

ANALYSIS OF *Paracoccidioides brasiliensis* EXOANTIGENS STABILITY

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ABSTRACT: Diagnosis by isolation of the microorganism from cultures provides the strongest evidence of infection by *Paracoccidioides brasiliensis* (*Pb*). However, isolation is not always possible and serological tests such as double immunodiffusion (ID) must be often employed. We analyzed the reaction profile of 75 serum samples from paracoccidioidomycosis (PCM) patients, by using ID, against nine different antigenic preparations of *Pb*: somatic antigen (SoAg), produced from *Pb*113 and *Pb*339 strains; cell-free antigen (CFAg), produced from *Pb*113 strain – both preparations were cultured in Fava-Netto's agar medium for 7 days at 36°C; metabolic antigen (MAg), produced from *Pb*113 and *Pb*339 strains cultured in liquid NGTA (neopeptone, glucose, thiamine and asparagine) medium for 20 days at 36°C; soluble components of the cell wall outer surface (SCCWOS), produced from *Pb*113 strain cultured in Fava-Netto's agar medium at 36°C for 5, 10, 15, and 20 days; and *Pb*113 Negroni and *Pb*113 NGTA antigens, cultured for 20 days at 36°C. Serum samples reactivity was 90% to AgSo and SCCWOS cultured for 5, 10, 15 and 20 days; 86.6% to CFAg; 83.3% to MAg; 80% to *Pb*113 NGTA antigen; and 76.6% to *Pb*113 Negroni antigen. Electrophoresis in 10%SDS-PAGE showed high complexity of the protein fractions of SCCWOS, *Pb*113 Negroni and *Pb*113 NGTA antigens, which presented molecular weight between 25 and 170 kDa. Specificity and sensitivity of SCCWOS against serum pool from patients with chronic and acute forms of the disease were confirmed by immunoblot, which demonstrated that 25, 43, 60, 70, 85 and 160-kDa antigenic fractions of SCCWOS cultured for 5 and 10 days showed intense reactivity. We could demonstrate that SCCWOS of *Pb* are stable and show highly preserved antigenic fractions, which was proved by their high reactivity pattern to sera from different forms of PCM, anti-*Pb* antigen and anti-gp43 antisera.

KEY WORDS: *Paracoccidioides*, serological assay, paracoccidioidomycosis diagnosis, antigen-antibody reaction.

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