Introduction

Bioprocesses like the cell-based production of recombinant proteins and monoclonal antibodies require optimal culture conditions to obtain a high yield of quality products. As the metabolic activity of the cells is very high during fermentation, the external and internal metabolite compositions vary tremendously throughout the process. The quantification of a wide range of metabolic substrates and products is a prerequisite to understand and optimize the underlying cell-based activities.

Furthermore, metabolite quantification reveals the composition of biologically derived cell culture supplements, thus serving as a tool to monitor supplement quality or providing the base for the formulation of a chemically defined medium supplement.

Methods and Materials

Utilizing a mass spectrometry platform (FIA-, LC-MS/MS, GC-MS), several hundred metabolites of different classes were concurrently analyzed either in small sample volumes of cell culture supernatants or in lyophilized cell extracts.

**Metabolite portfolio**

- Amino acids
- Energy metabolism-related metabolites (e.g., hexoses, pyruvate, lactate, succinate)
- Biogenic amines (e.g., histamine, putrescine)
- Phospholipids and sphingomyelins (e.g., sphingomyelin)
- Fatty acids
- Carnitine and acylcarnitines
- Vitamins and Vitaminoids

**Methods and Materials**

**Targeted Metabolomics**

- Quantification of predefined set of metabolites
- Coverage of several metabolite classes
- Medium- to high-throughput analysis procedures
- Small sample volumes
- Standardized data analysis
- Comprehensive data output format
- Short sample-to-result-time

**Applications**

**Analysis of medium supplements from biological sources**

- Composition of medium supplements

**Testing of**

- Supplement quality
- Lot variability
- Supplement suitability

Cell clone selection

Optimal cell & Optimal medium

- Efficient consumption of nutrients
- Minimal production of waste products
- No production of toxic by-products

Metabolism ➔ Growth ➔ Production

**Fig. 2:** Early identification of best producer cell lines with optimal metabolic characteristics using small sample volume

**Cell line characterization**

**Fig. 3:** Rapid identification of CHO cell line-specific differences (indicated in yellow) regarding extracellular amino acid concentrations during bioprocessing; absolute metabolite concentrations were obtained and served as base for the calculation of relative changes (see Acknowledgement) below

**Fermentation process and medium optimization**

**Fig. 4:** Estimated time line (working days) for targeted metabolite analysis applied in cell culture optimization

**Targeted Metabolomics**

- Optimized nutrient supply
- Metabolic waste products
- Unexpected nutrient utilization correlations

**Fig. 5:** From quantitative data to metabolic model: flow chart of principal approach

**Conclusion**

Targeted metabolomics comprises a rapid and comprehensive method to determine the composition of cell culture supplements of biological origin. The method is also well suited to rapidly characterize fermentational processes metabolically by monitoring changes in medium composition and cellular metabolite pools. The received quantitative data can be directly related to growth, vitality, and productivity of the cell.

**Acknowledgement:**

CHO culture experiments were done by Selexis SA, Geneva, Switzerland, the service provider for engineering high performance mammalian cell lines.

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