Rev. Inst. Med. trop. S. Paulo
47(6):332, November-December, 2005

SUMMARY OF THESIS*

FREITAS, Roseli Santos de - **Caracterização fenotípica de amostras de *Histoplasma capsulatum* var. *capsulatum* e padronização de antígenos para o diagnóstico da histoplasmose.** São Paulo, 2005. (Dissertação de Mestrado - Programa de Pós-Graduação em Ciências, Coordenadoria de Controle de Doenças, Secretaria de Saúde do Estado de São Paulo).

PHENOTYPIC CHARACTERIZATION OF *Histoplasma capsulatum* VAR. *capsulatum* ISOLATES AND ANTIGENIC STANDARDIZATION FOR HISTOPLASMOSIS DIAGNOSIS

Mycelial cells from twelve *H. capsulatum* var. *capsulatum* samples were cultivated at 27 ºC in potato agar and in Sabouraud-dextrose agar during 33 days in order to evaluate their macromorphological characteristics. The micromorphological aspects were analyzed growing the fungic samples at 27 ºC in agar potato for 45 days. The analysis of the macromorphological characteristics revealed that 100% of the studied samples presented cottony texture with pigments varying from white to brownish tones. In Sabouraud-dextrose agar, 91.7% of the samples showed cottony texture and only one presented mebranous/ coriaceous aspect. In this same medium, 83.3% showed white pigments, except two that displayed beige pigments. In relation to the micromorphological aspects, 75% of the fungic samples were in phase of intense sporulation. The growth kinetic of the samples was also evaluated. It was observed that the best period for the giant colonies development occurred between the 40th and 47th day of culture. After this period, we observed the majority of the samples entered into the stationary phase. The *H. capsulatum* antigens were obtained from the *H. capsulatum* samples cultivated at 27 ºC in Sabouraud-dextrose agar for 15 and 33 days. After the incubation period, the cultures were treated with merthiolate-borate solution (1:5,000) for 24 hours. After this they were filtered and concentrated by lyophilization procedure and stored at -20 °C. The specificity of the different *H. capsulatum* antigens were evaluated, by the double immunodiffusion (DI) technique against a panel of sera from: histoplasmosis (HP) patients (illness or infection); individuals with clinical suspicion of HP but non-reactive with *H. capsulatum* reference antigen by DI; paracoccidioidomycosis patients; aspergillosis patients; leishmaniasis patients; individuals of blood bank; rabbit polyclonal anti-*H. capsulatum, Paracoccidioides brasiliensis* and *Aspergillus fumigatus*; and reference positive control serum anti *H. capsulatum* (H and M fractions) were also employed. Moreover, the electrophoretic profile was analyzed by SDS-PAGE and the immunoreactivity, by immunoblotting. Through ID, it was verified that the 20-fold concentrated antigens presented reactivity only against serum anti-*H. capsulatum* and anti- H and M *H. capsulatum* fractions, being observed the presence of H and M bands, and also against sera from patients with HP infection or illness. The best pattern of reactivity was observed for antigens obtained with 33 days of culture from the isolates 200 and 406 and for the antigen 200 with 15 days of culture. The analysis of the electrophoretic profile by SDS-PAGE disclosed great proteic complexity, presenting antigenic components of apparent molecular mass from 17 to 119 kDa. Through the immunoblotting, it was observed intense reactivity of the sera from patients with HP (infection or illness) against epitopes with molecular mass from 74 to 119 kDa. It is noteworthy that the antigen from sample 200, with 15 or 33 days of culture, presented the best pattern of recognition against the homologous sera. The results suggest the employment of the antigen from sample 200 in the DI assay due to its good capacity to discriminate both sera from patients with HP illness and HP infection, besides its high specificity (100%) against heterologous sera.

Roseli Santos de FREITAS
Laboratório de Micologia Médica
Instituto de Medicina Tropical de São Paulo - LIM53
Av. Dr. Enéas de Carvalho Aguiar 500
05403-000 São Paulo, SP, Brasil
e-mail: roselsf32@yahoo.com

*This thesis is available at the Library of the Instituto de Medicina Tropical de São Paulo