TITLE: Evaluation of aspartyl proteases in the dimorphism, cell wall modulation, and metabolism in *Paracoccidioides brasiliensis*

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ABSTRACT:

Paracoccidioidomycosis (PCM) is the most prevalent systemic mycosis in Latin America and is caused by fungi from the *Paracoccidioides* genus. Proteases have been described as playing an important role in the host invasion process and immune modulation in many pathogenic microorganisms. Aspartyl proteases are virulence factors in many human fungal pathogens that play an important role in the host invasion process morphogenesis, cellular function, immunity, and nutrition. In the present study, we characterized the modulation of acid proteases from *Paracoccidioides brasiliensis*. In silico analyses were performed to evaluate the homology and structure of four aspartyl proteases identified in *P. brasiliensis* PADG_00634, PADG_03432, PADG_082082 and PADG_12056 and compare to aspartic protease from *Saccharomyces cerevisiae* PEP4. The homology was 57,4%, 26%, 28% and 26% respectively. When *P. brasiliensis* mycelium cells were exposed to an aspartyl proteases inhibitor (pepstatin A) and incubated at 37°C, there was inhibition in the dimorphic mycelium to yeast (M→Y) transition at pH 7 and 4 in presence and absence of BSA. In addition, treatment of *P. brasiliensis* yeast cells with Pepstatin A impacts the response of yeast to cell wall stressors compared to control. Then, we evaluated if pepstatin A modulates the expression of genes related to the synthesis and regulation of chitin, beta, and alpha glucans. We observed that the Amy gene showed decreased expression. This gene is essential for synthesizing this polysaccharide involved in α-(1-3) glucan. The gene expression of four aspartyl proteases identified in *P. brasiliensis* was evaluated in the presence or absence of dextrose or peptone, sources of carbon and nitrogen respectively. All four genes were up-regulated in the absence of dextrose and the presence of peptone. These data suggested that aspartyl proteases expression is modulated by environmental conditions and its ability to adapt, resist different types of stress and are essential for the transition from mycelial to yeast at 37ºC.

Keywords: Virulence factors, paracoccidiodomycoses, *Paracoccidioides brasiliensis*, aspartyl protease.

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