Microbial Cell Factories

Poster Presentation

Automation for higher throughput in protein expression: visions, facts and fictions

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Background

Down-scaling, parallelization and automation are new trends in the field of recombinant protein expression in the post genomic era [1-3]. During the past years many companies and academic institutions have heavily invested in process and automation technologies. Does this trend keep its promise? Can post genomic protein production issues be overcome with few automated processes?

This abstract wants to highlight two years of experience in running a Protein Production Center in an industrial environment applying the expression systems BEVS, *E. coli (*and transient HEK.EBNA). We describe the streamlined and partially automated processes, the automation equipment applied; discuss results from the past two years of experience and strategies to eliminate remaining bottlenecks.

Results

Proteins expressed in a generic way in the *E. coli* expression system are all his-tagged and often N-terminally fused to thioredoxin or other fusion partners in order to improve solubility. After small scale expression evaluation in 24-deepwell blocks recombinant proteins are produced in 1-L fermenter vessels using fully automated and unattended inductions and temperature shifts. The described method is applicable to all host strains and induction systems, providing the optimal induction time point and harvest for each construct.

To run the BEVS in an automated high throughput environment still represents a major challenge. In order to meet this challenge, we have developed robust automated protocols at various formats for the small scale BEV process on an epMOTION 5070 workstation and semi automated large scale protocols for 10-L wave bioreactors and purification on Akta 3D and Akta Express. The expression of DUB family and various other proteins were used as an example to validate these processes (Table 1).

During the validation of the technologies described above some weak points of the automated processes became obvious: the tendency of proteins to form soluble or insoluble aggregates at any stage of the process and the presence of proteases, which requires close control of the fermentation process and interferes with fully automated and unattended runs. While means to control the aggregation phenomenon are still under investigation, a generic method has been found to control the proteolysis issue in the BEVS. We demonstrate that the addition of 10 mM of the cysteine protease inhibitor E-64 during the course of production effectively reduces/prevents proteolytic degradation of various recombinant proteins over a minimum

Table 1: Expression of 30 DUB proteins in the BEVS at 10-L scale and processed in a semi-automated mode.

	Analysis Method	AVERAGE	RANGE	Av. Yield [%]
Small Scale Expression [mg/L]	ELISA	27	3 – 140	
Large Scale Expression [mg/l]	ELISA	43	0 – 270	
Large Scale Expression [mg]	ELISA	448	0 – 1400	100 %
Cross-flow yields [mg]	ELISA	169	0 – 825	41 %
Pure protein yields [mg]	HPLC	21	0 – 103	(16%)*

* different analysis method

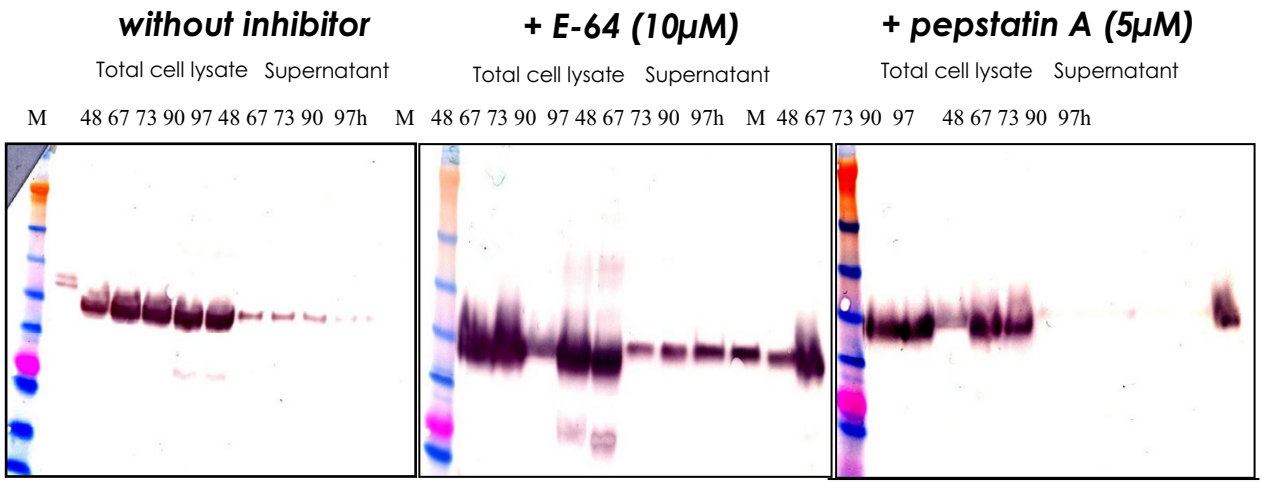

Figure 1
Effect of the addition of E-64 (cysteine protease inhibitor) and Pepstatin A (aspartic acid protease inhibitor) on the expression of a secreted wnt antagonist in Sf21 infected insect cells.(A): Western Blot (α-His) of cell lysates and medium supernatants taken at various time points (48, 67, 73, 90, 97 hours) post infection (expected size of protein: 28.1 kDa).

of 72 hours of culture time, thus improving the quality and yields of secreted as well as intracellular recombinant proteins (Figure 1).

Conclusion

We have demonstrated that recombinant protein expression can be evaluated, scaled up and proteins be purified in a parallel and (semi-) automated fashion using the BEVS and *E. coli* expression systems. Pre-requisites are that proteins are appropriately tagged and grouped. However, in order to attain optimal quality and yield of different recombinant proteins by generic processes the influence of protein destabilizing factors needs to be thoroughly understood and managed.

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