**TITLE:** FUNCTIONAL CHARACTERIZATION OF A PUTATIVE TRANSCRIPTION FACTOR INVOLVED ON CALCIUM SIGNALING PATHWAY IN *CRYPTOCOCCUS NEOFORMANS*

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**ABSTRACT:**

*Cryptococcus neoformans* is an opportunistic pathogen and the main cause of fungal infections related to death in immunocompromised patients. This yeast is able to disseminate from the lungs to the central nervous system (CNS), resulting in often fatal cryptococcal meningoencephalitis. The cryptococcal survival within the host requires a range of virulence factors, such as the ability to growth at 37°C, the production of a polysaccharide capsule and the secretion of enzymes as urease and phospholipase B1. The calcium ($Ca^{2+}$) is a cellular messenger that participates in calcineurin signaling, a pathway also important for cryptococcal virulence. Moreover, vacuolar $Ca^{2+}$ transporters (Vcx1 and Pmc1) coordinate the regulation of $Ca^{2+}$ intracellular levels. It has been shown that Pmc1 is required for proper virulence of *C. neoformans* since the null mutant strain (*pmc1*) could not cause death in mice. Our group carried out a RNA-Seq analysis to elucidate the global expression profile of *Δpmc1* cells. The disruption of *PMC1* gene leads to alterations in pathways involved in the paracellular mechanism of transmigration and calcium homeostasis and signaling. The purpose of this work is to functionally characterize a putative transcription factor potentially involved in the $Ca^{2+}$/calcineurin signaling pathway. From our RNA-Seq data, we selected genes that displayed differential expression profile in *Δpmc1* cells with potential transcription factor domains as predicted by bioinformatics. To assess the expression profile of these genes in condition of calcineurin inhibition, we performed a RT-qPCR of the H99 and *Δpmc1* strains treated or not with FK506. From this initial screening, we selected CNAG_03913 gene that is highly expressed on *Δpmc1* in the presence of FK506. There are two identified transcripts encoded by CNAG_03913 (T1 and T2), suggesting a regulation by alternative splicing. T2 showed to be highly expressed than T1 in the null mutant strain. To functionally characterize the role of CNAG_03913 gene in *C. neoformans* calcium homeostasis, a null mutant strain has been constructed and its pathogenicity and dissemination ability to CNS will be evaluated in a murine model of cryptococcosis. To differentiate the possible role of each CNAG_03913 transcript, we intent to overexpress the encoding sequences of T1 and T2 on the null mutant strain.

**Keywords:** Calcium transport, *Cryptococcus neoformans*, virulence.

**Development Agencies:** CNPq, CAPES, FAPERGS.