

QUANTITATIVE PROTEOMIC PROFILE OF CANDIDA ALBICANS INTERACTION WITH HUMAN MACROPHAGES BY MEANS OF AN EIGHT-PLEX ITRAQ STUDY

V. Vialas⁽¹⁾, J.A. Reales⁽¹⁾, M.D. Gutierrez⁽²⁾, F. Clemente⁽²⁾, C. Gil⁽¹⁾.

⁽¹⁾ Departamento de Microbiología II, Universidad Complutense de Madrid, ⁽²⁾ Unidad de Proteómica. Universidad Complutense de Madrid .

The first barrier *Candida albicans* faces during candidiasis is the cellular immunity mediated by macrophages. The study of this host-pathogen model is thus essential to understand the nature of the crucial initial steps of the infection. We present here a quantitative proteomic approach to assess which proteins and biological functions and to which extent are more relatively abundant or activated upon the interaction. The experimental design, consists of an 8-plex iTRAQ experiment which enables the inclusion of three biological replicates for the ratio interaction/control and one extra replicate for the same ratio composed of respective pools for both control and interaction samples. The mass spectrometry was performed with a 4800 MALDI-TOF/TOF instrument and identification and quantitation were acquired using ProteinPilot 3.0 software. Applying a false discovery rate threshold of less than 5% , and taking into account only those proteins supported by three or more peptides with at least 95% confidence in identification, we obtain a good quality subset of proteins for further analysis. Those were forced to pass additional quantitation filters to reach the subset of proteins that show statistically significant changes in each of the interaction vs. control ratios. To accomplish such task we selected only those proteins showing p-values of the ratios lower than 0.05 and the Error Factor parameter, a type of confidence interval, lower than 2.

We have also performed an additional normalization, in addition to ProteinPilot's bias correction, to make the distribution for each of the ratios constrain to a median of 0 in logarithmic scale, making thus, ratios comparable to study the overlap in proteins among them. The filtering procedure, followed by the normalization, leads to a quality subset of proteins. Their ratios are then combined for those proteins appearing in at least two replicates, and are subsequently checked for GO term enrichment in groups defined by quantiles in the combined ratio population.