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Oral Presentation

Design of transcriptional fusions of stress sensitive promoters and GFP to monitor the overburden of *E.coli* hosts during recombinant protein production

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Background

Nowadays a high number of recombinant proteins for therapeutical purposes in human and animal health care are produced by microbial systems, mainly in *E.coli* [1]. Basically *E.coli* is well studied, sequenced [2] and characterized but changes in the cell composition during heterologous protein expression are poorly understood. Due to the lack of appropriate sensors for monitoring alterations of *E.coli* cells and the huge complexity of cellular systems, many of the present protein production processes are still far from optimal. Aiming at optimal exploitation of the host cell enhanced knowledge of cellular reactions related to recombinant protein expression is required. Using current methods like DNA microarrays and 2-D-electrophoresis changes of transcriptional and translational activity in stress situations like heat shock, general stress response, nutrient limitation, and stress caused by overexpression of heterologues proteins can be monitored. However, acquisition of these data is time consuming; therefore the goal is to create new on-line systems to monitor metabolic shifts. The major advantage of on-line process monitoring derives from immediate intervention in a running cultivation process. 2D-multi-wavelength fluorescence spectroscopy (DELTA, Bioview) represents a powerful, non-invasive measurement principle for on-line monitoring providing direct acquisition of biologically active fluorophores e.g. NAD(P)H [3] and detection of the reporter protein GFP. Using GFP as reporter is superior in comparison to other reporters like luxAB-genes or CAT, because it does not need substrates, cofactors or additional stabilization to yield a fluorescence signal [4,5]. Therefore, GFPmut3.1 [6] was used as appropriate reporter by fusing stress relevant promoters and acquisition of resulting fluorescence [7].

Relevant promoters upregulated during the protein production process were derived from microarray data (Dürrschmid, Reischer unpublished data). For the construction of the promoter-reporter fusions the chaperone dnaK and the general regulator for stress response sigma32 (rpoH), which is feed back regulated by other stress genes, were used [8]. To gain an efficient monitoring system for metabolic load two different approaches were established. On the one hand the fusion of promoter and GFPmut3.1 were inserted into a low copy plasmid (pMMB67HE), kindly provided by Karaimann, [9] which is compatible to the expression plasmid pET30a containing rhSOD (superoxiddismutase). The resulting 2-plasmid *E. coliHMS174(DE3)* strains were tested in shake flask experiments and fed batch cultivations. On the other hand promoters-GFP cartridges were integrated in the *E.coli* genome, as described by Datsenko and Wanner [10]. The expression plasmid was transformed into the resulting monitoring host and the monitoring system was evaluated under protein production conditions.

Results

Fed batch cultivations (20 l) of 2-plasmid hosts were performed (μ = 0,15 h^{-1}). Tuning of recombinant gene expression was achieved by controlled feed of inducer, whereby the first generation in the feed phase was non-induced, followed by 3 generations in induced state with

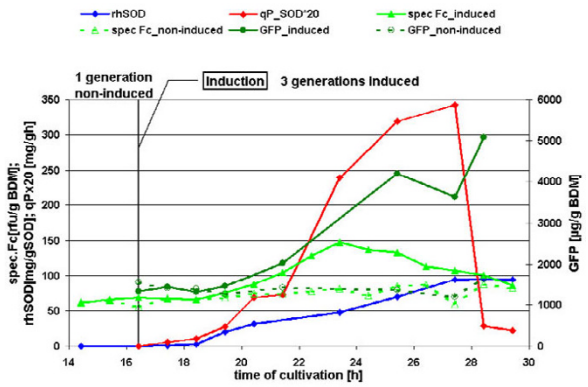

Figure 1
Comparison of *E.coliHMS174(DE3)* cultivations containing pMMB67HE:dnaKP:GFP and pET30arhSOD; amounts of SOD and GFP were determined by ELISA, spec. Fc = specific cellular off-line fluorescence by SPECTRAmax GeminiXS at ex488nm/em530 cut off filter 515 nm.

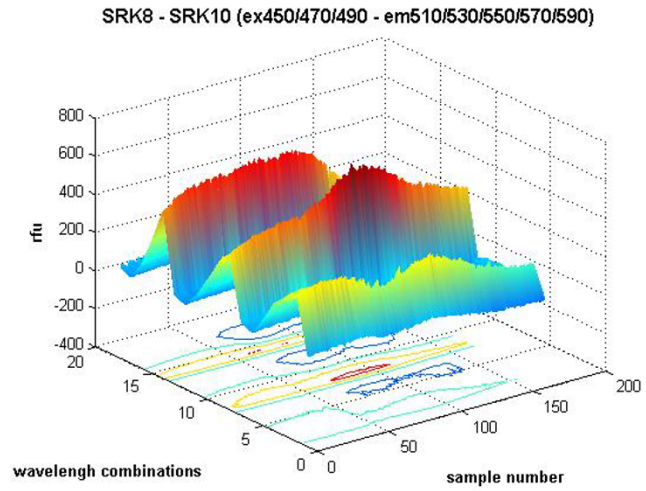

Figure 2
On-line fluorescence data of a *E.coliHMS174(DE3)* cultivation containing pMMB67HE:dnaKP:GFP and pET30arhSOD(SRK8) compared with a cultivation without monitoring plasmid (SRK10).

an increasing amount of IPTG (from 0,75 to 2,5 µmol/gBDM).

In the induced cultivation of *E. coliHMS174(DE3)* containing pMMB67HE:dnaKP:GFPmut3.1 and pET30arhSOD 95 mg SOD/g BDM were produced and compared with the non induced cultivation (see Figure 1). The off-line measured fluorescence was increased in comparison to the non-induced cultivation, which is in accordance with the amount of GFP determined by ELISA. In addition, the on-line fluorescence was increased during recombinant protein production and was compared with cultivation without monitoring plasmid to confirm the obtained fluorescence results from GFP (Figure 2). The system with the dnaK-promoter monitoring plasmid was able to show fluorescence due to the stress caused by recombinant protein production.

Our second approach for stress monitoring (genome integrated monitoring cartridges) shows lower fluorescence signals and less amount of GFP (data not shown). All induced cultivations show an increase in the amount of the stress alarmon ppGpp, indicating overburden of the cells.

Conclusion

The concept of fusing stress relevant promoters with GFP for monitoring the overburden of the cell was proven. The adoption of host cells with an additional monitoring plasmid for the evaluation of the stress caused by recombinant protein production was very successful. Regrettably the genome-integrated monitoring cartridges did not generate a significant fluorescence signal. To cope with the low fluorescence transcriptional amplification of the GFP signal is planned.

Acknowledgements

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