**Pichia pastoris** proteomic evaluation during HBsAg production

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**Introduction**  
Although the methylotrophic yeast *Pichia pastoris* has been used as a powerful and popular tool to express heterologous proteins, the current knowledge of the systematic metabolic and physiologic characterization of this yeast is still limited. To gain insights into the proteomic changes in *P. pastoris* GS115 during Hepatitis B virus surface antigen (HBsAg) production under the control of a methanol-regulated promoter, comparative analyses have 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); IPIphor™ Isoelectric Focusing System. IPG strip, pH 3-11, 12-16% linear gradient. gels were stained using colloidal Coomassie Blue G-250.

4. **In-gel trypsin digestion and peptide extraction**
   The spots were excised manually from 2-D gels, after destaining, reduction and alkylation, in gel digestion were carried out with trypsin. Obtained peptides were extracted and then desalted with reversed-phased C-18 ZipTips.

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**
   Peptide mass fingerprints obtained by the MALDI-TOF MS were processed using FlexAnalysis 2.0 (Bruker Daltonik GmbH, Germany) and used to search NCBInr database by using Mascot. All proteins with a Mouse score greater than 71 were regarded as significant (p < 0.05). Image analysis of the scanned gels was performed using Proteomweaver™ 3.0 (Definiens AG, Germany).

**MALDI-TOF data analysis**

**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 1). Notably, more than 30% of the total intracellular protein expressed after induction are related with methanol metabolism pathway (Figure 1 and 2). The intracellular protein expression profile throughout the cultivation of HBsAg production and few relevant changing are illustrated in figure 3 and 4 respectively. A profound analysis of these initially obtained experimental data are being done and the further comparison between different heterologous protein production in *P. pastoris* will be analyzed in order to better understand the systematic metabolism and physiology of this protein production system.

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