**TITLE** **THE ROLE OF DIMORPHISM REGULATING HISTIDINE KINASE (DRK1) IN *PARACOCCIDIOIDES BRASILIENSIS* CELL WALL MORPHOGENESIS**

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**ABSTRACT**

Fungi of *Paracoccidioides* genus are the causative agents of paracoccidioidomycosis (PCM), a systemic endemic disease in Latin America with high prevalence in Brazil. This fungus presents as infective mycelium in the soil (25°C), reverting to its pathogenic form when inhaled by mammalian host (37°C). The ability to switch from mycelium to yeast is essential to disease development. Among dimorphic fungus species, dimorphism regulating histidine kinase (Drk1) plays an essential role to morphological transition. Histidine kinases (HK) are very important as virulence and cellular survival regulators and are present in bacteria and fungi, but absent in mammalian cells. It was observed that *P. brasiliensis DRK1* (*PbDRK1*) expression during mycelium-yeast transition is predominantly expressed in pathogenic yeast phase. In addition, when fludioxonil, a Dkr1 pharmacological inhibitor (iDrk1), was incubated with mycelium at 37°C it alters the dimorphic switch. Hence, the purpose of this study was to investigate the role of PbDrk1 in modulation of *P. brasiliensis* cell wall. We observed that PbDrk1 participates in fungal resistance to different cell wall disturbing agents, such as Congo Red, Calcofluor White and sodium chloride, by reducing yeasts viability after treatment with iDrk1. In order to verify the role of *PbDRK1* in cell wall morphogenesis, we performed RT-qPCR analysis. The results indicated that samples previously exposed to iDrk1 presented higher expression levels in several genes related to cell wall modulation. Among the genes analyzed, we highlighted *FKS1*, a β-glucan synthase which was 3,6-fold increase when compared to no treatment control. Confocal microscopy analysis and flow cytometry showed higher β-glucan exposure in cell surface of *P. brasiliensis* after 24 h incubation with iDrk1. Accordingly, through phagocytosis assay, it was observed a significant higher phagocytic index in yeasts treated with iDrk1 than control group, evidencing the role of PbDrk1 in cell wall modulation, which becomes a relevant target to be investigated. The supernatant of phagocytosis assay was used to assess the immune response profile, revealing increased levels of pro-inflammatory cytokines IL-12p70 and TNF-α. Finally, our data strongly suggests that PbDrk1 modulates cell wall components expression such as β-glucan. The comprehension of this signaling pathway may be of great value to identify targets to antifungal molecular activity, since HK are not present in mammals.

**Key words:** *Paracoccidioides brasiliensis,* histidine kinase, Drk1, dimorphism, cell wall

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