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Mathematical model for change in diameter distribution of baculovirus-infected Sf-9 insect cells

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Abstract

Sf-9 insect cells were examined for diameter before and after baculovirus infection. The diameter distribution of uninfected Sf-9 insect cells was described by the normal distribution function with a mean cell diameter and standard deviation of 18.5 ± 1.5 μm. After synchronous infection at a high MOI and high cell density, the infected cells grew in diameter almost linearly with time for about 48 h to have a steady mean diameter 1.45 times larger than that of uninfected cells, though still normally distributed. The distribution of the infected cells began to broaden at around 6 h-post-infection and eventually had standard deviation twice as large as that of uninfected cells. A mathematical model was developed to describe the change in the diameter distribution of virus-infected insect cells, integrating the distributions of infected cell subsets that were generated according to the Poisson distribution function between time t and $t + \mathrm{d}t$ and individually characterized with a post-infection time. The growth of uninfected cells and post-infection events such as progeny virus replication and secondary infection of uninfected remained cells were simulated according to a previous report. The model calculation predicts the change in diameter distribution of Sf-9 insect cells that were infected under various conditions, giving an indication to assess the degree of virus infection.

Keywords: Baculovirus; Insect cell; Sf-9; Modelling; Diameter distribution

1. Introduction

The baculovirus expression vector system has been recognized as one of the most viable means for producing mammalian proteins not only in basic research field but also in the industrial production of vaccines and therapeutics, because of the ability to express a wide variety of proteins in high yield and of biological and immunological proper functionality of the produced proteins [1,2]. Several factors such as the infecting cell density (ICD) that is defined as the cell density at time of infection, multiplicity of infection (MOI), and dissolved oxygen (DO) have been revealed to significantly influence protein yields, not only by experimental evidence [3–8] but also by model calculation [9–16]. From an industrial perspective, these parameters must be determined properly so that the infection cultures yield a large amount of recombinant proteins. Sometimes, however, unexpected fluctuations in the progress of viral infection and recombinant protein production occur in the course of the cultures probably due to diversity in cellular physiology and medium composition and inaccuracy in determining virus titer and cell density. Therefore, a certain index parameter reflecting the physiological status of infected cells should be monitored during the cultures in order to operate them properly.

The decrease in viability of insect cells has been often used as a convenient indication to show the baculovirus infection [17]. After infection, cells stop growing and gradually die in the course of culture. However, there is a quite long time delay from infection until the decrease in cell viability becomes distinct. An apoptosis-suppressing ability assay [18] and an immunological assay [19,20] were reported to assess the infection of insect cells with baculovirus in early infection stage, while they required several preparatory steps to treat the cells with reagents. It was also reported that flow cytometry [21,22] and on-line monitoring of respiration [23] made it possible to follow the progress of baculovirus infection of Sf-9 insect cells, though these analyses require relatively expensive equipments.

Meanwhile, it has been widely recognized that insect cells increase in diameter after baculovirus infection. Braunagel et

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Nomenclature

d	cell diameter [μm]
$\bar{d}$	mean cell diameter of insect cells [μm]
$\bar{d}_U$	mean cell diameter of uninfected insect cells [μm]
$\bar{d}(\tau)$	mean cell diameter of virus-infected insect cells [μm]
f_{act}	dimensionless factor for physiological activity of virus-infected cells
$F(d;\bar{d},\sigma)$	normal distribution function
F_{app} (d)	apparent distribution function of infected insect cells
j	number of viruses infecting a cell [pfu]
k_D	intrinsic specific death rate of uninfected cells [h^{-1}]
k_V	intrinsic specific virus replication rate [pfu $cell^{-1}$ h^{-1}]
K_S	Monod saturation constant for glutamine consumption [mM]
$n(t,\tau, j\neq 0)dt$	concentration of cells infected by at least one virion during the time interval dt [cells ml^{-1}]
$n(t,0, j\neq 0)dt$	concentration of cells that are newly infected by at least one virion [cells ml^{-1}]
q_S	specific growth-limiting substrate consumption rate of cells [mM $cells^{-1}$ h^{-1}]
$r_d(\tau)$	dependence of mean cell diameter in the normal distribution function on time post-infection
$r_\sigma(\tau)$	dependence of standard deviation in the normal distribution function for cell diameter on time post-infection
$S(t)$	concentration of a growth-limiting substrate (glutamine) in the medium [mM]
t	culture time [h]
$V(t)$	virus concentration in the medium [pfu ml^{-1}]
$X_U(t)$	concentration of viable uninfected cells [cells ml^{-1}]
$Y_{X/S}$	growth yield of insect cells [cells mM^{-1}]

Greek

$\Phi(t,j)$	probability that uninfected cells are infected by j viruses between time t and $(t+dt)$
$\Omega(\tau,\rho,\sigma)$	cumulative Weibull distribution function
α	proportionality constant in virus infection by Poisson distribution
τ	time post-infection for individual cells [h]
τ_d	time post-infection for individual cells to stop growing in diameter [h]
$\tau_{\sigma E}$	time post-infection for cell distribution to start to broaden [h]
$\tau_{\sigma L}$	time post-infection for cell distribution to stop broadening [h]
μ_{max}	maximum specific growth rate of cells [h^{-1}]
ρ and ρ'	shape parameters of Weibull distribution function for virus-producing and dead cells, respectively
σ	standard deviation in the normal distribution function [μm]
$\sigma(\tau)$	standard deviation in the normal distribution function of diameter of virus-infected insect cells [μm]
σ_U	standard deviation in the normal distribution function of diameter of uninfected cells [μm]
υ and υ'	scale parameters of Weibull distribution function for virus-producing and dead cells, respectively [h]

al. [24] reported that Sf-9 cells were gradually arrested in G_2/M phase by baculovirus infection. Prikhod'ko and Miller [25] reported that the transfection of Sf-21 insect cells with *ie2* gene of AcNPV arrested the cell cycle, resulting in the accumulation of enlarged cells with abnormally high DNA contents. Since the cell enlargement can be determined without any laborious and/or costly pretreatments, it would be a good candidate as well to conveniently assess the status of virus infection. In fact, Palomares et al. reported that the cell size was used as a tool to predict the recombinant protein production [26]. Rosinski et al. demonstrated that rates of cell size increase are an important measure of success during the baculovirus infection process [27]. However, information concerning the dynamics of cell diameter increases after virus infection has been still limited. In the present study, Sf-9 insect cells are examined in detail for the trend of cell diameter distribution change after baculovirus infection. A mathematical model is developed to simulate the change in cell diameter distribution, which permits estimating the degree of virus infection.

2. Model for baculovirus-infected insect cell cultures

The mathematical models have been developed elsewhere for virus-infected insect cell cultures, describing the growth of uninfected insect cells, virus infection, progeny virus replication, and infection-induced cell death, whose profiles are influenced by many factors including MOI and ICD [14,28]. In brief, the viral infection is described by the Poisson distribution function, which defined the probability of cells receiving any particular number of viruses j as follows:

$$\Phi(t, j) = \frac{\exp\{-\alpha(V(t)/X_U(t))\}\{\alpha(V(t)/X_U(t))\}^j}{j!}, \quad (1)$$

where $X_U(t)$ and $V(t)$ are the concentrations of viable uninfected cells and viruses at any given time t, respectively, and α is a proportionality constant that describes infection efficiency. The concentration of cells that are infected between time t and $t+dt$ is thus described as

$$n(t, \tau, j)dt = \Phi(t, j)X_U(t), \quad (2)$$

where $n(t,\tau,j)$, a frequency function, characterizes a population subset of infected cells with a post-infection time τ.