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論　文　内　容　要　旨

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学位論文題目	Studies for development of hyper osmotic resistant mammalian cells for industrial scale production of recombinant protein（組換えタンパク質の工業生産のための高浸透圧耐性細胞開発に関する研究）		

内容要旨

Due to the increase in demand for the biopharmaceutical manufacturing and marketing, the cell culture process with high productive cell lines generation has been developing. Chinese Hamster Ovary (CHO) cells are widely used as a host to produce various therapeutic proteins including recombinant monoclonal antibodies (mAbs). A fed-batch cell culture process with concentrated nutrient feeding throughout the culture period is commonly used for the industrial scale production. There are two types of feeding strategy for fed-batch cultures: i) continuous feeding; and ii) bolus feeding. The cells in a fed-batch process with continuous feeding will have less stress from environmental conditions including osmolality during the culture period compared with bolus feeding. Currently, the fed-batch process with bolus feeding is the most widely used method for industrial large-scale mammalian cell culture because of its simple operation.

In our previous study, a fed-batch process with continuous feeding for mAb production by CHO cells was developed. This platform process maintained high cell density and produce high antibody concentrations. However, a more simplified process is required for industrial production to reduce the risk of contamination and human error. To establish a simplified platform process, the feeding method was changed from continuous feed to bolus feed. The bolus feeding method lead to a greater increase in medium osmolality compared with the continuous feeding method because of the large amount of feed volume required to be added in a single administration. The increased osmolality suppressed cell culture growth. The Integral Viable Cell Concentration (IVC) with bolus feeding was 54% of the control culture with continuous feeding. However, the increased osmolality in the bolus feed method led to a 40% increase in cell-specific productivity (q_p) compared with the continuous feeding. The increase of q_p did not result in a substantial increase in the final mAb concentration because of decrease of IVC over the culture duration. The final mAb concentrations in the bolus-fed and continuous-fed cultures were 5.6 g/L and 7.3 g/L, respectively. The final mAb concentration was 77% of the control culture product concentration. It is well known that hyper osmotic pressure suppresses cell growth. However, few studies have investigated the development of host cells that do not suppress cell growth in hyperosmotic cultures. In this study, hyper osmotic resistant CHO host cells were developed to overcome growth suppression effects by hyper osmotic pressure. To establish the hyper osmotic resistant CHO host cells, CHO-S host cells were passaged long-term in a hyper osmotic basal medium. After 10 passages with hyper osmolality medium, the osmotic resistant CHO host cells, named CHO-S-OR host cells were established. To evaluate the differences in cell growth between CHO-S-OR host cells and CHO-S host cells, batch cultures under osmotic stress conditions were performed. There were marked differences in cell growth of the CHO-S host cells and established CHO-S-OR host cells under iso- (328 mOsm/kg) or hyper-osmolality (over 450 mOsm/kg) conditions. Cell growth of the CHO-S host cells was markedly decreased by the induction of osmotic stress, whereas cell growth of the CHO-S-OR host cells was not affected. The maximum viable cell concentration of CHO-S-OR host cells was 132% of CHO host cells after the induction of osmotic stress. Detailed metabolic analyses of CHO-S host cells and CHO-S-OR host cells were performed with or without osmotic stress. The results of metabolic analysis between CHO-S host cells and CHO-S-OR host cells suggests that CHO-S-OR host cells have a greater capacity to generate osmolytes and handle osmotic stress compared with CHO-S host cells. Moreover, stability of the CHO-S-OR host cells with iso-osmolality basal medium was evaluated. The hyper osmotic resistant characteristic was maintained in CHO-S-OR host cells even after seven passages in iso-osmolality basal medium.

The new approach by using the hyper osmotic resistant host cells could be a powerful tool to improve final concentration of products in fed-batch culture. The recombinant protein production hyper osmotic resistant host cells will not be suppressed in response to increased osmolality in fed-batch culture. The IVC will increase throughout the culture period. Moreover, the q_p might increase under increased osmolality medium. Further experiments are required to validate these hypotheses.