Construction of *Cryptococcus neoformans* reporter strains containing GFP or mCherry fluorescent proteins for pathogen-host interaction studies

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Abstract

Cryptococcus neoformans is the etiological agent of cryptococcosis, one of the major invasive fungal diseases across the world. *C. neoformans* is known for its remarkable ability to parasitize immune system phagocytic cells, exploiting them as a site for replication, protection from host recognition and immune response, and/or even as a vehicle for dissemination from the lungs, the primary site of inoculation, to secondary infection organs such as the brain, promoting cryptococcal meningitis, generally with fatal outcomes. In this context, the improvement of methodologies for the study of the interaction between *C. neoformans* and phagocytes of the mammalian host are essential for the clarification of the mechanisms of the disease. The aim of this work was to construct and characterize *C. neoformans* reporter strains containing GFP or mCherry fluorescent proteins to better investigate the dynamics of *C. neoformans*-host cells interaction both *in vitro* and *in vivo*.

Methodology

Results

Fig. 1. Intracellular distribution of fluorophores and fluorescence intensity of the engineered transformants.

Fig. 2. Phenotypic analysis of the transformant strains under stress conditions.

Fig. 3. Analysis of mating hyphae formation.

Fig. 4. Evaluation of virulence factors.

Fig. 5. Virulence of the transforming strains in *Galleria mellonella* and in primary murine macrophages infection models.

Figure 6. Fluorescence microscopy and flow cytometry analysis of macrophage phagocytosis.

Conclusion

Our results indicates a promising use of engineered *C. neoformans* *HIS3* and *PABP* fluorescent reporter strains coupled to flow cytometry and fluorescent microscopy for the improvement of investigation of pathogen-host interplay.

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