

TITLE: BIOFILM FORMATION BY *CRYPTOCOCCUS NEOFORMANS* AND *CRYPTOCOCCUS GATTII* CLINICAL ISOLATES

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ABSTRACT

Cryptococcus neoformans and *Cryptococcus gattii* are the etiological agents of cryptococcosis, a disease with a high incidence among severely immunocompromised patients. These fungi present yeast morphology during infection in humans and express mainly the following virulence factors: capsule; melanin production; enzymes; biofilm formation and thermotolerance. These virulence factors are known to contribute to the resistance of *Cryptococcus* spp. to antifungal drugs and the expression of its pathogenicity. In this way, the present work aims to evaluate the biofilm formation of 5 clinical isolates of *C. neoformans* and 5 clinical isolates of *C. gatti* and compared with standard strain *C. neoformans* H99 and *C. gatti* ATCC 56990. The yeasts were cultured in Sabouraud dextrose broth for 72 h at 35 °C to standardize the fungal inoculum 1×10^7 CFU/mL in RPMI 1640 medium buffered with 0.16 M MOPS. An aliquot of 100 μ L of fungal suspension was dispensed into the wells of a flat bottom 96-well microplate and incubated at 35 °C without agitation. The biofilms were analyzed in different times (24, 48, 72 and 96 h) of incubation by assessing metabolic active cells utilizing the colorimetric tetrazolium salt reduction assay (XTT). Regarding the biofilm formation our results shows that for *C. neoformans*, H99 strain produced a higher amount of biofilm reaching maximum peak in 72 hours. On the other hand, the 5 clinical isolates of *C. neoformans* did not produced significant amounts of biofilm when compared to the reference strain H99. For *C. gattii*, the clinical isolates #616A, #652A and #551A produced a higher amount of biofilm when compared to the reference ATCC 56990, and other 2 isolates produced less biofilm. Comparing the two species groups, both produce similar amounts of biofilm with optical density ranging from 0.05 to 0.15, with the exception of the H99 that reached values higher than 0.45. Nevertheless, further investigations about virulence factors are needed to get at a more concrete conclusion regarding the virulence of these clinical isolates.

Keywords: Cryptococcosis, virulence factors, biofilm