ANALYSIS OF MEMBRANE RAFTS FROM Histoplasma capsulatum AND Sporothrix schenckii

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Membrane rafts (or lipid rafts) are subdomains of the plasma membrane that contain high concentration of cholesterol and (glyco)sphingolipids. In association with specific proteins, they form specialized membrane microdomains in which glycosil epitopes have been implicated in cellular events such as cell adhesion and signalling transduction. The packing of lipids and phase separation in cell membrane are probably responsible for the insolubility in nonionic detergents. To determine the lipid and protein compositions of membrane rafts, yeast forms of Histoplasma capsulatum and Sporothrix schenckii were homogeneized in TNE buffer at 4°C. The cleared lysate was incubated with Brij 98. After solubilization, the lysate was submitted to sucrose density gradient. The samples were ultracentrifuged and twelve fractions of equal volume were collected from top to **HPTLC** bottom. The fractions were analyzed by in solvent chloroform:methanol:CaCl₂ (60:40:9), and it was observed an enrichment of glycoinositol phosphorylceramide and ergosterol in membrane rafts fractions. By SDS/PAGE and Western-blot, different protein components were also detected in this fraction. It was verified the enrichment of a known membrane raft marker (Pma1p) in fractions corresponding to membrane rafts. These results demonstrate the existence of membrane rafts in fungi, and their possible roles in fungi pathogenicity is discussed.

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