IDENTIFICATION OF PROTEINS POTENTIALLY EXPRESSED DURING INFECTION CAUSED BY *Paracoccidioides brasiliensis,* USING *IN VIVO*-INDUCED ANTIGEN TECHNOLOGY

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Paracoccidioides brasiliensis, is the ethiologic agent of paracoccidioidomycosis (PCM) a human systemic mycosis most prevalent in Latin America. *P. brasiliensis* is a dimorphic fungus that grows as mycelia at room temperature and as yeast cells in host tissues. Mycelium infects the mammalian host through inhalation into the lungs, where it differentiates into the pathogenic yeast phase. The objective of this work was to screen immunogenic proteins of *P. brasiliensis* potentially expressed during human infection using the *In Vivo* Induced Antigen Technology (IVIAT). Sera were collected from patients with paracoccidioidomycosis. Equal volumes of sera were pooled and reacted with whole yeast cells and yeast cell lysates of the fungal isolate *Pb*01. We constructed a cDNA expression library with RNAs of *P. brasiliensis* yeast cells recovered from livers of infected mice. Sera adsorbed with *P. brasiliensis* yeast cells were used to screen the library. We identified 35 clones which cDNAs encoded proteins related to cell metabolism, transport, energy, transcription, protein fate, signal transduction, biogenesis of cellular components. Of all positive clones identified by IVIAT we selected two encoding the sera reactive aromatic - L- amino acid decarboxylase (DDC) and lumazine synthase (LS) of *P. brasiliensis* for further studies. We characterized the complete cDNAs of *Pb*ddc and *Pb*ls that were overexpressed in an *Escherichia coli* host to produce high levels of recombinant fusion protein with GST. The recombinant proteins DDC and LS were recognized by sera of patients with confirmed PCM and not by

sera of healthy individuals. Real time RT-PCR was used to analyze the expression of the *Pb*ddc and *Pb*ls in the two forms of *P. brasiliensis* and in yeast cells infecting macrophages. The accumulation of both transcripts was higher in the yeast form and in yeast cells infecting murine macrophages. The results suggest that *Pb*DDC and *Pb*LS can be considered immunogenic proteins up-regulated during the infective process.

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