P. 96

USE OF 2-D DIGE AND PREPARATIVE DENATURING IEF TO ENHANCE SENSITIVITY IN THE DIFFERENTIAL PROTEIN EXPRESSION ANALYSIS OF *CANDIDA ALBICANS* YEAST-TO-HYPHA TRANSITION

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Candida albicans is one of the leading causes of opportunistic fungal infections in immunocompromised individuals. The yeast-to-hypha transition has been considered one of the primary causes of *C. albicans* pathogenicity. In order to quantify protein expression changes related to this transition, we performed a 2-D DIGE analysis of cytoplasmic protein extracts obtained from *C. albicans* yeast and hyphal cells after growth for 6h in Lee's Medium at different pH values. Four biological replicates were obtained, labelled with Cy3 or Cy5 and pooled with a Cy2 labelled internal standard before 2-DE. To obtain a global picture of protein expression changes, IPG strips with a pH gradient of 3-11NL were used in 2D-DIGE gels. DeCyder analysis gels allowed us to detect 2500 spots, 106 of which showed a significant variation in their expression (standardized average volume ratios ≥ 1.3, *t*-student $\leq 0{,}05$). Forty-five differentially expressed proteins were identified by MALDI-TOF/TOF. They are proteins mainly involved in sugar and purine metabolism, response to stress, protein folding and filamentous growth

To get more information, a preparative denaturing isoelectric focusing separation of *C. albicans* yeast and hyphal cytoplasmic extracts was carried out using a Rotofor cell. Colleted fractions with pH from 4.5 to 5.5 and from 5 to 6 were mixed and analysed in preparative narrow pH gradient 2-DE gels of yeast or hypha forms. Before 2-DE, acidic fractions were mixed with yeast and hyphal total extracts labelled with different Cy dyes to be able to detect new differentially expressed proteins at these pH ranges. More than ten new proteins were identified.

Data integration and network interaction analysis of all over- and under-expressed proteins are currently underway what allow us to identify different processes taking place in *Candida albicans* yeast-to-hypha transition.