TITLE: CHARACTERIZATION OF ALPHA-GALACTOPYRANOSYL EPITOPES IN PARACOCCIDIOIDES AND OTHER PATHOGENIC FUNGAL SPECIES

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ABSTRACT:

Our group has previously characterized the presence of terminal alpha-linked galactosyl epitopes (alpha-Gal) in *Paracoccidioides brasiliensis* cell wall, vacuoles, and extracellular vesicles with the use of anti-alpha-Gal IgG isolated from patients with Chagas disease (Ch-anti-alpha-Gal). We also showed that alpha-Gal stimulates the expression of anti-alpha-Gal antibodies in paracoccidioidomycosis patients. Considering that alpha-Gal epitopes and their role in fungal systems are poorly studied, we presently used confocal microscopy to test the Ch-anti-alpha-Gal IgG reactivity in cells from the yeast and mycelium phases of *Paracoccidioides* Pb18, Pb3, Pb01, *Histoplasma capsulatum,* and also *Aspergillus fumigatus, Candida albicans*, *and Cryptococcus neoformans*. In general, confocal images revealed the localization of Ch-anti-alpha-Gal IgG in the yeast cell wall, especially in the buds and bud base, and also in intracellular compartments (except for *C. neoformans*). The fluorescence had a punctuated pattern for most images. Pb18 hyphae had surface label, while *A. fumigatus* filaments were intensely labelled in conidia and phialides. These results suggested that alpha-Gal epitopes are expressed in many pathogenic species both in yeasts and mycelia. However, these observations should be confirmed by electron microscopy and, importantly, with the use of control samples treated with alpha-galactosidase. In order to indirectly show that alpha-Gal epitopes may be found in the cells of the aforementioned species, we used a total of ten 1,2- and 1,3-alpha-galactosyltransferase (alpha-GTs) gene sequences from *Schizosaccharomyces pombe* to search for paralogues. We found three alpha-GTs in each species that shared over 30% of protein sequence identity with two alpha-1,2-GT (GMH5 and GMH4) and one alpha-1,3-GT (OTG2). Quantitative real-time PCR analysis showed higher mRNA accumulation of the Pb18 GMH5-like gene during the mycelium phase, while it seemed to be more highly expressed in the Pb3 and Pb01 yeast phase instead. The GMH4-like mRNA had much higher accumulation in the yeast than in the mycelia from Pb18, Pb3, and Pb01. Further studies to confirm the presence and localization of alpha-Gal epitopes in pathogenic fungi include Transmission Electron Microscopy (TEM) and flow cytometry, while quantitative real-time PCR analysis will help analyse the expression profiles of putative alpha-galactosyltranferase genes in the studied species.

Keywords: Paracoccidioides, pathogenic fungi, alpha-Gal epitopes, anti-alpha-Gal IgG, alpha-galactosyltransferases.

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