GROWTH LIMITING FACTORS INFLUENCING HIGH DENSITY CULTURE OF INSECT CELLS IN GRACE'S MEDIUM

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SUMMARY

Growth limiting factors influencing the high density cultivation of insect cells were studied. A stationary phase in Grace's medium was found to be due to the deprivation of the active form of FBS components. Various compounds were added in the early stationary phase to observe the recovery of cell growth. With the addition of yeastolate, final cell densities were 4-fold and 3.5-fold higher in monolayer and suspension cultures, respectively. Pluronic F-68 increases the specific growth rate and the growth yield of the cell as well as protects the cell from the shear damage.

INTRODUCTION

Insect cell-baculovirus system has wide applicability as an alternative to classical bacterial or yeast systems for the production of recombinant proteins. Insect cells are capable of most of the posttranslational modifications performed by mammalian cells including correct signal peptide cleavage, proteolytic processing, glycosylation, phosphorylation, palmitylation, and myristylation (Shuler, 1992). The posttranslational modification processes result in the expression of proteins that are functionally and immunogenetically similar to the authentic protein. Insect cell-baculovirus system is increasingly used for the production of recombinant proteins and insect pathogenic viruses. Many biotechnology companies are in the process of evaluating or are actually using this system for the synthesis of bioproducts of medical and agricultural significance (Agathos et al., 1990). The high density culture of insect cells is essential to the maximization of productivity. To increase cell density, various techniques such as periodic replacement of medium, perfusion culture (Klopping et al., 1990) and immobilization (King et al., 1988) have been used. Immobilized insect cell cultures may improve productivity in comparison to suspension cultures; however, there appear to be biocompatibility problems (Wu et al., 1989). The process developments for large-scale suspension culture of insect cells have been reported by Maiorella et al. (1988) and Murhammer and Goochee (1988).

This work is concerned with growth limiting factors influencing the high cell density culture in Grace's medium. The effects of seeding density, medium compounds,
and Phuronic F-68 on cell growth were investigated in monolayer and suspension cultures.

MATERIALS AND METHODS

Cell Line and Medium

*Spodoptera frugiperda* (Sf9) cell line was obtained from Dr. J.M. Yang, Department of Biology, Sogang University, Seoul, Korea. Cells were grown in Grace's medium (Gibco) supplemented with 0.35 g/L NaHCO₃ (Sigma), 5% fetal bovine serum (FBS, Hyclone) and antibiotic-antimycotic (Gibco, 600-5240AG).

Culture Condition and Assay

Cells were grown in 25-cm² tissue culture flasks (Falcon) containing 6mL of medium for the monolayer culture. Temperature was maintained at 28°C. For the suspension culture, 100mL spinner flasks (Bellco) were used and the medium was supplemented with 3% Phuronic F-68 (Gibco). The agitation rate was set at 80 rpm (Lindsay and Betenbaugh, 1992). The cell concentration was measured using a hemocytometer and cell viability was determined by trypan blue exclusion test (Summers and Smith, 1987). Since dead cells absorb trypan blue (Sigma), they could be identified under light microscopy.

RESULTS AND DISCUSSION

Cell Growth in Tissue Culture Flask

Sf9 cells grow as a monolayer in a tissue culture flask. The effect of seeding density on cell growth is shown in Fig. 1. The logarithmic value of cell concentration is linear to culture time. The lines have similar slopes, which means that seeding density does not affect the specific growth rate. However, higher final cell concentration can be obtained up to a certain value with higher seeding density. About 160 h after inoculation, the cultures reach a stationary phase. Although cultures with different seeding densities reach the stationary phase at a similar time, the final cell concentration for the culture with lower seeding density is significantly lower. It seems unlikely that this phenomenon is due to the accumulation of growth-inhibitory compound, since it appears to be a time dependent phenomenon, independent of the final cell concentration. One explanation for this phenomenon may be the inactivation of a critical nutrient or growth factor over time under the culture conditions. Therefore, various compounds were added in the early stationary phase to observe the recovery of cell growth.

Seven days after inoculation, glutamine, FBS, or Grace's medium components were added to make the concentrations same as fresh medium. Or the waste medium was replaced with fresh medium supplemented with FBS. The results are shown in Fig. 2. The addition of glutamine or Grace's medium components did not stimulate the cell growth. The addition of vitamins was also not helpful. The glucose concentration was measured and it was not limiting. The addition of FBS and the medium replacement with fresh Grace's medium containing 5% FBS did stimulate cell growth, however. Thus, the stationary phase is considered to be due to the deprivation of the active form of FBS components. The growth promoting activity of FBS was found to degrade