A TWO-COMPONENT SIGNAL TRANSDUCTION SYSTEM REGULATES THE DIMORPHISM IN *Paracoccidioides brasiliensis*

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ABSTRACT

Paracoccidioides brasiliensis and *P. lutzii,* thermally dimorphic fungi, are the causative agent of paracoccidioidomycosis. *Paracoccidioides* infection occurs when conidia or mycelial fragments are inhaled by the host, which causes cell transition to the yeast form. The development of disease requires conidia inside host alveoli to differentiate into yeast cells in a temperature-dependent manner. This fungus is a facultative intracellular pathogen able to survive and replicate inside non-activated macrophages. Therefore, the survival of *P. brasiliensis* inside the host depends on the ability to adapt to oxidative stress induced by immune cells, especially alveolar macrophages. We have previously reported that low reactive oxygen species concentrations cause cell proliferation in the human pathogenic fungus *P. brasiliensis*. In the present report, we investigated the influence of phosphorylation events in that process. Using a mass spectrometry-based approach, we mapped 440 phosphorylation sites in 230 *P. brasiliensis* proteins and showed that phosphorylation at different sites determines fungal responses to oxidative stress. Furthermore, we described the presence of a two-component signal transduction system in *P. brasiliensis* by expression analysis of a hypothetical protein gene (PADG_07579) that showed high similarity with the histidine kinase (*DRK*) of *Blastomyces dermatitidis*. This gene was sensible to environmental redox changes, which was evidenced by 2.5-fold decreased levels of the transcript after 0.1 mM peroxide stimulation and 4.4 and 5.9-fold decreased levels of the transcript after 1 mM or 10 mM H_2O_2, respectively. In the mycelium-yeast (M-Y) transition, *DRK1* gene displayed increase of 14-fold in the mRNA expression after 24 h incubation at 37°C. These results suggest that *DRK* gene is differentially expressed during dimorphic M-Y transition. Still when *P. brasiliensis* mycelial cells were exposed to a histidine kinase inhibitor and incubated at 37°C was observed a delay in dimorphism M-Y transition. Finally, *P. brasiliensis* yeast cells were susceptible to 1 µg histidine kinase inhibitor treatment, evidencing the active role for histidine kinases in the *P. brasiliensis* metabolism. These findings will help us to understand the phosphorylation events involved in the oxidative stress response as well as dimorphism-associated pathways.

Palavras-chaves: Phosphoproteomics, dimorphic fungi, *Paracoccidioides*

Financial Support: CAPES, FAPESP and CNPq