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QUANTITATIVE ANALYSIS OF DIFFERENTIAL PHOSPHORYLATION IN A *PKC1* OVEREXPRESSION STRAIN OF *SACCHAROMYCES CEREVISIAE*

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In *S. cerevisiae*, protein kinase C (Pkc1p*)* is involved in the control of actin polarization and morphogenesis. Pkc1p acts upstream of the cell integrity MAPK pathway. A protein kinase C overexpression strain of *S. cerevisiae* was investigated for differential protein phosphorylation as compared to an isogenic wild type strain.

We have used a phosphoproteomic approach based on quantitative mass spectrometry based on stable isotope labeling with amino acids in cell culture (SILAC).

The *PKC1* overexpression strain was labeled by growth in media containing stable isotopic amino acids, i.e C13 – arginine and C13-lysine, to do differential analysis in a 1:1 protein mixture of both strains using mass spectrometry.

Several phosphopeptide enrichment tecniques have been used, and all fractions were analysed by nano – HPLC-MS/MS and neutral loss dependent MS3 on a LTQ mass spectrometer that allowed identification of phosphopeptides using Mascot scoring and quantification with MSquant, a freely distributed program for SILAC quantification.

Of 299 non-redundant phosphopeptides identified and quantified, 93 were upregulated more than 2-fold (average ratio).

The proteomic work was done at the Proteomics Facility of UCM-PCM, a member of ProteoRed Network