

TITLE: CHARACTERIZATION OF THE ANTIGENIC PROFILE OF PROTEINS SECRETED BY HISTOPLASMA CAPSULATUM USING IMMUNOPROTEOMIC APPROACHES

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ABSTRACT: The histoplasmosis is considered the most common respiratory fungal infection with a worldwide distribution caused by the fungus *Histoplasma capsulatum*. The currently available forms of treatment are drugs such as amphotericin B and itraconazole. However, patients need to undergo long periods of treatment that do not exclude recurrences of the disease and side effects resulting of the high toxicity of these antifungal drugs and interaction with other drugs. Vaccine models have been developed for histoplasmosis, however, there is still no vaccine that shows good efficacy. In this context, our study aims to expand the knowledge and establish new tools for the development of therapeutic vaccines, based on the selection of new antigens derived from *H. capsulatum*. We used the virulent G-217B isolate of *H. capsulatum* (ATCC 26032) in this study. Cells were cultured in BHI medium supplemented with 0.5% cysteine and 1% glucose, incubated under stirring at 37 ° C for 7 days. Posteriorly, the cell mass was separated from the culture medium by centrifugation and successive washes with ultrapure water. About 1.5 g of wet mass of yeast were mechanically lysed followed by extraction of proteins with 3 distinct buffers in sequence, Tris-MgCl₂/CHAPS (Buffer 1) Urea I (Buffer 2); Ureia-Thiourea (Buffer 3) pH 8.0. Proteins extracted from each buffer were maintained separately. The protein content was quantified by the Bradford method and the protein content was monitored by SDS-PAGE 12%. Proteins were purified and concentrated by Chloroform/methanol precipitation and resuspended in sample buffer. C57BL/6 mice (CEUA nº1169061218) were infected with *H. capsulatum*. After 10 days of infection the polyclonal serum of the mice was obtained and tested by immunoblotting against the purified proteins. The results of bradford demonstrate that approximately 1 mg/ml of proteins were extracted with Buffer 1, followed by 0.5 mg/ml of proteins with the other buffers. SDS gel band profiles confirm that most of the protein content was extracted with buffer 1 and immunoblotting revealed 15 reactive bands with the polyclonal serum. Buffer 2 and 3 did not present reactive proteins. From these results we conclude that the extraction with buffer 1 is sufficient to continue the proteomic studies and characterize the major antigens of *Histoplasma capsulatum* in the search of a suitable target for vaccine development.

Keywords: antigen; yeast; vaccine.

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