

論文内容要旨

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学位論文題目	Strategies to improve recombinant protein production using CHO cell culture system (CHO細胞を用いた組換えタンパク質生産における生産性向上法に関する研究)		
<p>内容要旨</p> <p>【Background and objectives】 Chinese hamster ovary (CHO) cell lines are widely used for therapeutic protein production. Recently, with the dramatic increase in demand for therapeutic antibodies, CHO cell culture systems have made significant progress by cell line development, clone screening and isolation, and optimization of basal media and feed supplements, as well as advances in cell culture process. However, the demands of the ever-increased markets for therapeutic antibodies still require cells to be more highly productive and to be grown at higher cell densities. Moreover, overcoming instability of cellular productivity is also important to maximize the effects of developed culture process. The purpose of this study is improving CHO cell production system by approaches through medium development and expression vector engineering.</p> <p>【Results and discussion】 In this study, we evaluated the effects of deoxyuridine addition to fed-batch cultures of antibody-expressing CHO cell lines. Furthermore, we investigated the effects of combined addition of deoxyuridine, thymidine, and deoxycytidine. Our results suggest that addition of these pyrimidine nucleosides can increase CHO cell growth, with no significant change in the specific production rate. As a result of the increased cell growth and prevention of decrease in viability in the death phase the antibody concentration was elevated and we were able to achieve more than 9 g/L during 16 days of culture. Similar effects of nucleoside addition were observed in fed-batch cultures of a Fab fragment-expressing CHO cell line, and the final Fab fragment concentration was more than 4 g/L. This nucleoside addition strategy is expected to provide us simple and reproducible technology for antibody production. Meanwhile, despite the progress of media development, the instability of specific production rate, which often occurs during long-term culture, is still crucial issue. We isolated DNA regulatory motifs from CHO genome sequence for stable protein production and determined whether these motifs act as an insulator. In previous study, a bacterial artificial chromosome (BAC) library from stable and highly productive CHO cell line was constructed for genome-wide analysis. BAC library has provided a useful tool for obtaining sequence data for gene discovery and functional sequence annotation. Although the vector engineering and cell engineering to CHO cell culture has various benefits, the limited success in its application may be caused by the insufficient genomic information of CHO cells. In this regard, our approach has advantage and would be a powerful tool for therapeutic protein manufacturing. Our results suggest that stable expression of a transgene can be promoted by the CHO genome sequence. We could find that regulatory motifs isolated from the recombinant CHO genome sequence can act as an insulator and have positive effect for high and stable transgene expression. We isolated the insulator sequence in the CHO genome sequence, which was expected to have effective activity in a CHO cell expression system.</p> <p>The technologies developed in this study covers monoclonal antibody manufacturing from stable cell line development to production culture. As a future work, the new-finding genome sequence should be used to generate highly stable expressing CHO cell lines; and established CHO cell lines should be performed fed-batch culture with nucleosides addition condition. It could be beneficial platform technology for recombinant protein production in CHO cell culture system.</p>			