

TITLE: ZRG1 IS A CRYPTOCOCCAL SPECIFIC GENE INVOLVED IN ZINC HOMEOSTASIS REGULATION

AUTHORS: DIEHL, C; GARCIA, AWA; SCHNEIDER, RDO; DOS SANTOS, FM; VAINSTEIN, MH; KMETZSCH, L; STAATS, CC.

INSTITUTION: PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA CELULAR E MOLECULAR, CENTRO DE BIOTECNOLOGIA, UFRGS, PORTO ALEGRE, RS.

ABSTRACT:

Cryptococcus gattii is one of the etiological agents of cryptococcosis, a life-threatening disease that is generally characterized by meningitis and/or pneumonia. Metal homeostasis in pathogens must be well regulated, as excess or deprivation of metal levels can produce a toxic environment for the cell. Zinc is an essential element for all organisms and is required for the functions of many proteins with catalytic and structural roles. In the present work, we have identified a new gene involved in zinc homeostasis in *C. gattii*. The gene coded by CNBG_1485, which we named *ZRG1*, encodes a hypothetical protein restricted to cryptococcal species was identified from a screening for zinc deprivation hypersensitive phenotypes mutants in library of T-DNA insertional mutants. Bioinformatic analysis revealed the absence of any conserved domain in the primary sequence. To evaluate the role of *C. gattii* *ZRG1* in zinc homeostasis, we constructed null mutants and the sensitivity of the constructed strains to zinc-limiting conditions was evaluated (YNB containing DTPA or TPEN). Metal deprivation resulted in decreased growth of the $\Delta zrg1$ null mutant compared to WT. Expression analyses demonstrated a significant increase in the transcript levels of *ZRG1* when WT cells were cultured in the presence of DTPA or TPEN. In addition, we found that Zrg1 could be involved in the transcriptional regulation of genes involved in zinc homeostasis, as the transcript levels of the zinc transporter coding gene *ZIP1* gene and the zinc-homeostasis master regulator coding gene *ZAP1* transcription factor are reduced in $\Delta zrg1$ compared to WT cells. No differences in the production of the most studied virulence factors (melanin, capsule production, and growth at mammal host temperature) could be observed. Virulence assays employing murine macrophages are being conducted to evaluate the participation of such protein in the pathogenic potential of *C. gattii*. In conclusion, these results suggest that *ZGR1* gene is involved in the regulation of zinc homeostasis in *C. gattii*.

Keywords: *Cryptococcus gattii*, cryptococcosis, zinc, null mutant

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