

Title: Evaluate the susceptibility of *Cryptococcus gattii* in the presence and absence of fluconazole to macrophage phagocytosis activity

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Cryptococcus gattii causes a systemic infection that primarily infects healthy individuals. The disease is difficult to treat, and fluconazole is antifungal of choice. Phagocytic cells represent the first line of nonspecific host defense against this pathogen and the interaction of macrophages with yeast plays a central role in cryptococcosis. Thus, the aim of this study was evaluate the susceptibility of the strains in the presence or absence of fluconazole to macrophage phagocytosis activity and the resistance of the strains to the antifungal action of phagocytes. Therefore, phagocytosis assays were conducted with 1×10^6 J774.1 macrophage cells in DMEM supplemented was seeded into 12-well culture plates for 18h at 37 °C with 5% CO₂. The *C. gattii* cells were opsonized with anti-GXM antibody 18B7, and resuspended in a fluorescein isothiocyanate (FITC) solution. 1×10^7 cells/ml was added to the wells containing macrophage cells and incubated for 2h at 37 °C with 5% CO₂. After the incubation period, the cells were collected by scraping and analyzed with a Guava easyCyte Flow Cytometer (Merck Millipore) by measuring the green fluorescence of 5000 events. A second plate under identical conditions was incubated for 24h to evaluate antifungal activity. After incubation, the wells were plated on YPD plates for CFU determination. The survival index was obtained by normalizing the CFU counts after 24h of interaction to the fluorescence units obtained in the flow cytometric analysis following 2h of interaction. A GraphPad prism t-test was used to determine the statistical significance of the observed differences in fungal survival. The strains cultured in the presence of fluconazole displaying a mean fluorescent intensity (MFI) of 267, whereas the strain without FLC exhibit a MIF of 79 ($p < 0,05$). In CFU determination, strain of FLC retrieved $33,7 \times 10^2$ CFU/ml while the strain without FLC retrieved 1500 CFU/ml, obtaining an survival index of 127 and 19, respectively ($p < 0,05$). The strains cultured in the presence of fluconazole were internalized by macrophages compared to the strain without FLC. In addition, the capability of *C. gattii* to survive and replicate inside macrophages was increased in strains with fluconazole based on the recovery of a higher number of CFUs from the infected macrophages. In conclusion, this report contributes to better understanding of *Cryptococcus*-host interaction in the presence of antifungal.

Keywords: *Cryptococcus gattii*, fluconazole, phagocytosis assays, cytometric

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