Proteomic and metabolomic characterization of CHO DP-12 cell lines with different high passage histories

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Introduction

For industrial pharmaceutical protein production fast growing, high producing and robust cell lines are required. To select more pH shift permissive and fast growing sub-populations, the CHO DP-12 (ATCC clone #1934) cell line was serially subcultured at high viability (>90 %) for more than four hundred days in shaker flasks and chemically defined media. Cell samples were cryopreserved at four different time points, after 21, 95, 165 and 420 days. The effects of long-term passaging before cryopreservation with an increase in specific growth rate were examined in parallel bench-top bioreactor cultivations.

Please consinder poster number 1.40, Heinrich *et al.* for further information concerning long-term cultivation.

Methods

Samples for metabolome and proteome analysis were taken from parallel bioreactor cultivations of the four sub-populations (SP) during exponential growth phase.*

Proteome analysis was performed using 2D DIGE. Image processing and statistical evaluation was carried out with Delta 2D 4.2 software. All differentially expressed protein spots were successfully identified using a MALDI-TOF-TOF mass spectrometer.

The analysis of intracellular metabolites was performed with our in-house developed fast-filtration quenching procedure. Generated samples were analyzed by GC-MS and LC-MS.

Conclusions

- for several proteins the abundance increased with the number of passages of the sub-populations. Among these were proteins involved in stress resistance and folding. Proteins participating in transcription regulation, mRNA processing, folate and purine metabolism, protein expression as well as glycolysis also showed similar profiles
- increasing intracellular pool sizes of glycolysis intermediates and higher nucleotide phosphorylation ratios indicate higher metabolic activity in the high passaged sub populations
- merging proteomic findings with the measurements of intracellular metabolite pools led to a better understanding of factors which make a production cell line faster growing and more robust against pH shifts

* Find further informations to the cell line history on poster number 1.40, Heinrich *et al.*

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Results

Proteome

- the four different sub-populations could be clearly separated from each other by their proteome profiles as shown in PCA plot (fig. 1)

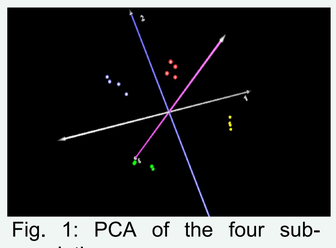


Fig. 1: PCA of the four sub-populations

- 43 out of 1377 overall detected protein spots show a significantly different protein expression for at least one sub-population (ANOVA, $\alpha \leq 0.05$, a false significant proportion ≤ 0.01) (fig. 2)
- the detected differences between the analyzed groups increase with their number of passages (fig. 3)
- for several proteins the expression correlates with the number of passages before cryopreservation
- among others, anti-stress proteins or proteins involved in transcriptional regulation and mRNA processing were upregulated in long-term sub-populations

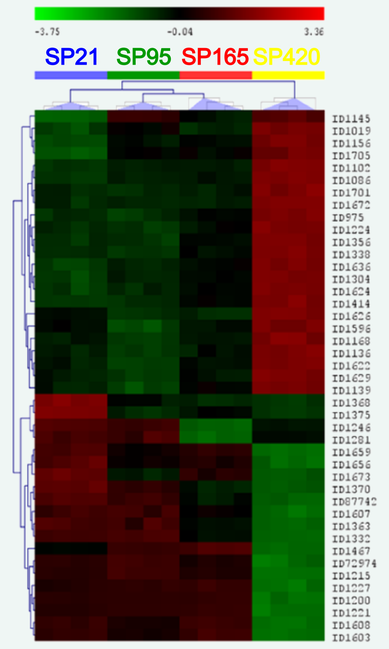


Fig. 2: Heatmap with hierarchical clustering of significantly different expressed protein spots

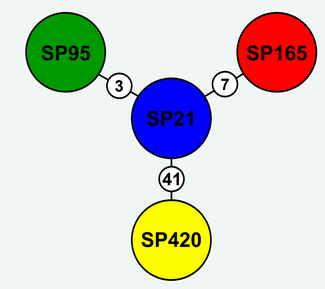


Fig. 3: Significant differences with a fold change ≥ 2 between the sub-populations

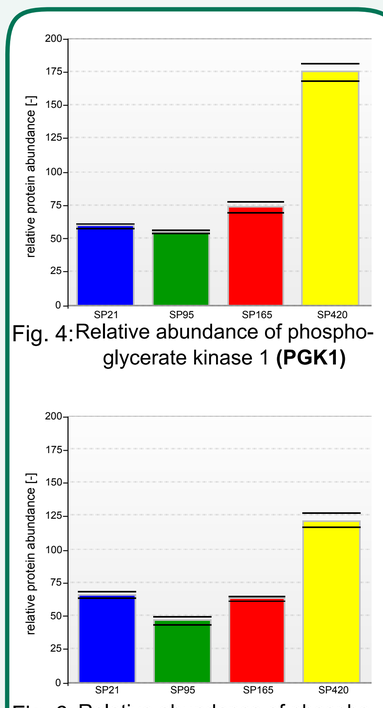


Fig. 4: Relative abundance of phospho-glycerate kinase 1 **(PGK1)**

Fig. 6: Relative abundance of phospho-glycerate mutase 1 **(PGAM1)**

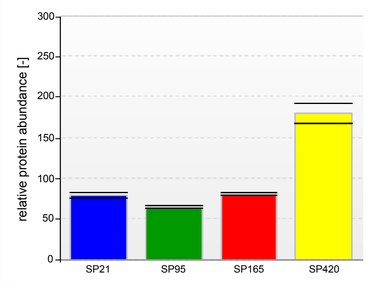
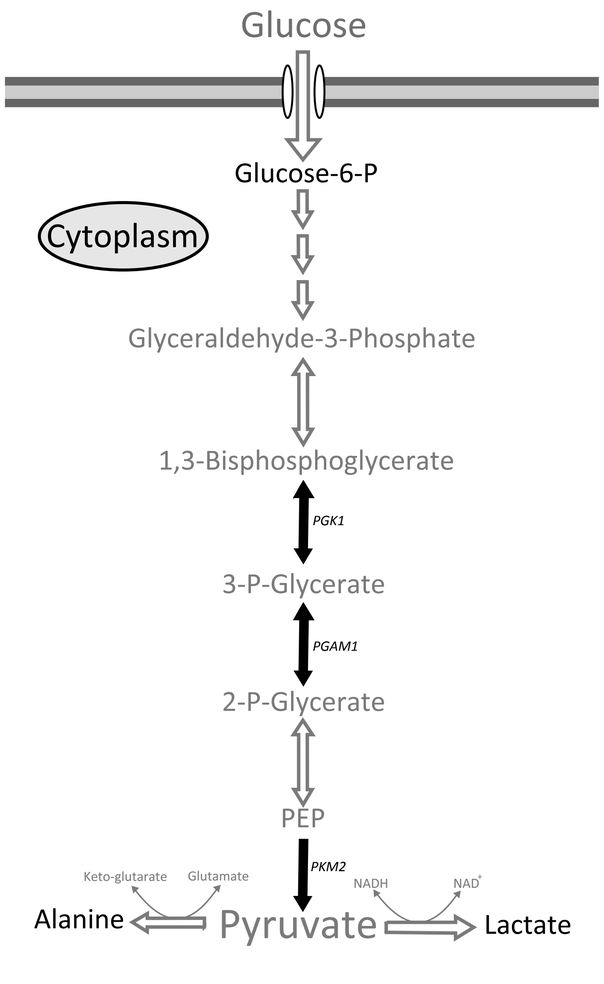


Fig. 8: Relative abundance of pyruvate kinase **(PKM2)**



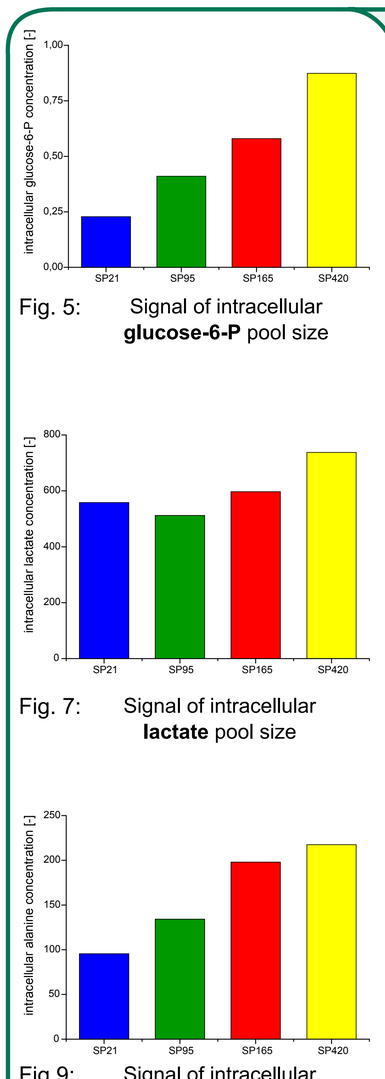


Fig. 5: Signal of intracellular **glucose-6-P** pool size

Fig. 7: Signal of intracellular **lactate** pool size

Fig 9: Signal of intracellular **alanine** pool size

Metabolome

Intracellular metabolomics give additional information to extracellular consumption patterns:

- the consumption rate of extracellular aspartate decreases while intracellular pool sizes increase

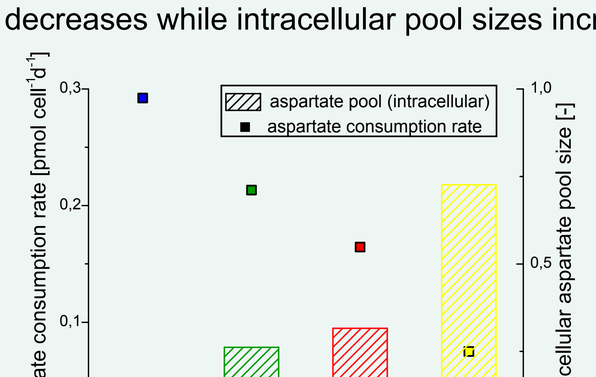


Figure 10: Comparison of intracellular aspartate pool sizes and cell specific aspartate uptake rates of the high passage cell lines

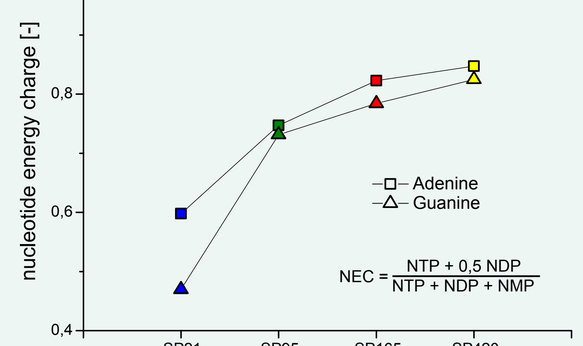


Figure 11: Adenylate energy charge (AEC) and guanylate energy charge (GEC) of the four subpopulations during exponential growth

- high number of passages lead to an increase in adenylate energy charge and guanylate energy charge
- high posphorylation rates of adenine and guanine nucleotides support an increased metabolic activity