Cell Culture Optmization for the Baculovirus Expression Vector System (BEVS)

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ABSTRACT

An experiment was carried out to study the fundamental factors that affect the growth of *Spodoptera frugiperda* Sf-9 insect cells. Initial cell density, spent medium carry over and inoculum phase withdrawal significantly influenced the growth kinetics of Sf-9 cells. The percentage of cells infected with *Autographa californica* multiply-enveloped Nuclear Polyhedrosis Virus (AcMNPV) was obviously affected by spent medium carry over. On the other hand, initial cell density and Multiplicities of Infection (MOI) have minimal influence on infectivity percentage.

Keywords: *Spodoptera frugiperda* (Sf-9), Multiplicities of Infection (MOI), *Autographa californica* multiply-enveloped Nuclear Polyhedrosis Virus (AcMNPV).

1. INTRODUCTION

The baculovirus expression vectors system (BEVS) has attracted wide attention as vectors for high level expression of a variety of heterologous proteins from prokaryotic and eukaryotic origin in insect cells (J M Vlak, 1997). As a prerequisite of successful cell growth and later cell infection with baculovirus, a good quality inoculum is necessary. For the first part of this study, a few parameters that optimize the growth rate of Sf-9 cells culture had been studied including initial cell density, effect of phase withdrawal and spent medium carry over. For the mock baculovirus infection, the interaction of the infection parameters also had been reviewed especially Multiplicities of Infection (MOI) with the above culture parameters.

2. METHODOLOGY

2.1 INSECT CELL LINE HOST

Spodoptera frugiperda (Sf-9) insect cells (ATCC cat. No. 1711, Rockville, MD) were maintained in 25 cm^3 tissue culture flasks (Techno Plastic Products(TPP), Switzerland) in a humidified 27^0C incubator (Memmert, Switzerland). Cell viability was determined using the trypan blue exclusion test and cell counts were performed using an inverted microscope (Zeiss Instruments, Germany).

2.2 WILD TYPE BACULOVIRUS EXPRESSION VECTOR

The wild type baculovirus AcMNPV was a gift from Universiti Kebangsaan Malaysia.

2.3 VIRUS TITER

In order to calculate the concentration of the infectious virus particles in wild type baculovirus stock, end point dilution was performed by using the method developed by Reed and Muench (1938) and modified by Nielsen *et al.* (1992).

2.4 INFECTION WITH BACULOVIRUS

Infection of Sf-9 insect cells involved inoculation of exponential stage cell cultures with a small volume of viral stock of known viral titer.

2.5 SAMPLING AND STORAGE

Samples were withdrawn at regular 24 hour intervals and centrifuged at room temperature (1000g, 10min) in a microcentrifuge (Desaspeed MH-2, Germany). The supernatant was separated from the cell pellet and directly stored at 4 ^{0}C. The cell pellet was lysed in lysis buffer (50mM Tris-HCl, pH 7.5, containing 1% Triton X-100, 10mM EDTA, 150m*M* NaCl, 1mM PMSF) at 4 ^{0}C for 60 min, followed by centrifugation and storage of the supernatant fraction at 4 ^{0}C. Samples were kept in -20 ^{0}C freezer for long term storage.

3. RESULTS AND DISCUSSION

3.1 Sf-9 CELL GROWTH OPTMIZATION

The effect of inoculum cell density was investigated at three different cell densities- 0.20 x 10^5, 1.20 x 10^5 and 2.33 x 10^5 cells/ml. As shown in Fig 1, the lowest cell concentration resulted in achieving the lowest maximum viable cell number. This observation is in contrast with Kioukia N. (1995), which found that the maximum cell number achieved was highest for the lowest density. However, the maximum growth rate, μ, was similar in all three cell concentrations.

Another factor regarding phase withdrawal of the inoculum (Fig 2) was also studied. Three inocula at fixed density of 1.6 x 10^5 cells/ml were seeded at three different phases – early exponential, late exponential and stationary phase. Early exponential phase gave the fastest growth rate (0.014/h), while those from stationary phase were obviously unsatisfactory (growth rate and maximum viable cell number were 0.006/h and 12.55 x 10^5 cells/ml). In a related work (Kiokia N., 1995), it has been shown that there were higher proportions of G1 and S phase cells in the early exponential than in the other growth phases. It was reported that in insect cells, the resting phase is G2 where most cells are accumulated when nutrient is depleted (Fertig G *et al.*, 1990). And this could explain why the cells from the early exponential phase set off faster and achieved the highest growth rate.

The effect of spent medium carry over on cell growth pattern was also studied. All cell cultures were inoculated at fixed density of 1.5 x 10^5 cells/ml as shown in Fig 3. It is obvious that the effect of medium carry over was significant for the spent medium ratio 1:1 and 1:2, which resulted in reduction in growth rate and maximum cell number compared to the negligible ratio. The result is expected because cells prefer to survive in a rich nutrient culture.

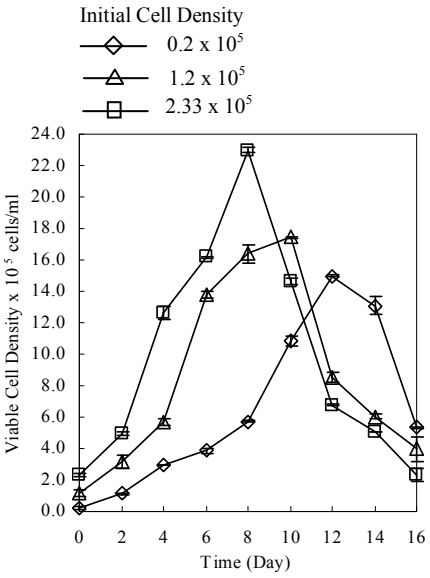

Figure1: The Effect of Initial Cell Density on the Growth of Sf-9 Insect Cells

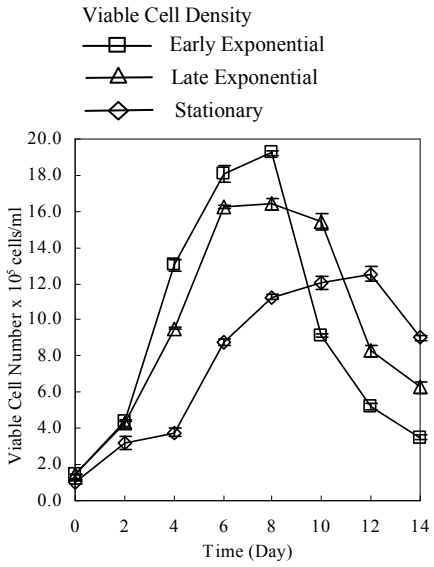

Figure2: The Effect of Phase Withdrawal of the Inoculum on the Growth of Sf-9 Insect Cells

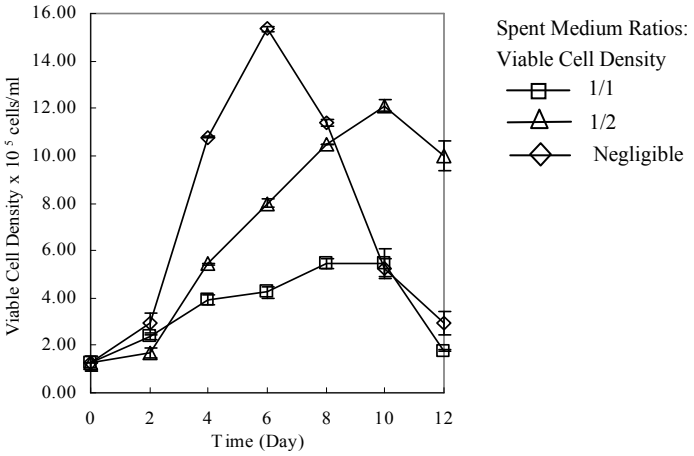

Figure3: The Effect of Spent Medium Carry Over on the Growth of Sf -9 Insect Cells

3.2 ESTABLISHMENT OF BACULOVIRUS EXPRESSION VECTORS SYSTEM (BEVS)

3.2.1 Mock Infection Optimization

The effect of cell density at the time of infection in fresh medium was examined by infecting cells from the early exponential phase with wild type baculovirus (AcMNPV) at MOI 10 at three different densities, 0.95 x 10^5, 2.05 x 10^5 and 5.13 x 10^5 cells/ml. The cell densities used in this experiment were not too high in order to avoid any oxygen and nutrient limitation possibly caused in the small flasks. As can be seen in Fig. 4, the rates of infection were similar and reached a maximum infectivity of 98.5%, 99.5% and 100% for 0.95 x 10^5, 2.05 x 10^5 and 5.13 x 10^5 cells/ml respectively by 120 h post-infection.

As for the spent medium effect on the infectivity, it significant effects were observed for spent medium ratio of 1:1 and 1: 2. The infectivity percentages for the spent media ratio 1:1, 1:2 and negligible were 72.5%, 93.8% and 99.8% respectively by 120 h post infection. The result is observed on Fig 5.

It is expected that increasing the added amount of virus in cultures can intensify the process of cell infection. Therefore, by increasing the number of virus per cell (MOI), a reduction in the time of cell infection can be achieved. This experiment was carried out

using different MOI to infect the stationary phase cell culture using fresh media. This is because cell infectivity and viral yields in stationary phase are strongly dependent on the MOI (Licari R *et al.*, 1991). The behavior is different to that when cells were infected in the exponential phase (Maiorella B *et al.*,1988 , Schorp B *et al.*,1990). Fig 6 shows that in the stationary phase, higher MOI will enhance the rate of infection (rate of polyhedra development as observed microscopically). However, the final infectivity for each MOI was similar because the infections were carried out in fresh media and most probably the availability of nutrient allowed the cells to survive long enough to be infected from viruses released from primary infected cells.

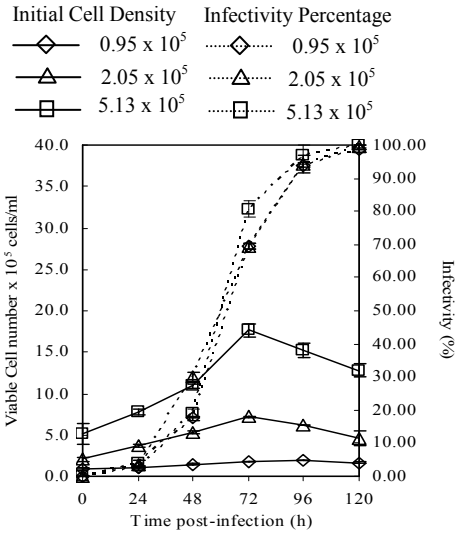

Figure4: The Effect of Initial Cell Density on Sf-9 Insect Cells Infected with Wild Type AcMNPV Virus at MOI 10

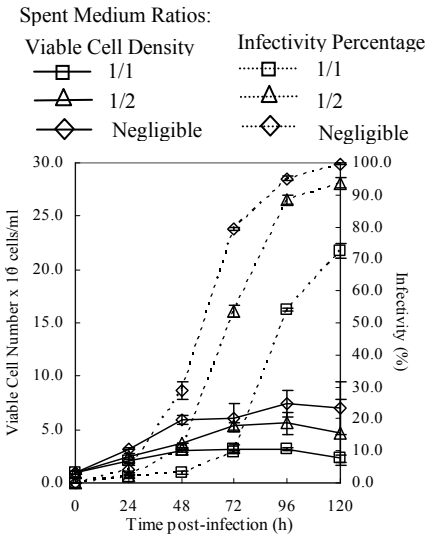

Figure5: The Effect of Spent Medium on Sf-9 Insect Cells Infected with Wild Type AcMNPV Virus At MOI 10

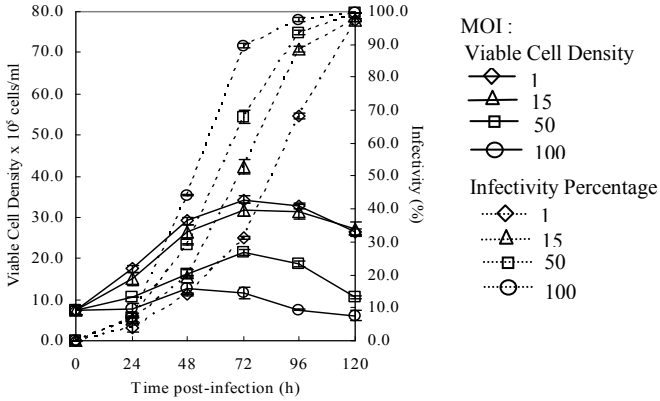

Figure 6: The Effect of Initial Cell Density on Sf-9 Insect Cells Infected with Wild Type AcMNPV Virus at MOI 10

4. CONCLUSIONS

A set of fundamental study regarding the physiological and environmental factors that affect the growth of insect cells and infection with baculovirus had been carried out early in this research. These data provided a initial understanding that can influence the growth, cell infection, viral multiplication and protein expression in Sf-9 insect cells.

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