Heterologous expression and functional characterization of a hypothetical mannoprotein from *Cryptococcus gattii*

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Cryptococcus neoformans and *Cryptococcus gattii* are encapsulated yeasts and the ethiological agents of cryptococcosis. While *C. neoformans* normally infects immune compromised patients, *C. gattii* has the capability to infect healthy individuals. Mannoproteins correspond to approximately 1% of the composition of *Cryptococcus* capsule and are highly immunogenic. These proteins drive immune responses mediated by T cells during the infection in murine model. *In silico* analysis of *C. gattii* genome revealed the presence of several mannoprotein-coding genes. One of such genes is highly expressed during infection of mouse lungs. This gene product, with a predicted molecular mass of 43kDa, has all the essentials features of a mannoprotein: (i) a region rich of the amino acids serine and threonine, where the mannoses are added; (ii) one C-terminal domain of GPI anchoring and one N-terminal domain with a signal peptide. In this context, the aim of this study is to evaluate the immunotherapeutic potential of this recombinant predicted mannoprotein. For this, trials of experimental infection will be conducted with the recombinant protein and virulence assays employing *C. gattii* null mutant strains for this gene. As a primary strategy, the ORF from this gene was cloned into *P. pastoris* expression vector pHIL-S1, and the heterologous expression was conducted with *P. pastoris* GS115 and KM71 strains. In addition to low levels of expression, the recombinant protein was associated to cell wall. The apparent molecular mass of the cell wall associated recombinant protein was near 100 kDa, suggesting that it was glycosylated. A new heterologous expression experiment is being conducted with *P. pastoris* lineage X-33. We have cloned the coding sequence of the hypothetical mannoprotein into the plasmid pPICZαC for its secretion and confirmed the construction by restriction enzyme cleavage and sequencing. The electroporation of the linearized plasmid in *P. pastoris* resulted in 57 transformants that are being tested for the expression of *C. gattii* mannoprotein. In addition, we have been constructing the vectors that will be used for the construction of *C. gattii* null and overexpressing mutants to characterize the function of this mannoprotein in the host-pathogen interaction. After the expression and purification of the recombinant mannoprotein, assays will be conducted to evaluate the immunotherapeutic potential in a murine model of cryptococcosis.

Key-words: mannoprotein, *Cryptococcus gattii*, *Pichia pastoris*, immunotherapy