Microbial Cell Factories

Poster Presentation

Improvement of the energy metabolism of recombinant CHO cells by cell sorting for reduced mitochondrial membrane potential

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Background

Mammalian cells, when cultivated in-vitro, are characterised by an inefficient glucose metabolism, which leads to the production of lactic acid. Specific glucose uptake rates and corresponding lactate production rates are dependent on the cell line as well as on glucose concentration and growth rate. To ensure sufficient supply of energy, cells metabolise glutamine, which improves viability and growth rates, but results in the release of ammonium into the medium. High ammonium concentrations, however, have been shown to impair product glycosylation. As the mitochondrial membrane potential was shown to correlate to cell specific glucose uptake rates, Rhodamine 123, a lipophilic cationic dye was used for cell sorting. Two recombinant CHO cell lines with known differences in lactic acid production rate were used as model cell lines and subclones obtained by sorting for low and high mitochondrial membrane potential, respectively.

Results

The two cell lines C2F5 and C2G12, which both produce a human monoclonal antibody and which were obtained by a similar transfection and amplification protocol, differ in their specific glucose uptake and lactate production rate, as well as in their growth rates and maximum cell counts. To isolate cells with an altered energy metabolism, C2G12 cells were stained with Rh123 and sorted both for a lower and a higher mitochondrial membrane potential into 96 well plates. Batch cultures in Spinner bottles were run of the best subclone of each sort.

Conclusion

Using sorting protocol based on a simple staining method for mitochondrial membrane potential we were able to isolate subclones from an established monoclonal antibody production cell line with significantly altered physiological properties. The subclone sorted for lower mitochondrial membrane potential had a faster growth

Table 1: Numbers in brackets are percent of the corresponding value of the parental cell line

	C2F5	C2G12	C2G12 low	C2G12 high
Inoculation cell concentration [cells/ml]	0,2 × 10^6	0,2 × 10^6	0,2 × 10^6	0,2 × 10^6
Cell count after 4 days [cells/ml]	2,0 × 10^6	0,8 × 10^6	1,4 × 10^6 (170)	0,5 × 10^6 (61)
Specific growth rate [μ/day]	0,61	0,39	0,51 (131)	0,26 (67)
Specific Glucose uptake [pg/cell/day]	525	900	660 (73)	1170 (130)
Specific Glutamine uptake [pg/cell/day]	130	185	165 (88)	245 (132)
Specific Lactate production [pg/cell/day]	505	700	420 (60)	1075 (154)
Specific mAb production [pg/cell/day]	6,3	3,7	7,3 (195)	4,4 (118)
Rh123 fluorescence at 2 g/l glucose [rel.U]	300	455	360 (80)	510 (112)

rate, attained higher final cell concentrations in batch cultures, had lower glucose and glutamine uptake and lactate production rates as well as a higher specific production rate. The subclone sorted for high mitochondrial membrane potential on the other hand had a lower growth rate and final cell count, increased glucose and glutamine consumption and lactate production rates. These subclones can now be used for genomic or proteomic analysis of properties that characterise a cell line with efficient or inefficient metabolism. In addition, the method described is a valuable tool for cell line development and optimisation, offering the possibility to isolate subclones with both superior and inferior properties. Future cell line development programs can now be extended from merely looking for a cell line with a high specific production rate, to one, which in addition has optimised metabolic properties.