Development of a CHO Production Medium Utilizing Proteomic and Metabolomic Analysis

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ABSTRACT

Traditional CHO cell medium development relies heavily on the stoichiometric analysis of metabolites in spent medium with emphasis on amino acid, glucose and select water soluble vitamin consumption. Analysis of these basic metabolites in an empirical design of experiment (DOE) has consistently resulted in incremental increases in titer generally through increased viable cell density (VCD) and viability of the culture. It has been long hypothesized that an upper limit to titer and product quality would be reached using traditional medium development techniques. Through the use of advanced cellular analytics we are developing a hypothesis based design of media through proteomic and metabolomics analysis of critical pathways focused on specific productivity. Metabolomic and proteomic analysis was conducted on two medium formulations with disparate growth and production characteristics. Medium formulation 1 (M1) demonstrates moderate peak VCD with a high specific productivity (qP) over a 14 day growth performance assay utilizing a recombinant IgG producing CHO-S cell line and DG44 cell line. Medium formulation 2 (M2) demonstrates a high peak VCD with moderate qP under the same conditions with the same cell lines. A comparative analysis of metabolite abundance and enzyme regulation identified that M1 had greater flux in the sorbitol pathway verses glycolysis and the TCA cycle was upregulated to a greater degree than M2. A Multi-Omics based Design of Experiment (DoE) study was developed to increase the specific productivity of M2 without decreasing the VCD to M1 levels resulting in a superior volumetric titer. Simultaneously, we utilized traditional empirical approaches to increase the qP of M2 in a parallel set of experiments. We describe here the path to develop the medium, metabolic and proteomic pathways which were found to be important, and a comparison of results based on the traditional empirical path verses the hypothesis based advanced cellular analytics path.

INTRODUCTION

Traditional CHO cell medium development relies heavily on the stoichiometric analysis of metabolites (primarily amino acids) in spent medium with multiple screens of components to identify interactions and concentrations that enhance performance. Analysis of metabolites in an empirical DOE has consistently resulted in incremental increases in titer. Despite this, the time required to reach product goals using an empirical DOE approach is lengthy, and improvements in product titer and quality using traditional medium development techniques has limiting returns in today's CHO base media which are 20-50 times more concentrated than classical media. Utilization of these traditional approaches resulted in a prototype medium which performed only moderately better than the original formulation. Through the use of advanced cell signature analysis (ACSA) we employed a multi-omics approach to rationally design media by focusing on critical metabolic pathways designed to increase specific productivity.

Metabolomic and proteomic analyses were conducted on two media with disparate growth and production characteristics. A comparative analysis of metabolite and enzyme abundance identified that the higher performing media had differential flux through specific pathways compared to Medium 2. Immediately following analysis, 9 components were identified that improved performance in a prototype medium to 30-70% over Medium 1. The improvements resulting from the rational approach accelerated the timeline to reach target goals by 30%, identified new raw materials to use in media design, and established a novel mass-spectrometry based process for medium design. ACSA based techniques have been used to improve and accelerate two medium development projects.

RESULTS

Figure 1. Examination of Differentially Performing Medium

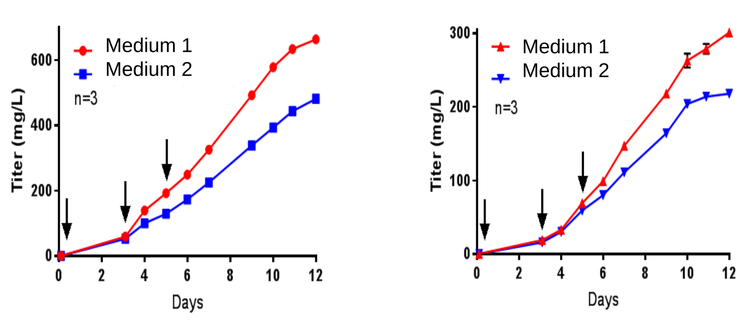

Figure 1: Medium 1 produced higher titers than Medium 2 in CHO-S and DG44 cells producing a human recombinant mAb. Samples were taken at times indicated (arrows) to measure proteomic differences in the cells, and daily to measure changes in media and cellular metabolites. Mean titer +/-SEM, n=3.

Figure 2. Proteomic Analysis of Cells

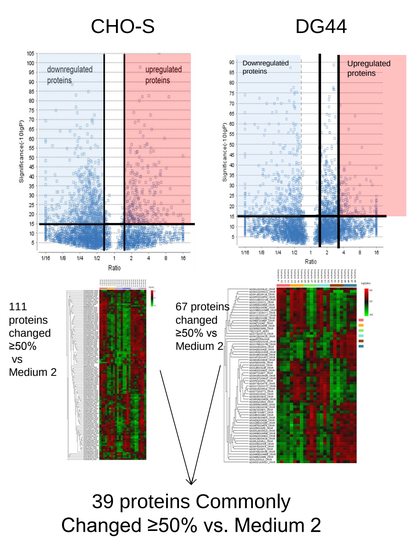

Figure 2: Label free quantitation of proteins detected 111 changed proteins (>50%) in CHO-S and 67 in DG44 between the two media. Of these, 39 proteins were commonly changed in both cell lines.

Figure 3. PCA Analysis of Cellular Metabolites

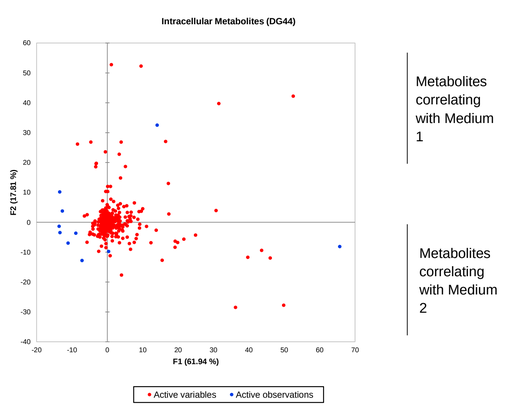

Figure 3: Principal Component Analyses (PCA) demonstrate time-dependent changes in metabolites that correlate with titer (x-axis) and separate DG44 cells cultured in the two media studied along the y-axis.

Figure 4. Pathway Analysis

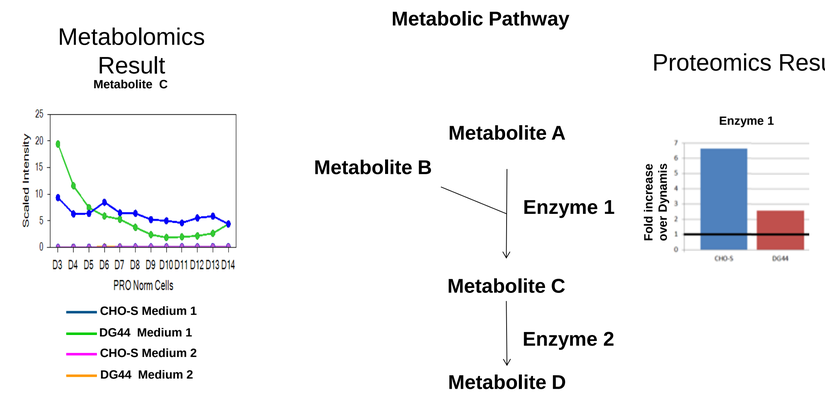

Figure 4: A metabolic pathway was shown to be upregulated in Medium 1 as evidenced by increased expression of the enzyme (right panel) and higher levels of metabolite in cells and media in CHO-S and DG44 cells (left pane) indicating a possible critical role.

Figure 5. Rational Screens and Fractional DOEs Combine to Meet Project Goal

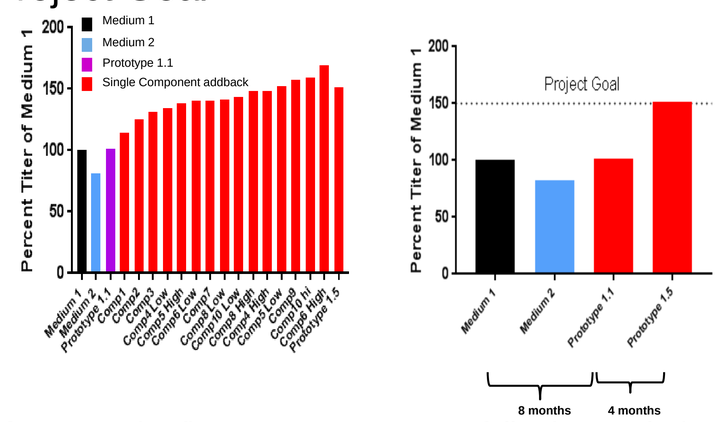

Figure 5. The first component screen following analysis of proteomics and metabolomics data generated 15 conditions that significantly improved titer, with some components supporting or exceeding project goals. Subsequent fractional factorial DOEs met project goals within 4 months for both CHO-S and DG44 cells. Traditional empirical approaches were also utilized with a modest increase in performance despite 8 months of effort.

Figure 6. Application of ACSA for Specific Cell Lines

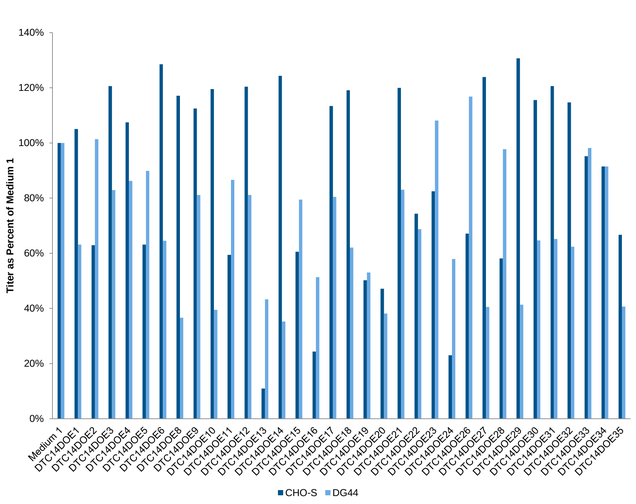

Figure 6. Multi-factorial DOE of components identified in initial screens demonstrated a wide range of responses between CHO-S and DG44 cell lines. This indicates the ability to use ASCA to optimize a medium or process to specific cell lines.

CONCLUSIONS

Rational design of CHO media using a multi-omics approach enabled the development of a competitive medium in less time than conventional methods (Figure 5). Several new raw materials were identified, and information gained from these studies has led to a titer doubling from current medium offerings. Utilizing ACSA capabilities would be beneficial for the development of platform and custom medium applications.

REFERENCES

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ACKNOWLEDGEMENTS

We thank Yang Wu, Erica Wehling, Mary Reynolds and Alexis Freeland for their support of experiments conducted by the team. We appreciate helpful discussion and support from Atul Joshi, Scott Jacobia and Steve Gorfien.

TRADEMARKS