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Direct monitoring of the unfolded protein response in *Pichia pastoris*

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High productivity, safety and the ability to secrete high quality heterologous proteins make the methylotrophic yeast *Pichia pastoris* a popular microbial host system for the production of recombinant proteins. However, inappropriate process conditions, as well as heterologous protein production itself induce stress in the lumen of the endoplasmic reticulum. Consequently, a cascade of signalling processes called the Unfolded Protein Response (UPR) pathway is triggered. Because the pathway results in the decrease of proteosynthesis or even protein degradation, and thus is the main factor affecting protein quantity and quality (cell productivity). It would be of great significance to monitor the UPR-activity directly during a cultivation process. Nowadays, UPR is monitored by time-consuming and expensive methods (PCR, electrophoretic methods). To analyse the state of UPR in a real time, we designed a *Pichia pastoris* strain in which the UPR-activity was detected by flow cytometry. A green fluorescent protein gene was used as a marker of the activity of six selected upstream regions (i.e. promoters) of the UPR-related genes (*PDI*, *ERO*, *JEM*, *SCJ*, *CAL* and *BiP*). The UPR pathway was induced by dithiothreitol addition and the response of the promoters was evaluated as a fluorescence signal by flow cytometry. *BiP1* was assessed as the most suitable promoter for stress monitoring, since the five-fold increase of its activity was observed after the treatment. The *BiP1* promoter is currently being characterised. We suggested a promising method straightforward monitoring of the cellular stress that could be applied to optimize a biotechnological production of recombinant proteins.

Biography

Raschmanova H completed her Master's degree in the Department of Biotechnology of the University of Chemistry and Technology Prague in 2014 at the age of 24 years. Since 2014, she has been the student of PhD degree at the same institute. She specializes in the research of the Unfolded Protein Response in *Pichia pastoris* and its relationship to the production of recombinant proteins.

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