TITLE: INCREASED *Candida albicans* BIOFILM FORMATION AFTER *IN VIVO* SYSTEMIC INFECTION IS RELATED TO DIFFERENTIAL PROTEIN EXPRESSION

AUTHORS: ARITA, G.S.; SAKITA, K.M.; FARIA, D.R.; MENEGUELLO, J.E.; PILAU, E.J.; GHIRALDI-LOPES, L.D.; CAMPANERUT-SÁ, P.A.Z.; KIOSHIMA, E.S.; BONFIM-MENDONÇA, P.S.; SVIDZINSKI, T.I.E.

INSTITUTION: UNIVERSIDADE ESTADUAL DE MARINGÁ, MARINGÁ, PR (AVENIDA COLOMBO, 5790, CEP 87020-900, MARINGÁ – PR, BRAZIL)

ABSTRACT:

Candida albicans is the major pathogen isolated from nosocomial bloodstream infections, which are associated with high mortality rates. This species is related to virulence, such as adherence, yeast-to-hypha transition, secretion of hydrolytic enzyme, phenotypic switching and biofilm formation, which contributes to the establishment of infection. In addition, many proteins related to these virulence factors are expressed during the infection. This study aimed to correlate proteins detected after serial systemic candidiasis in murine model with phenotypic factor expression. Four female BALB/c mice were inoculated with an inoculum of 3.5×10^5 wild-type *C. albicans* SC5314 (WT) cells via lateral tail vein. The animals were euthanized five days post-infection, the kidneys were removed, homogenized in lysis buffer, plated on Sabouraud dextrose agar, and incubated for 24 h at 35 °C. With the colonies recovered, labelled as P1, proteins were extracted and biofilm formation assay was performed. The animals experiments were approved by the Ethics Committee on Animal Use in Experimentation (CEUA) from the State University of Maringa (protocol number 7261020418). The proteins were analyzed by LC-MS/MS using ultra-high-performance liquid chromatography (Shimadzu, Nexera X2, Japan) coupled to high-resolution mass spectrometry (Impact II, Bruker Daltonics Corporation, Germany). The data were processed in MaxQuant software for label-free quantification and in Perseus for statistical analysis. The biofilm formation was evaluated through colony form unit and biomass quantification by crystal violet. The results showed that some proteins associated with biofilm formation, such as cystathionine gamma-lyase (Cys3), yeast-form wall protein 1 (Ywp1), elongation factor (CEF3), Mir1p, and Ifd6p were detected in increased abundance from P1 when compared to protein profile from WT strain. Regarding biofilm assay, there was statistically significant increase in both, CFU count and total biomass from P1 in relation to WT. Altogether, the results from proteomics demonstrated a good correlation with the phenotypic assay performed. Thus, these proteins could have contributed to the infection, and could be further analyzed as new targets for the development of antifungal therapies.

Keywords: *Candida albicans*, proteomics, biofilm, virulence factor

Development Agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)