TITLE: VARIATION IN THE CELL WALL ORGANIZATION AND GENE EXPRESSION IN RESPONSE TO OXIDATIVE STRESS IN *Candida tropicalis*

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ABSTRACT:

In healthy hosts, phagocytic cells are the first defense line against microbial pathogens. These cells produces reactive oxygen species (ROS) creating a toxic environment. In response to host oxidative stress, pathogen fungi such *Candida* engage transcriptional, post-translation and enzymatic responses. Physiological changes that allow the emergence of strains more resistant to ROS may bring adaptive advantage to individuals that manifest them. The aim of this study was to analyze the effect of phenotypic switching on *Candida tropicalis* response to oxidative stress. A clinical isolate (strain 49.07) and its switched strain (Crepe morphotype) were exposed to 5 mM of H_2O_2 for 10 minutes. After exposure, the cell viability (CFU counting), quantification of cell wall components using the dyes calcofluor white (chitin) and FITC-ConA (mannan) (Fluorimetry assay), cell wall porosity (polycation-induced leakage of UV-absorbing) and expression of *HOG*1, *WOR*1 and *EFG1* genes (qPCR) were analyzed. The crepe morphotype was more resistance to H_2O_2 ($p<0.05$) with lower reduction of cell viability (80 % viability) than its clinical isolate counterpart (60% viability). After oxidative stress, chitin content (from 1.5×10^3 to 2.5×10^3 Fluorescence units (FU) ($p<0.05$) was increased for both strains; increased in mannan content (from 2.1 to 3×10^3 FU) was observed only in clinical isolate, and decrease in cell wall porosity was observed also only clinical strain (80 to 60 %, $p<0.05$). Crepe morphotype do not presented decrease in cell wall porosity and alteration in mannan content. Under control conditions (absence of H_2O_2) the expression of *WOR*1 gene (transition to filamentous forms) by the crepe morphotype was lower than that observed for the strain 49.07. Differently, the expression of *EFG*1 gene (morphological switch transitions) was higher for the crepe morphotype than that observed for the clinical strain. After oxidative stress, all genes were upregulated in both strains (except *WOR*1 for 49.07), furthermore, the Crepe morphotype showed higher expression to all genes compared to the clinical strain. The cell wall is the first contact point of the yeast cell with the host defense cells. Our data show that oxidative stress promotes alteration at cell wall level and expression of genes associated with morphological transitions in *C. tropicalis*. Besides, phenotypic switching provides to *C. tropicalis* strain more persistence under adverse host conditions.

Keywords: Oxidative stress, *Candida tropicalis*, phenotypic switching, virulence.

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