Title: EFFECT OF FLUCONAZOLE RESISTANCE ON THE METABOLISM OF *Cryptococcus gattii*

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Abstract:

Cryptococcus gattii is one of the etiologic agents of cryptococcosis, a disease that affects lungs, and that can also migrate to the central nervous system and cause meningoencephalatis. This pathogen is found in soil and associated with trees, mainly eucalyptus, and it is known for causing cryptococcosis in healthy individuals. Fluconazole is the first-choice antifungal, but treatment of the disease is difficult. Furthermore, the number of cases of fluconazole resistance has been increased. However, the metabolic alterations related to this resistance are still unknown. Thus, the goal of this study was to compare the effect of fluconazole-resistance on the metabolism of *Cryptococcus gattii*. For this, the strain L27/01 of *C. gattii*, susceptible to fluconazole, was previously submitted to a process of resistance induction to this antifungal. Even after being subcultured 170 times in the absence of the antimicrobial, L27/01 still was resistant, being then named as L27/01F. The two strains, susceptible (L27/01) and resistant (L27/01F), were compared for their ability to grow in media under starvation or excess of the following nutrients: glucose, $FeSO_4$, KH_2PO_4, $NaNO_3$, KCl and $MgSO_4$. It was also analyzed the influence of the presence and excess of inositol on fungal growth. Inocula of L27/01 and L27/01F, in the concentrations of 10^2, 10^3 e 10^4 cells, were plated on media presenting the different conditions. The plates were incubated at 37º C for 96 hours, being then determined the number of colony forming units (CFU). It was observed that five conditions were favorable for the growth of the strain resistant to fluconazole: excess of KH_2PO_4 (1% more growth), shortage (5% more) and excess (5% more) of $NaNO_3$, shortage of glucose (7,6% more) and the presence (12,8% more) of inositol, whereas only the presence of inositol (1% more) was favorable for the growth of the susceptible strain, when compared to the ideal concentrations of the nutrients in the minimal medium. The results obtained suggest that there are metabolic alterations in L27/01F strain, when compared to the original strain, indicating that the induction of fluconazole resistance is also related to the physiological metabolism of *C. gattii*. Future studies at the genome level will be performed for understanding those alterations.

Keywords: *Cryptococcus gattii*, fluconazole, resistance, metabolism

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