EVOLUTION OF rCHO CELLS UNDER MILD ER STRESS TO MAKE THEM SUPER PRODUCERS

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To increase the productivity of rCHO cells, many cell engineering approaches have been demonstrated that over-express or knockout a specific gene to achieve increased titers. This single-gene approach has resulted in mixed outcomes, as productivity is a function of many genes and pathways, as also demonstrated by various omics analysis. In this work, we present an alternate approach, based on the concept of evolution, to achieve cells with higher titers.

We had earlier demonstrated an increase in productivity of CHO cells even after brief exposure to an ER stress inducer, tunicamycin. However, the increase in productivity is not sustained over the entire course of batch culture, ultimately leading to lower titers due to increased cell death. To harness the beneficial effect of ER stress, we have evolved rCHO cells producing a monoclonal antibody under tunicamycin pressure. The rCHO cells were adapted for more than 25 passages, first under mild tunicamycin concentrations, and later to sustain higher concentrations of tunicamycin. The evolved clones have been characterized in detail in culture.

A sustained higher productivity of at-least 2-fold was achieved in all the clones, in a concentration dependent manner. Similarly, a 1.5-2 fold increase in final titers was also achieved in the batch culture. Intracellular IgG analysis using FACS demonstrated higher secretion efficiency of these adapted cells, correlating with up-regulation of the UPR pathway in the adapted cells.

Metabolic analysis of the adapted cells in the batch culture revealed higher consumptions rates of key nutrients (glucose and Amino acids) as well as limitations in the late stage of the culture. Upon culturing these adapted cell lines in non-nutrient limiting conditions (i.e. Fed-batch), we observed significantly higher titers (~2g/l) and cumulative productivity (~50 pg/cell/day) as compared to control. Valproic acid, a small molecule demonstrated to could increase product titers of adapted cells further.

Our work illustrates how process modifications, cell engineering and use of small molecules can be used in synergy to drive up product titers. Future efforts will focus on extending this strategy to develop generic host cells with high secretory capacity for subsequent transfections.

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