MOLECULAR CHARACTERIZATION OF MEMBRANE RAFTS IN *Paracoccidioides brasiliensis*

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Membrane rafts are membrane microdomains involved in cell signaling and enriched in cholesterol and sphingolipids, formed by the tight packing of cholesterol and saturated fatty acids of (glyco)sphingolipids in association with specific proteins. The insoluble property in cold nonionic detergents allows the separation of membrane rafts by flotation in sucrose density gradient (SDG). Recent works have shown that membrane rafts are present in non-pathogenic yeast such as *Saccharomyces cerevisiae*. In order to investigate the presence of microdomains in *Paracoccidioides brasiliensis* cell membrane and determine their composition, cell extracts of yeast forms of *P. brasiliensis* were homogeneized at 4°C. The lysate was incubated with Brij-98 and submitted to SDG. Twelve fractions were collected from top to bottom. Phospholipids, sterols and (glyco)sphingolipids were analyzed and quantified by HPTLC-densitometry. About 30% of Pb-1 and Pb-2 GIPCs, 44% of CMH and 30% of ergosterol were found in fractions 5-6, corresponding to low density fractions. By SDS/PAGE and Western-blot, it was detected protein markers for membrane rafts such as Pma1p and Gas1p, along with unknown proteins. These results demonstrate for the first time the existence of microdomains constituted by antigenic structures such as Pb-1, suggesting possible roles of this specialized membrane regions in the pathogenicity of *P. brasiliensis*.

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