BEYOND EXPONENTIAL PHASE: METABOLIC PHENOTYPES IN THE STATIONARY PHASE OF CHO CELL CULTURES

Jerneja Štor, Austrian Centre of Industrial Biotechnology, Graz, Austria; University of Natural Resources and Life Sciences, Austria
jerneja.stor@boku.ac.at
David Ruckerbauer, Austrian Centre of Industrial Biotechnology, Graz, Austria; University of Vienna, Austria
Diana Széliová, University of Vienna, Vienna, Austria
Sarah Sacco, Vanderbilt University, Nashville, Tennessee, USA
Jamey Young, Vanderbilt University, Nashville, Tennessee, USA
Nicole Borth, University of Natural Resources and Life Sciences, Austria
Jürgen Zanghellini, University of Vienna, Austria

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With respect to recombinant protein production, the metabolism of Chinese Hamster Ovary (CHO) cells is sub-optimally regulated. The excessive supply of amino acids (AA) in the media introduces unbalanced uptake behavior, which leads to (undesirable) by-product formation. Some of these by-products, such as ammonia, are toxic, and their accumulation in the media negatively affects cell growth, productivity, and product quality. Here we use carbon-13 metabolic flux analysis (^{13}C-MFA) coupled with flux balance analysis to gain insight into intracellular flux distributions and to analyze differences in AA metabolism across different CHO cell lines and growth phases.

A producer and a non-producer cell lines were grown in fed-batch culture with temperature shift. Cultures were frequently sampled throughout exponential and stationary phases for cell density, viability, cell diameter, productivity, cell dry mass, metabolite consumption/production rates, and labeling with ^{13}C glucose or glutamine tracers.

During the exponential phase, the producer cell line had significantly higher consumption rates for most of the amino acids. Specifically for glutamine consumption the producer showed a higher rate compared to the non-producer, which coincided with higher ammonia and glutamate production. Higher extracellular rates for the producer cell line, compared to the non-producer, in the exponential phase were also seen in the ^{13}C-MFA simulation. Despite the producer cell line showing higher consumption rates, differences on the intracellular level between the cell lines are not significant. Intracellularly, the non-producer cell line has higher rates for the phosphoenolpyruvate (PEP)-pyruvate-oxaloacetate node (PPO-node) at the junction between glycolysis and the TCA cycle. Producing PEP is important for the cell to produce energy as this is one of the two reactions that produce ATP in glycolysis. On the other hand, the producer shows a higher rate for the reaction between oxaloacetate (OAA) and malate, which is part of the malate-aspartate shuttle, the process that is involved in generation of ATP in the TCA cycle.

After the transition to the stationary phase, we observed significantly lower exchange rates for both cell lines. This is expected since cells are also exposed to a temperature shift that slows down their metabolism. Compared to the non-producer, the producer cell line showed higher consumption rates for the majority of metabolites particularly for the amino acids most abundant in the production of the mAb. Interestingly, mAb production dropped significantly in the stationary phase compared to the exponential phase. Referring back to glutamine, compared to exponential phase, its consumption decreased for both cell lines, while the production of glutamate increased for the non-producer without showing a higher glutamine consumption. We hypothesize that this results from the non-producer having lower flux from glutamate to alpha-ketoglutarate, which was confirmed by fluxes estimated for the exponential phase by ^{13}C-MFA simulation.

Taken together, based on the current data metabolic profiles differ significantly between exponential and stationary phases and the differences between the cell lines can be observed on the intracellular extracellular levels. We are currently in the process of verifying the underlying mechanisms and changes in the stationary phase with further analysis using ^{13}C-labeling data.