Comprehensive analysis of *Pichia pastoris* proteome expressing Hepatitis B surface antigen

Vanz AL*, Adnan A*, Gurramkonda C*, Nimtz M*, Khanna N*, Scheper T* and Rinas U* c

a Institute of Technical Chemistry, Leibniz University of Hannover, Hannover, Germany  
b Department of Chemistry, Government College University Lahore, Lahore, Pakistan  
c Helmholtz Centre for Infection Research, Braunschweig, Germany  
d International Centre for Genetic Engineering & Biotechnology, New Delhi, India

Introduction
Although the methylotrophic yeast *Pichia pastoris* has a wide application in research and biotechnology industry, this yeast is still not adequately characterized at the physiological and proteome level, especially in relation to its response to the stressful growing environment in bioreactor and heterologous protein production. To gain insights into the proteomic changes in *P. pastoris* Gs115 during high level production of Hepatitis B virus surface antigen (HBsAg) under the control of a methanol-regulated promoter, comparative analyses are been done.

Method
1. **Pichia pastoris** cultivation  
   - Defined medium with low salt and high glycerol concentration as carbon source,  
   - Induction with methanol,  
   - Sample were taken from different time points.

2. **Cell disruption and protein extraction**
3. **2D Gel electrophoresis**  
   - Isoelectric focusing (IEF); Immobiline strip, pH 3.5-11.  
   - 12%-16% linear gradient gel. Gels were stained using colloidal Coomassie Blue G-250.

4. **In-gel trypsin digestion and peptide extraction**
5. **MALDI-TOF MS analysis**  
   - The molecular masses of the tryptic peptides were determined on a Bruker Ultraflex time-of-flight mass spectrometer.

6. **Database search and identification of proteins**

MALDI-TOF data analysis

Figure 1. Methanol pathway: protein expression level along the cultivation. Magnified regions of 2D image of specific enzymes from methanol metabolism and its respective expression level before induction and after 4, 16, 72 and 114 hours after induction.

Figure 2. Distribution of the yeast proteins before and 114 hours after induction according to their functional categories. Before induction (red) and 114 hours after induction (green).

Protein expression profile

Final considerations
Out of the total picked spots, 104 were matched to database sequences which represent 79 different proteins. Most of the high abundance identified spots before induction corresponds to proteins involved in glycolysis, TCA cycle, ethanol metabolism and heat shock response (Figure 2). Notably, more than 30% of the total intracellular protein expressed after induction are related with methanol metabolism pathway (Figure 1). It was also observed up-regulation of proteins related to ER stress, probably in response to the HBsAg accumulation in ER. A profound analysis of these obtained experimental data are being done in order to better understand the systematic metabolism and physiology of this protein production system. These results will provide new insights on the transient responses of cells growing on changing environment and can serve as a valuable basis for future rational strain engineering.

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