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Abstract

CHO cells primarily utilize amino acids for three processes: biomass synthesis, recombinant protein production and catabolism. In this work, we disrupted 9 amino acid catabolic genes participating in 7 different catabolic pathways, to increase synthesis of biomass and recombinant protein, while reducing production of growth-inhibiting metabolic by-products from amino acid catabolism.

Key message

CHO cells primarily utilize amino acids for three processes: biomass synthesis, recombinant protein production and catabolism. In this work, we disrupted 9 amino acid catabolic genes participating in 7 different catabolic pathways, to increase synthesis of biomass and recombinant protein, while reducing production of growth-inhibiting metabolic by-products from amino acid catabolism.

Background

Amino acid catabolism produces a wide range of growth inhibiting compounds1, amongst these ammonium and lactate. Ammonium is produced by transamination and deamination reactions2, whereas lactate is produced by either amino acid and catabolic pathways fueling glycolysis or by NAD+-producing catabolic pathways, which forces the cell to regenerate NADH through lactate synthesis3. Disruption of amino acid catabolic pathways may reduce production of growth-inhibiting metabolic by-products.

Physiology of single gene disrupted CHO cells

To study the physiological impact of disrupting single amino acid catabolic pathways, we characterized single gene-disrupted clones in triplicate shake flask cultures in batch mode. We monitored physiological changes in terms of maximum specific growth rate ($\mu_{max}$), integral of viable cell density (IVCD) and secretion of lactate and ammonium.

Single gene disrupted clones generally showed an increased growth phenotype with 8 of 9 clones displaying increased $\mu_{max}$ (up to 115% of WT), while 6 of 9 clones had increased IVCD (up to 136% of WT). Specific secretion of lactate was reduced in 4 of 9 clones (down to 81% of WT), while specific secretion of ammonium was reduced in 5 of 9 clones (down to 91% of WT). Monoclonal antibody titers increased proportionally to IVCD (data not shown).

To exclude that the improved phenotypes are caused by clonal variation, we characterized multiple clones with different mutations in gene 4 and 6, and found a strong link between genotype and phenotype.

Validation of functional gene knock-out

Functional gene disruptions were validated using deep sequencing of the targeted genomic loci, gene expression analysis, western blots and proteomics. All genes displayed out-of-frame mutations (A) and generally reduced transcription (B). Western blots indicated potential wildtype proteins in some clones (C), to proteomic analysis and mRNA sequencing was applied to verify functional knock-out of target genes (ongoing work).

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References

3. Thomsen K. et al. (2012), Biotechnology and Biomanagement, 112(11), pp. 2379-2397.
4. Ley & Kazemi et al. (2015), Biotechnology and Bioengineering, 114(8), pp. 1779-1790.