TITLE: *IN VITRO* ANALYSIS OF GAMMA-GLUTAMYL TRANSPEPTIDASE AND UREASE DURING NITROGEN STARVATION IN *Paracoccidioides brasiliensis*

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Paracoccidioides spp are fungi that cause paracoccidioidomycosis (PCM), an endemic human systemic mycosis in Latin America. These organisms grow as mycelium in temperatures below 28 °C and as yeast form in temperatures around 37 °C. Nitrogen is an important element in this microorganism's nutrition that participates in the synthesis of proteins, nucleic acids and others biomolecules. In this regard, nitrogen uptake and metabolism are essential to growth and fungal establishment in host milieu. When nitrogen levels and preferential sources such as glutamine and ammonia concentration are limited, pathogenic fungus use a regulation system called Nitrogen Catabolic Repression that induces the expression of genes encoding permeases and enzymes required for the catabolism of secondary nitrogen sources, such as formamidase, gamma-glutamyl transpeptidase and urease. Gamma-glutamyl transpeptidase (Ggt) is an enzyme that catalyzes the first reaction of glutationa degradation and it has been the target of several studies about nitrogen starvation in various fungi. It has been observed that the expression of this enzyme is induced in limiting conditions of nitrogen and repressed when the availability of nitrogen was high. Urease (Ure) is an enzyme that catalyzes the degradation of urea in ammonia and carbonic acid and is known as virulence factor in some fungi, such as *Cryptococcus neoformans*. Also, it has been aim of studies about nitrogen starvation. In this study we aim to express gamma-GT and urease of *Paracoccidioides brasiliensis*, *Pb*18, in *Escherichia coli* bacterial heterologous system and characterize the recombinant proteins regarding its function in nitrogen starvation. The gene coding for Ggt and Ure were obtained by PCR, cloned in pET32a expression vector and transformed into *E. coli* pLysS cells. Proteins expression was optimized using different concentration of IPTG and they were used for production of polyclonal antibodies. Furthermore, enzymatic assays were performed and the recombinant proteins produced were shown to be catalytically active. Additionally, we started the construction of mutants for *ggt* through antisense RNA technology. In conclusion, characterization of Ggt and Ure may help to elucidate its role during nitrogen starvation, add knowledge about these enzymes and contribute to the understanding of host-pathogen relationship, biology and virulence of this important pathogenic fungus.

Keywords: Nitrogen Catabolite Repression, cloning, enzymatic activity.

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