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Oral Presentation

Monitoring of transcript regulation and protein production related stress responses in *Pichia pastoris* secreting Fab antibody fragments

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Background

Protein production processes having the methylotrophic yeast *Pichia pastoris* as heterologous production host became increasingly important in the last decade. Although *Pichia pastoris* is known as a highly efficient expression system, there is only little knowledge about the physiology and the genetics lying underneath. During the recent years, it has become evident that a variety of intrinsic, metabolic and environmental stresses may have a strong impact on recombinant protein production.

Especially the production of complex proteins has turned out to have a very low success rate. Several physiological studies have demonstrated that many physiological processes, including stress responses to environmental factors, and protein folding/aggregation and secretion are highly interrelated. Among the environmental factors influencing protein expression and secretion, pH, osmolarity, oxygen availability and temperature appear to be particularly important.

For the production of heterologous proteins it seems implausible to cultivate the host cells at higher temperatures than the growth optimum, as the products naturally are heat sensitive. Lower temperatures, however, are often applied, usually based on empiric data based on improved product formation or stability.

Therefore a deeper understanding of the physiological and molecular links between protein folding and temperature (-adaption/-stress) appears useful. While a lot of data have been collected regarding the regulatory events as a reaction to temperature changes, there is not much information on the true physiological reaction of cells particularly in context of heterologous protein expression.

Results

The rapid transcriptional profiling method VTT-TRAC has been applied to monitor the levels of a subset of mRNAs coding for UPR-regulated and stress-connected genes in chemostat cultivations of a *P. pastoris* strain secreting the 2F5 antibody fragment. Specific marker genes have been chosen to deliver insights into the general physiological status of the cells under production conditions (including growth, protein synthesis, oxygen and nutrient limitation responses) with the main focus on secretion stress connected genes (UPR, ERAD, posttranslational processing).

As product formation is known to be strongly dependent on cultivation conditions, the influence of different cultivation temperatures has been analysed.

Transcript formation rates of the two respective product genes (for Fab light chain and heavy chain mRNA) have been set in correlation to the mRNA levels of folding related genes such as KAR2 and PDI1, and additionally to the specific product formation rate of secreted Fab.

Interestingly, although the transcriptional levels of the product genes were reduced at lower temperature, specific productivity of the 2F5 Fab protein was significantly increased. Thus it is tempting to speculate that at lower temperature a reduced amount of folding stress is imposed on the cells, consequently leading to a higher rate of correctly folded product. Also the chaperone KAR2/BiP, which is commonly seen as a marker of unfolded protein stress appeared among the genes down-regulated at lower temperature. Additionally, the levels of intracellularly retained antibody fragments and the UPR marker protein BiP (Kar2) were analyzed by immunofluorescence and flow cytometry.

Conclusions

The robust, sensitive and cost-efficient transcriptional profiling method VTT-TRAC could be set up for *P. pastoris*. Apart from being useful for bioprocess monitoring and control of fermentation conditions, it was possible to obtain new and valuable information regarding the physiological regulation of protein production in *P. pastoris* under altering culture conditions and to reveal key regulatory pathways determining the efficiency of protein production.

The induction of UPR-target genes due to heterologous protein production could be shown for the first time in *P. pastoris*. From the technological point of view, connections between growth temperature as an example for environmental conditions and specific productivity could be revealed at a transcriptional level, once again confirming that a release of folding stress can lead to higher product secretion rates.