TITLE: HETEROLOGOUS EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF PEROXISOMAL CATALASE AND 30 KDA IDENTIFIED IN THE FUNGI WALL OF THE *PARACOCCIDIOIDES SPP.*

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ABSTRACT:

The genus *Paracoccidioides* comprises thermodymorphic ascomycetes fungi, which grow in the yeast form at 36º C in infected tissues and grow at temperatures between 22 and 27º in vitro in the mycelial form on the soil. This fungi is the causative agent of Paracoccidioidomycosis (PCM) that is an endemic granulomatous systemic mycosis in Latin America. The ability of the pathogen to interact and adhere to host surface structures is crucial for the colonization, invasion, growth and spread of the fungus to tissues. When spores are inhaled and come into contact with the respiratory tract, epithelial cells and resident macrophages are the first line of defense against fungal cells. This pathogen-host interaction is a set of actions between host defense mechanisms and the effort of microorganisms to overcome them and cause infection. Besides this fungi can use a variety of surface molecules in order to bind to defense cells such as macrophages and therefore maintain its survival and growth. A total of 214 cell wall proteins of the fungus *Paracoccidioides spp.* which interact with macrophages were identified through mass spectrometry studies. In this sense, the production of these possible adhesins via heterologous expression and analysis of the adhesion and survival capacity of the fungus by indirect immunofluorescence technique to confirm the function and localization of these proteins becomes relevant. Therefore, 30 kDa and peroxisomal catalase of *Paracoccidioides brasiliensis*, isolated Pb18, were expressed in a bacterial heterologous *Escherichia coli* system. The 30 kDa and peroxisomal catalase coding gene were cloned into pGEX-4T3 expression vector and the respective clones were used in the transformation of *E. coli* pLySs cells. The resulting recombinant proteins were used in the production of polyclonal antibodies by mice. The antibodies will be used in the functional characterization to block the protein present in the wall of the fungus, and later the immunofluoride technique will be performed using polyclonal antibodies labeled with fluorescein isothiocyanate (FITC) and analyzed by fluorescence microscopy to visualize the presence or absence of adhesion between the fungus and macrophages. The study of the virulence factors of *Paracoccidioides*

spp. is important for the understanding its role in the pathogenesis of the disease and may allow the development of drugs that act by reducing the virulence of the pathogen.

KEYWORDS: adhesins, 30 kDa, catalase peroxisomal, *Paracoccidioides sp.*

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