PROTEOME ANALYSIS OF WILD *SACCHAROMYCES CEREVISIAE* AND POTASSIUM TRANSPORT-AFFECTED MUTANT STRAINS

Miguel Curto Rubio[1], José Ramos Ruíz[2] and Jesús V. Jorrín Novo[1]

[1]Agricultural and Plant Biochemistry Research Group, Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, Córdoba, España;
[2]Departamento de Microbiología; Universidad de Córdoba, Córdoba, España

We have been using *Saccharomyces cerevisiae* as a model system to study homeostasis of potassium, the most abundant intracellular cation. For this purpose, the proteomes of the wild type BY4741 and potassium transport-affected mutant *trk1,2* strains have been compared, either under optimal or limiting potassium concentrations. This work is part of the multidisciplinary international research project "Gene interaction networks and models of cation homeostasis in Saccharomyces cerevisiae" (TRANSLUCENT, http://www.sysmo.net/index.php?index=61).

By using a differential expression proteomics approach, we have been analyzing differences in the protein profile between wild and *trk1,2* mutants under the following conditions: i) optimal growth potassium concentration (20 mM), exponential and stationary phase; ii) absence of or limiting (5 mM) potassium concentration, 30 min, 1, 3, and 5h. The following workflow has been used: i) protein extraction (buffer homogenization of the cells and TCA-acetone precipitation of the proteins); ii) protein separation by two-dimensional (IEF, SDS-PAGE) electrophoresis; iii) Coomassie gel staining, densitometer image capture, and spot quantitation; iv) statistical analysis of the data, and identification of qualitative or quantitative differential spots; v) trypsin digestion of the protein spots; vi) mass spectrometry analysis of the tryptic peptides (MALDI-TOF-TOF); vii) protein identification from MS and MS^2 spectra.

Comparative analysis of the 2-DE protein map of wild and mutant types grown under non limiting potassium concentrations revealed the existence of qualitative and quantitative differences between strains and growth phases. Taking as a reference the 2-DE map of the wild cells at the exponential phase, we have observed 10, 40, and 65 qualitative and 112, 122 and 131 quantitative variable spots, for, respectively, wild type stationary ones, and mutant exponential and stationary ones. Mass spectrometry analysis of these variable spots is in progress. Changes in the protein profile of both strains under K^+ starvation are being analyzed. In the wild type, the absence of potassium caused a decrease in the protein content of the cells, this being manifested by a reduction in the number of 2-DE spots detected.