPV/Br-CL2 in HZ-A11 stationary and suspension cultures is shown in Fig. 2. The maximum titers attained for HzSNPV/Br-CL2 in cells grown in stationary and suspension cultures were $1.8 \times 10^6$ TCID$_{50}$/ml at 120 h and $1.5 \times 10^7$ TCID$_{50}$/ml at 168 h, respectively. Although there was a short lag time in the growth of the virus in suspended cells, no such lag time was observed in cells grown in stationary culture.

In considering large scale *in vitro* production of baculoviruses, the substrate, medium and virus are very important parameters in the successful development of such a process. It is first necessary to select a suitable cell line (substrate) which can be grown in suspension culture in large fermenters designed to grow cells in suspension. This investigation demonstrated that HZ-A11 cells grow to higher densities in suspension cultures than in stationary cultures and also produce approximately a 10 fold higher virus titer in suspension cultures than cells in stationary culture. The OB count from HZ-A11 in stationary culture was $5 \times 10^5$/ml in a total volume of 5 ml whereas the OB count from HZ-A11 in suspension culture was $6 \times 10^7$/ml or a total of $3 \times 10^9$ in a total volume of 50 ml.

The reasons for better growth in suspension culture is unknown but may involve better aeration as well as better presentation of required nutrients and possible avoidance of localized build up of inhibitory cellular metabolites. This study shows that the suspension culture system is better than the stationary conventional method for laboratory-scale production of both HZ-A11 cells and HzSNPV. HZ-A11 cells grew in serum-free medium but may need further adaptation before it can attain the cell density observed in medium containing serum. Since serum is the most expensive component of cell growth medium, its concentration from 10% could be gradually reduced if cells failed to attain high densities in serum-free medium. Further studies would be necessary in scale-up bioreactors to determine whether the same or better productivity could be achieved.

**ACKNOWLEDGEMENTS**

The authors thank Mr. Steve Long for technical assistance.

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that also may be suitable.

All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

**REFERENCES**


Goodwin, R. H., G. J. Topkins, R. R. Gettig and J. R. Adams


