Journal of Microbiology and Biotechnology
Vol. 4, No. 3, 200-203, 1994

Insect Cell Culture for Recombinant β-galactosidase Production Using a Spin-filter Bioreactor

KIM, HAK-RYUL, KI-WOONG LEE, TAE-YONG KIM, JAI-HYUN OH, JAI-MYUNG YANG[1], SEOK-KWON KANG[2] AND IN-SIK CHUNG*

Department of Genetic Engineering, Kyung Hee University, Suwon,
[1]Department of Biology, Sogang University, Seoul,
[2]Department of Agricultural Biology, Seoul National University, Suwon, Korea

Spodoptera frugiperda IPLB-SF-21-AE cells were cultivated in a spin-filter bioreactor with continuous perfusion for the recombinant β-galactosidase production. At the perfusion rate of 0.06 hr^{-1}, the maximum cell density of insect cells in this bioreactor system reached 3.5×10^6 viable cells/m*l* using the Grace media containing 5% FBS and 0.3% Pluronic F-68. The recombinant β-galactosidase production of 8,100 units per reactor volume was also achieved at this perfusion rate.

Protein expression systems based on the *Autographa californica* nuclear polyhedrosis virus (AcNPV) have wide applicability as an alternative to prokaryotic or other eukaryotic expression system (6, 7, 9, 12, 13). However, the application of the baculovirus expression systems has been limited by difficulties in the scale-up of insect cell culture (10, 15). Therefore, the scale-up of insect cell culture is a scientific and technological challenge for ensuring the efficient production of various significant products for human health.

The major obstacle to scale-up lies in obtaining high cell density in culture vessels. The most effective way to achieve high cell density is to retain insect cells in the bioreactor by suitable methods.

Cell retention can be problematic, if the cells are separated outside the cultivation vessel. Filtration and centrifugation steps may pose problems of contamination and cell lysis. The separation of the aqueous medium from the cells within the cultivation vessel would be advantageous as this would eliminate the need for pumping the fragile cells and should alleviate the problems with aseptic operation. Internal cell retention has been achieved by several investigators through cell precipitation or spin-filtration (14, 16). But it has been reported that cell retention using precipitation works only over a narrow range of perfusion rate (5). Therefore, it appears that the cell retention using internal spin-filtration needs to be further investigated.

*Corresponding author
Key words: *Spodoptera frugiperda*, spin-filter bioreactor, perfusion, recombinant β-galactosidase production

In this study, *Spodoptera frugiperda* IPLB-SF-21-AE cells were cultured in a spin-filter bioreactor in order to obtain information for future development of large suspension culture. This work was also to examine the feasibility of recombinant protein production by cells with a genetically-modified baculovirus.

MATERIALS AND METHODS

Cell Line and Culture Conditions

The cell line used in this study was *Spodoptera frugiperda* IPLB-SF-21-AE (Sf 21) cells. The cells were maintained in 25 cm^2 and 75 cm^2 tissue culture flasks to provide cells for perfusion reactors. A recombinant AcNPV expressing *E. coli* β-galactosidase was propagated and amplified on monolayer Sf 21 cells and kept at 4°C in the form of supernatant (11). The medium used was Grace's insect medium (Sigma), which was supplemented with 50 μg/m*l* gentamycin sulfate, 2.5 μg/m*l* fungizone, 0.35 g/*l* sodium bicarbonate and 5% (v/v) fetal bovine serum (Gibco).

Perfusion Bioreactor Operations

The spin-filter bioreactor was a 300 m*l* spinner flask equipped with 10 μm stainless steel filter element which was a part of the impeller assembly (Fig. 1). Insect cells were first grown batchwise in a spin-filter bioreactor with 150 m*l* of complete medium containing 5% FBS in Grace medium. After the cell density reached $5\sim10\times10^5$ cells/m*l*, the perfusion was started with Grace medium containing 5% FBS under the conditions of the initial pH 6.2, 28°C, 80 rpm and surface aeration,

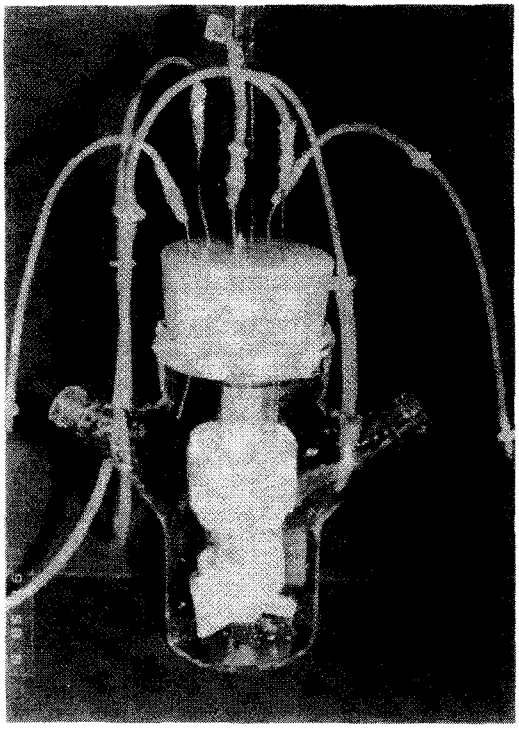

Fig. 1. Photograph of a spin-filter bioreactor.

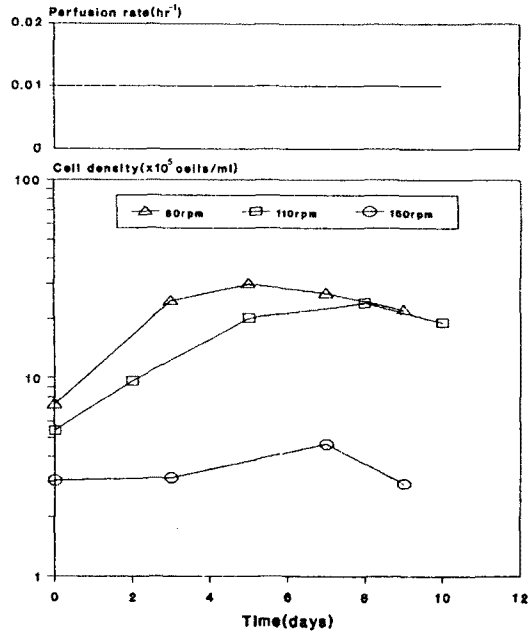

Fig. 2. Effect of agitation rate on cell growth.

unless otherwise specified.

Analytical Methods

The cell number was counted with a hemacytometer under the microscope. The cell viability was determined by the dye exclusion method with 0.4% trypan blue solution. The recombinant β-galactosidase activity was determined by the procedure described as elsewhere (8). One unit of activity is defined as 1.0 mM of ONPG cleaved per minute at 37°C and pH 7.3. One mg of pure β-galactosidase contains approximately 300 units of activity (Sigma, G-6008).

RESULTS AND DISCUSSION

The experiments were carried out in a spin-filter bioreactor to investigate the effect of agitation rate on insect cell growth. As shown in Fig. 2, viable cell density decreased abruptly as the agitation rate was increased from 80 rpm to 150 rpm. Agitation at 80 rpm had no apparent harmful effect on cell proliferation for up to 5 days and then viable cell density started to decrease. At this condition lower hydrodynamic damage to the cells would be expected.

It is well known that surfactant Pluronic F-68 protects animal cells against hydrodynamic forces. Therefore, the insect cell growth was tested using a media containing 0.1% Pluronic F-68. The specific growth rate in the media containing 0.1% Pluronic F-68 was no better than that in the media without Pluronic F-68 (Data not shown). However, The addition of 0.3% Pluronic F-68 to the media improved insect cell growth in the spin-filter bioreactor. Evidently, medium supplemented with 0.3% Pluronic F-68 protected the cells from detrimental hydrodynamic forces. The specific mechanism of Pluronic F-68 protection is not clear at present time, although Handa *et al.* (3-4) hypothesized that the protection is due to formation of a stable foam layer on the medium surface. Fig. 3 shows the time course changes of the perfusion culture using Grace media with 0.3% Pluronic F-68. It is noted that during the perfusion culture a stationary level of 3.5×10^6 cells/ml was obtained, and that the specific production rate of recombinant β-galactosidase (β-gal/10^6 cells) was decreased. Similar observations were made with other researchers (1-2).

Since insect cell growth and recombinant protein production may have been influenced by the medium composition, the comparison of the culture kinetics under three different media was made. Fig. 4 shows the cell growth and β-galactosidase production in Grace, TNM-FH, and IPL-41 media, respectively. In both IPL-41 and TNM-FH media, the cell growth and β-galactosidase production were relatively poor compared to those in the Grace media. In all cases, β-galactosidase productivity(β-gal/10^6 cells) decreased dramatically as the perfusion progressed. It appears that the β-galactosidase productivities decrease gradually during the perfusion culture. This may be because the metabolic activities of cells decrease with limited oxygen supply due to surface aeration or with cell density effect.

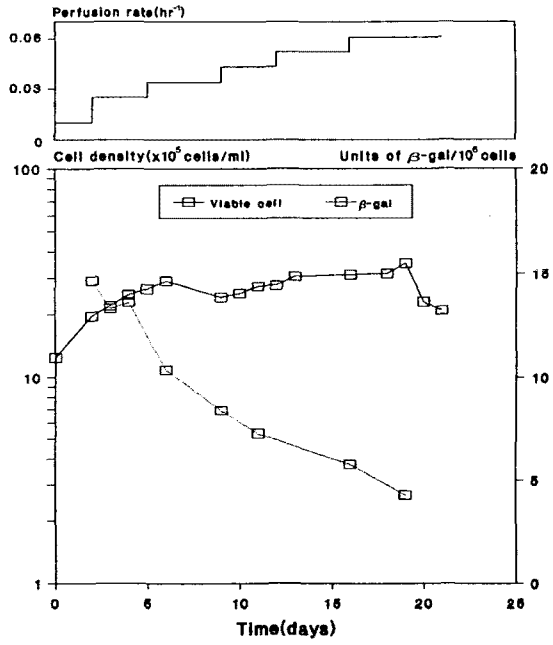

Fig. 3. Time course changes of cell growth and recombinant β-galactosidase production.

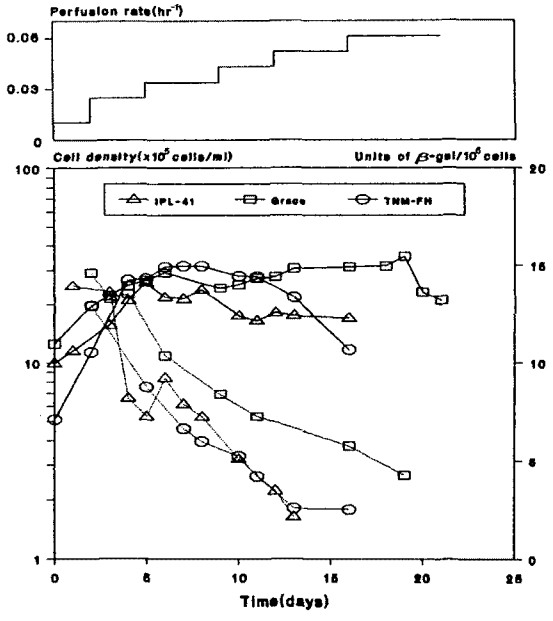

Fig. 4. Effect of media composition on cell growth and recombinant β-galactosidase production.
Cell density (—); activity of β-galactosidase (----).

To find out the effect of aeration on insect cell growth and β-galactosidase production, bioreactor experiments under both surface aerated and sparged conditions were carried out using the Grace media containing 0.3% Pluronic F-68. In the culture at sparged conditions, maximum cell density reached to about 3.05×10^6 cells/ml

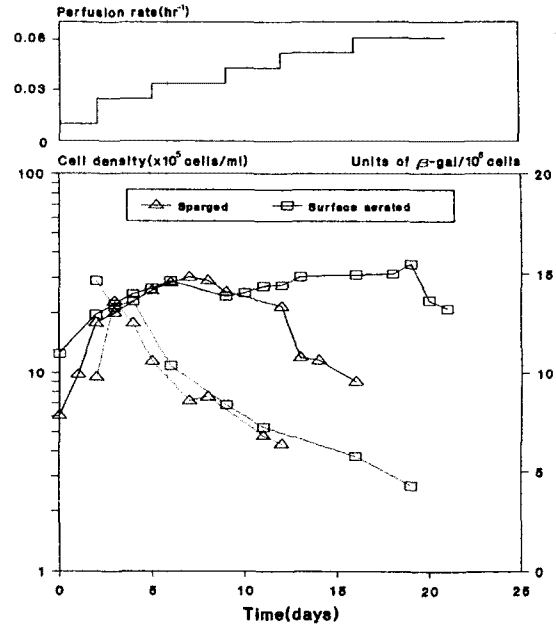

Fig. 5. Profiles of recombinant β-galactosidase production at surface aerated and sparged conditions.
Cell density (—); activity of β-galactosidase (----).

and the trend of β-galactosidase production was similar to that obtained from previous experiments (Fig. 5). Therefore, oxygen limitation due to surface aeration may not be appropriate to explain reduced β-galactosidase production during the perfusion culture in a spin-filter bioreactor. These results suggest that cell density effect is the important factor causing reduced β-galactosidase production in a spin-filter bioreactor.

Improvements may still be made by further optimization of other operating conditions. Provided that the conditions which presently limit cell and viral growth are well identified, a high density of insect cells and the maximum productivity of recombinant β-galactosidase could be obtained in a spin-filter bioreactor.

Acknowledgement

This work was supported by the Korea Science and Engineering Foundation (Grant No. 911-1005-018-2 and 91-0500-13) and by the Research Center for New Bio-Materials in Agriculture.

REFERENCES

1. Cavegn, C. and A. Bernard. 1992. A perfusion process for high density insect cell cultures. p. 262-273. *In* Vlak, J.M., E.J. Schlaeger and A.R. Bernard(eds.), *Proceedings of the baculovirus and recombinant protein production workshop*, Interlaken, Switzerland.

2. Deutschmann, S. and V. Jager. 1991. High density suspension culture of insect cells in a stirred bioreactor. p. 151-158. *In* Sasaki, R. and K. Ikura(eds.), *Animal cell culture and production of biologicals*, Kluwer Acad. Pub., Dordrecht.
3. Handa, A., A.N. Emery and R.E. Spier. 1987. On the evaluation of gas-liquid interfacial effects on hybridoma viability in bubble column bioreactors. *Dev. Biol. Stand.* **66**: 241-252.
4. Handa-Corrigan, A., A.N. Emery and R.E. Spier. 1989. Effect of gas-liquid interfaces on the growth of suspended mammalian cells: mechanisms of cell damage by bubble. *Enzyme Microb. Technol.* **11**: 230-235.
5. Kim, H.R., B.H. Chung, C.H. Kim and I.S. Chung. 1991. Continuous culture with cell precipitation for recombinant protein production. p. 81-86. *In* Sasaki, R. and K. Ikura(eds.), *Animal cell culture and production of biologicals*, Kluwer Acad. Pub., Dordrecht.
6. Luckow, V.H. and M.D. Summers. 1988. Trends in the development of baculovirus expression vectors. *Bio/Technology* **6**: 47-55.
7. Maiorella, B., D. Inlow, A. Shauger and D. Harano. 1988. Large-scale insect cell-culture for recombinant protein production. *Bio/Technology* **6**: 1406-1410.
8. Miller, J.H. 1972. Assay of β-galactosidase, pp. 352-355. In Experiments in molecular biology, Cold Spring Harbor Lab, New York.
9. Miller, L.K. 1988. Baculoviruses as gene expression vectors. *Ann. Rev. Microbiol.* **42**: 177-199.
10. Murhammer, D.W. and C.F. Goochee. 1988. Scale up of insect cell cultures: protective effects of Pluronic F-68. *Bio/Technology* **6**: 1411-1418.
11. Park, Y.M. 1991. Development of mass production system of useful proteins in insect cells, M.S. Thesis, Kyung Hee University, Korea.
12. Smith, G.E., G. Ju, B.L. Ericson, J. Moschera, H.W. Lahm, R. Chizzonite and M.D. Summers. 1985. Modification and secretion of human interleukin-2 in insect cells by baculovirus expression vector. *Proc. Natl. Acad. Sci.* **82**: 8404-8408.
13. Smith, G.E., M.D. Summers and M.J. Frazer. 1983. Production of human β-interferon in insect cells infected with a baculovirus expression vector. *Mol. Cell. Biol.* **3**: 2156-2165.
14. Takazawa, Y. and M. Tokashiki. 1988. High cell density perfusion culture of mouse-human hybridomas. *Appl. Microbiol. Biotechnol.* **32**: 280-284.
15. Tramper, J., J.B. Williams and D. Joustra. 1986. Shear sensitivity of insect cells in suspension. *Enz. Microb. Technol.* **8**: 33-36.
16. Weiss, S.A. and J.L. Vaugh. 1986. Cell culture methods for large-scale propagation of baculovirus. p. 63-87. *In* Granados, R. and B.A. Federici(eds.), *The Biology of Baculovirus*, CRC Press, Florida.

(Received May 26, 1994)