Recombinant Protein Production in immobilized Insect Cell Culture System

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캡슐화된 곤충세포 배양시스템을 통한 유전자 재조합 단백질 생산

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The baculovirus expression vector system (BEVS) in insect cell culture has been widely used for expression a wide range ofheterologous proteins due to advantages such as high expression levels, limitless size of the expressing of proteins, post-translational modifications, simultaneous expression of multiple genes, and safety for vertebrates[1]. Most common BEV can be created by replacing the polyhedrin gene with a target gene under the transcriptional control of the polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcNPV). Then, they are expressed during the very late stages of infection in insect cells.

Although the insect cell culture has been usually employedfor a high production level by the BEV system, only a few desired products have been commercially produced. The main problem is associated with the scale-up of a virus infected insect cell culture, which is the desirable process toward the commercial production. At large cultures, the common method for supply oxygen to cells like sparging and agitation can damage the cells that are extremely shear-sensitive. In addition, the oxygen demand of the insect cells has been reported to be higher than that of mammalian cells, and the oxygen uptake increases upon virus infection. As well as, due to the lytic behavior of baculovirus infected cells, it is

difficult to achieve high cell densities and high production levels.

Cell immobilization systems have been shown as one of the solutions to overcome the disadvantages above. Immobilized cells are protected from any shear stresses, while oxygen and nutrients are supplied to the cells sufficiently, and the cells are retained in the hollow microspheres completely during the exchange of cultured medium. Hence it is possible to reach higher cell densities and product concentrations as compared to suspension culture[2]. Green fluorescent protein (GFP) isolated from jellyfish *Aequaorea victoria*is a protein consisting of 238 amino acids, with a molecular weight of 27kDa. The protein has a maximal absorption peak at 395nm and a minor peak at 470nm with anemission peak at 509nm[3]. Its fluorescence is very stable, independent, and requires no substrate, cofactor, or additional proteins for illuminating green light. At these reasons, GFP is highly attractive as a visual marker for gene expression[4),5)].

In this study, Sf21 insect cells were infected with the recombinant Ac-omega-GFP baculovirus for production of green fluorescent protein (GFP) as a sign of recombinant protein production in an immobilized culture system. *Spodoptera frugiperda* (Sf21) insect cells were grown in the hollow microspheres by using sodium-cellulosesulfate (NaCS) and poly-dialyldimethylammoniumchloride (PDADMAC) polymers and infected with the Ac-omega GFP baculovirus that was a recombinant *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) encoded GFP gene for expression of green fluorescent protein.

Immobilized Sf21 cell density achieved was 1.02×10^8cells/ml in hollow microspheres, which was about 30 fold higher than in the suspension culture. The produced green fluorescent protein was obtained 159μg/ml in the hollow microspheres as compared to 4.8 μg/ml in the suspension culture.

References

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