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"Genetic Complementation of the EDE1 Endocytosis Mutant in the Pathogenic fungus *Cryptococcus neoformans*"

Introduction. *Cryptococcus neoformans* is an opportunistic fungal pathogen and a leading cause of life-threatening meningitis, especially in people with weakened or immunocompromised immune systems. One process that helps this fungus grow and survive is endocytosis, in which cells take up materials from their surroundings. Its capacity to cause disease depends on endocytosis and intracellular membrane trafficking, which support growth, morphogenesis, signal transduction, and virulence. The *EDE1* gene encodes a multi-modular endocytic scaffold protein that organizes clathrin-mediated endocytosis and actin remodeling. Previous studies showed that deleting *EDE1* (creating a *Δede1* mutant) reduces uptake of the fluorescent dye FM4-64, suggesting that endocytosis is impaired. In this project, we aim to restore a normal copy of *EDE1* to the *Δede1* mutant strain to determine whether normal endocytosis can be restored.

Hypothesis. We hypothesize that the endocytic defect of the Δede1 mutant results specifically from the loss of EDE1, and that reintroducing a wild-type copy of EDE1 into the mutant will restore normal FM4-64 dye uptake. Therefore, the complemented strain is predicted to recover wild-type endocytic function, while the uncomplemented Δede1 mutant remains impaired.

Methods. The wild-type *EDE1* gene was amplified from *C. neoformans* genomic DNA using PCR with primers designed to amplify the promoter and coding regions. PCR products were analyzed by agarose gel electrophoresis. DNA fragments of the expected size were cloned into plasmid vectors, propagated in *Escherichia coli*, and verified by DNA sequencing. The verified construct will then be introduced into the *Δede1* mutant using biolistic (gene gun) transformation for genetic complementation.

Results. We first compared wildtype and *Δede1* strains using FM4-64 dye uptake and observed delayed uptake in the *Δede1* mutant, consistent with impaired endocytosis. To generate the complementation construct, we designed PCR primers based on publicly available *EDE1* sequences. While we successfully amplified a fragment of approximately 3 kb from the 3′ region of the gene, repeated attempts to amplify the remaining regions were unsuccessful until our last day of data aggregation. Attempting with the reference strain, H99, we successfully amplified the first fragment, completing a fully functional gene. Verifying the fragment with base specific primers, we can finally proceed with the complete fungal complementation.

Discussion. If reintroducing the wild-type *EDE1* gene restores FM4-64 dye uptake, it would confirm that loss of *EDE1* is responsible for the observed endocytosis defect. These findings would improve our understanding of how endocytosis contributes to the biology of *C. neoformans* and may provide insight into cellular processes that support fungal disease.