

TITLE: IDENTIFICATION AND CLASSIFICATION OF EXOANTIGENS PRESENT IN ISOLATES OF PARACOCIDIROIDES SPP. USING IMMUNOPROTEOMIC APPROACHES

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Fungi of the *Paracoccidioides* genus are the etiological agents of paracoccidioidomycosis (PCM). Currently, the genus encompasses two species: *Paracoccidioides lutzii* and *Paracoccidioides brasiliensis*. PCM is acquired by inhalation of conidia or fragments of fungal. The main diagnostic method of the disease is through serological tests, however diagnosis can also be performed by the presence of budding fungal cells present in the lesion biopsy material. In the pathogen-host interface, exoantigens occupy a prominent place, nevertheless the global characterization of exoantigens present in the *Paracoccidioides* complex has not yet been performed. In this study we characterized the antigenic profile of proteins secreted by isolates of different phylogenetic species of *Paracoccidioides*. For the production of exoantigens the isolates *Pb01* (*P. lutzii*), *Pb18* (S1b), *Pb02* (PS2), and *PbEpm83* (PS3) were employed. Initially the yeast cells were cultured separately in *Fava-Netto*'s liquid medium during 4 days. Subsequently, the cells were centrifuged, washed and inoculated in *McVeigh & Morton* liquid minimal medium. All these steps were performed at 37 °C pH 7,2. After 24 hours of exoantigen production, the samples were centrifuged and the supernatants were filtered. The obtained secreted proteins extracts were concentrated, washed and protein quantification was determined by the method of Bradford. Mice were divided in different groups and inoculated with three doses of 50 µg of exoantigens from each isolate were inoculated. On the 15th day after the last dose, blood serum from the animals were collected and tested by using immunoblotting. Preliminary results demonstrated differences in the antigenic profile of isolates *Pb01* and *PbEpm83*. In order to elucidate these differences, *NanoUPLC-MS^E* will be employed for the identification of these exoantigens. The analysis of the antigenic profile of the isolates *Pb02* and *Pb18* is in progress. As PCM is the most important systemic mycosis in Latin America, it is extremely important to characterize the antigenic profile of proteins secreted by isolates of different phylogenetic species of *Paracoccidioides*. These analyzes will contribute to a better understanding of the biology of the fungus, as well as the identification of possible candidates for the diagnosis of the disease.

Keywords: *Paracoccidioides* spp., Exoantigens, Proteomic

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