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Bioengineering M.S. Defense

Investigating Low Culture Temperature and Low Media pH of Two Chinese Hamster Ovary (CHO) Cell Lines with Next Generation RNA Sequencing (RNA-Seq) Technology

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Biologic drugs, or large molecule drugs, such as monoclonal antibodies, are proteins that are manufactured in living cells. Many biologics, such as Humira and Enbrel, provide thousands of patients worldwide with lifesaving therapies. In order to produce these drugs, different expression systems, like Chinese hamster ovary (CHO) cells, are used. CHO cells are the most widely utilized mammalian cell line for recombinant protein production due to a long history of regulatory approval for producing safe biologic drugs and have the ability to produce therapeutic proteins at an industrial scale. CHO cells have many advantageous characteristics, like producing the protein with desired post-translational modifications. Many methods over the last 30 years have been used to improve productivity, including strain selection, media improvements, and physiologic studies. Yet despite significant improvements in yields and productivity, a major current drawback to using CHO cells is high production costs due to low product yields.

The recent increased availability of CHO genome information has provided fundamental knowledge needed to conduct studies to better understand CHO cell physiology. Transcriptomic studies allow for quantitative measures of gene expression changes when cells are grown in different culture conditions. In this study, the effects of culture temperature and pH for two CHO cell lines, a non-recombinant CHO K1-PF cell line and a recombinant rCHO DP-12 cell line was quantified using RNA-Seq to characterize the transcriptome. The culture temperatures compared were 37°C and 33°C, where 37°C represents the standard culture temperature. The 33°C culture temperature represents a reduced culture temperature used to increase cell viability and sustain productivity in industry. The media pH levels examined were pH 6.95, a control level, and pH 6.70, a low pH level. At lower pH levels, lactate consumption is often observed to occur more readily.

The impact of pH on the transcriptome for both cell lines was determined to not be significant. The impact of temperature on the transcriptome, however, was determined to be significant and similar between the two cell lines. A total of 4184 genes were identified as temperature sensitive for the union of differential expressed genes for each cell line (FD ≤ 0.05). Several genes related to glycosylation, apoptosis, and cell cycle were identified as temperature sensitive. The gene ontology analysis identified additional biological processes that could explain improved glycosylation at reduced temperatures. Specifically, glycosylation genes, like Slc35d1 and Slc9a9, had higher expression for both CHO K1-PF and rCHO DP-12, and are linked to improving protein glycosylation at lower temperatures.

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