

TITLE: PHOSPHOLIPASE ACTIVITY OF *Candida albicans* RECOVERED FROM SERIAL EXPERIMENTAL CANDIDEMIA

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ABSTRACT

Candida albicans is part of normal human microbiota. However, an imbalance of the immune system can lead from superficial to invasive candidiasis. Furthermore, virulence factors expressed by this yeast, such as biofilm formation, filamentation, and secretion of phospholipases and proteinases enzymes, contribute for the development of the disease. The serial infection provide important insights about virulence evolution. This study aimed to analyze phospholipase activity from yeast recovered from murine model of serial candidemia. Four female BALB/c mice were inoculated with 3.5×10^5 *C. albicans* cells via the lateral tail vein. After five days, they were euthanized and colonies recovered from kidney were labelled P1. Thus, those colonies were used to fungal burden determination and to prepare a new inoculum for the subsequent infection of four animals. This scheme was repeated until obtaining colonies P5. All animals experiments were approved by the Institutional Ethics Committee of State University of Maringá (UEM), the Ethics Committee on Animal Use in Experimentation CEUA/UEM (protocol number 7261020418). For phospholipase assay, a loopful of the samples was inoculated onto the plates containing Sabouraud dextrose agar, 1 M NaCl, 0.005 M CaCl₂ and 8% egg yolk. They were incubated for 7 days at 37°C. The Pz value was determined by the ratio of the diameter of the colony to the diameter of the colony plus zone of precipitation. Thus, the lower Pz value, the higher enzymatic activity. Statistical analysis was performed by GraphPad Prism version 5.00 program, using one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison test. The fungal burden ranged from 2 to 5 log₁₀ CFU/g organ (previous data), presenting significant difference in the last two passages ($p < 0.05$). In relation to phospholipase activity, P1 and P2 showed weaker activity (Pz value 0.82 and 0.76 respectively). Furthermore, from P3 to P5, an increase of enzymatic activity was observed (Pz value 0.44, 0.45 and 0.41 respectively) that was statistically significant in comparison to P1 and P2 ($p < 0.05$). Thus, the higher enzymatic production could have contributed to the development of invasive candidiasis, which reflected to an increase of fungal burden over passages.

Keywords: *Candida albicans*; Candidemia; Phospholipase Activity; Virulence Factors

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