TITLE: Identification of antigenic targets of *Histoplasma capsulatum* with potential use in the immunological diagnosis of histoplasmosis

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ABSTRACT:
Histoplasmosis is a cosmopolite systemic mycosis and one of the most frequent opportunistic diseases in HIV patients. In this population, histoplasmosis has high rates of morbidity/mortality, being often fatal if diagnosis and treatment are delayed. The gold standard diagnosis, based on a fungal culture from clinical specimens, has limitations, making presumptive immunodiagnosis an attractive option for clinical decisions. Despite the continuous development of immunodiagnostic tests, improvements to reach higher efficiency are still required. Notwithstanding, studies on the characterization of putative antigenic proteins participating in the host humoral response have been underexplored in *Histoplasma capsulatum*. In the present study, we performed a co-immunoprecipitation assay between a protein extract from the yeast form of *H. capsulatum* and a pool of sera from patients with histoplasmosis, followed by liquid chromatography coupled to mass spectrometry, in order to identify potential antigenic targets. Control samples (pool of sera from patients with other pulmonary infections or from healthy individuals living in an endemic area of histoplasmosis) were also assayed. The primary structures of *H. capsulatum* immunoprecipitated proteins were evaluated using the DNAStar Protean 7.0 software by the Jameson-Wolf, Kyte-Doolittle, and Emini algorithms. In parallel, the online epitope prediction server, BCPREDS, was used to complement the B-epitope prediction analysis. Our approach detected 134 reactive proteins against histoplasmosis serum; however, only three proteins (M antigen, catalase P, and YPS-3) reacted only to sera from patients with histoplasmosis and, therefore, may be potential specific antigenic targets. In previous studies of our group, the M antigen had its biological nature identified, was structurally characterized and demonstrated its expression on the yeast cell surface, besides being characterized as useful in the diagnosis of histoplasmosis. However, catalase P and YPS-3 protein (previously studied and characterized as virulence factors in *H. capsulatum*) had not yet been described as antigenic targets. Overlapping the results obtained with the two bioinformatics tools (BCPREDS and DNAStar), nine regions are proposed as mostly immunogenic in M antigen, eight in catalase P, and two in YPS-3 protein. These data indicate the possibility of the use of these three proteins as potential antigens in new methods for the diagnosis of histoplasmosis.

Keywords: Immunoproteome, *Histoplasma capsulatum*, Diagnosis.
Development Agency: Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro