METABOLIC ENGINEERING OF CHO CELLS TOWARDS REDUCED NOVEL GROWTH INHIBITOR PRODUCTION AND AMINO ACID PROTOTROPY

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Chinese hamster ovary (CHO) cells are currently the workhorses for recombinant therapeutic protein production. In fed-batch processes, these cells consume large amounts of nutrients and convert a significant fraction of them to inhibitory byproducts that accumulate in the culture. Various methods have been devised to control the accumulation of classical mammalian cell culture byproducts, namely lactate and ammonia. We employed one such method, called HiPDOG[1] (High-end pH-controlled Delivery of Glucose), which controlled lactate levels effectively in fed-batch cultures allowing cells to grow to higher cell densities. However, even under this control scheme, cells eventually stopped dividing suggesting that another level of growth-inhibitory metabolic byproducts were accumulating. Omics approaches were employed to identify and quantify these novel byproducts accumulating in the cultures. A significant fraction of these compounds were identified to be intermediates or byproducts of amino acid catabolism pathways. Optimizing the supply of specific amino acids in lactate-controlled HiPDOG cultures led to reduced production of corresponding toxic byproducts resulting in significantly higher peak viable cell densities and titers[2].

In addition to the nutrient optimization strategies, genetic engineering methods were also explored to modulate intrinsic metabolic pathways in order to reduce the channeling of the amino acid carbon flux towards inhibitory byproduct formation. Two pathways were considered as part of this effort, the phenylalanine-tyrosine catabolic pathway and the branched chain amino acid (BCAA) catabolic pathway. Generation of growth inhibitory byproducts, including 4-hydroxyphenyllactate and 3-(4-hydroxyphenyl)pyruvate, from the phenylalanine-tyrosine catabolic pathway was determined to be mainly due to low to negligible transcript expression of four endogenous enzymes including phenylalanine hydroxylase (PAH), 4-hydroxyphenylpyruvic acid dioxygenase (HPD), homogentisate 1,2-dioxygenase (HGD) and pterin-4 alpha-carbinolamine dehydratase 1 (PCBD1). Mouse orthologs of these four enzymes were heterologously overexpressed in CHO cells. PAH and PCDB1 enzymes together catalyze the conversion of phenylalanine to tyrosine. Cells overexpressing the above mentioned four enzymes were conferred with tyrosine prototrophy, viz. growth in tyrosine-free culture conditions, and in addition, produced lower levels of the pathway related growth inhibitors in fed-batch cultures.

In case of the BCAA catabolic pathway, production and accumulation of the pathway related inhibitors, isovalerate, 2-methylbutyrate and isobutyrate, was ascertained to be an outcome of high catabolic rates of leucine, isoleucine and valine amino acids, respectively. Branched chain aminotransferase 1 (BCAT1) enzyme catalyzes the first enzymatic step in the catabolic pathway of all three BCAAs. A siRNA knockdown strategy was employed to reduce levels of BCAT1 enzyme in CHO cells. In fed-batch cultures, the BCAT1 knockdown cells had reduced consumption rates for all the three BCAAs and reduced production rates of the above mentioned inhibitory byproducts, which resulted in enhanced culture performance. The presentation will showcase the results from the above mentioned metabolic engineering efforts and discuss how such an enhanced understanding of CHO cell amino acid metabolism can be employed in development of novel host cell lines with optimized nutrient metabolism.

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