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Oral Presentation

Monitoring the Dynamics of Transcription and Translation Within the Time Course of Recombinant *E. coli* Cultivations

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Background

Among the key objectives for the optimization of recombinant protein production on industrial scale high yield of the product is one of the most important issues. To achieve high quantities of recombinant protein strong host/vector systems are utilized, generally leading to an overburden of the host cell metabolism. To cope with different types of stress cells have evolved complex regulatory entities acting on the highest level of metabolic regulation, such as the stringent response network. Highly specific signal molecules of these regulatory networks, like sigma factors and ppGpp, as well as global regulators like ArcA, Crp and Fis enable accurate up-and downregulation of specific genes [1,2]. The thereby altered expression profile confers increased resistance to adverse conditions. As the exploitation of the cell factory will always approach the physiological limits of the cellular machinery the short- as well as the long-term impact of high recombinant gene expression rates have to be investigated. Therefore key variables of metabolic stress to define and thus quantify the actual metabolic load of host cell metabolism can be inferred from variations of genome and proteome patterns. Due to the availability of whole genome *E. coli* microarrays this technology provides an efficient tool for screening overall transcriptional changes in the course of fermentation processes. 2D-Fluorescence Difference Gel Electrophoresis (DIGE, Amersham Biosciences) and MALDI-MS are established to identify changes on proteome level. A combination of these three methods (microarrays, DIGE and MS) paired with the application of time series experiments during a recombinant fermentation process provide an insight into the black box of cell metabolism and improve the understanding of cellular physiology.

Results

The design of expression profiling experiments, measuring the rate of transcription of mRNA and expression of proteins, is a very important issue in bioprocessing. Most of these studies have been carried out in flask cultures, whereby non defined conditions were applied [3]. In accordance with good experimental design it is important to ensure that the changes in expression profiles are due to the perturbing event. To fulfil these requirements chemostat cultivation is performed in our study to monitor the behavior of the production strain *E. coli* HMS 174(DE3)(pET11a) under stress conditions. Thus homogeneous samples and steady state conditions can be guaranteed and aimed perturbation is applied. Different induction strategies are used to exert different metabolic loads on the production organism and two soluble pro-

teins are expressed in order to detect the impact of the recombinant protein on the production organism.

Microarray and DIGE data show a significant increase in the number of altered genes and respectively proteins, whereof a dramatic change in the cell during this kind of stress condition could be derived. Evaluation of these data was performed according to their assignment to metabolic pathways as well as to regulatory profiles.

Conclusion

Microarray analysis as well as DIGE combined with MS proved to be well suited tools for monitoring changes on transcriptome and proteome level of time-series experiments during recombinant cultivations. The acquired data provide the basis to identify interactions and bottlenecks between metabolic pathways and regulatory circuits of the production organism and should enable reverse engineering of host cell for the optimal exploitation for recombinant protein production.

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