TITLE: THE TWO-COMPONENT HISTIDINE KINASES DRK1 REGULATES THE EXPRESSION OF CELL WALL GENES IN *Paracoccidioides brasiliensis*

AUTHORS: [1]NAVARRO, M.V., [2]BARROS, Y.N., [1]CHAVES, A.F.A., [1]CASTILHO, D.G., [1]CALADO, J.C., [2]CONCEIÇÃO, P.M., [2]FURUE, B., [1]SILVA, R.S., [1,2]BATISTA W.L.

INSTITUTION: [1]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil. [2] Departamento de Ciências Farmacêuticas, Universidade Federal de São Paulo, Diadema, São Paulo, Brazil.

ABSTRACT:

The dimorphic fungi of *Paracoccidioides* spp. genus are the causative agents of Paracoccidioidomycosis (PCM), an endemic systemic mycosis in Latin America with high prevalence in Brazil. On the environment (25°C) this fungus presents as infectious mycelium and when inhaled by the host (37°C), reverts to the pathogenic yeast form. This ability of switching from mycelium to yeast is essential for the development of PCM. It is known that different genes are expressed according to the fungus phase and, in the last years, genes related to mycelium-yeast transition (M-Y) were identified. In dimorphic fungi species, the dimorphism-regulating histidine kinase (*DRK1*) is mainly expressed in yeast phase. Recently, we characterized the expression of *PbDRK1* gene, the study showed its role in dimorphism over iprodione (a histidine kinase inhibitor). It was demonstrated that the fungus remained as mycelium even when cultivated at 37°C. Histidine Kinases (HK) represents great importance regulating virulence and cell survival, and the investigation of associated signaling pathways may aid the discovery of potential molecular targets resulting on the development of drugs with antifungal activity, once HK proteins are present in prokaryotes, plants, bacteria and fungi but absent in mammalian cells. Studies with *Sporothrix schenckii* demonstrated that *SsDRK1* is also required for cell wall composition and integrity. A spot assay, in which yeast cells were first incubated for 24 h, was performed with varying concentrations of Drk1 inhibitor (iDrk) and subsequently cultivated on YPD agar. The results showed that iDrk alone is not responsible for reducing cell viability. Next, we used an intermediate concentration of iDrk (25 µg/mL) and incubated *P. brasiliensis* yeast cells for 24 h, followed by spotting on YPD agar containing varying cell wall disturbing agents, such as Congo Red, Calcofluour White and sodium chloride. Interestingly, we observed reduced cell viability on samples submitted to iDrk, suggesting that *PbDRK1* is related with cell wall morphogenesis. In order to evaluate genes involved with cell wall morphogenesis we performed qPCR analysis and observed differences between iDrk treated samples and non-treated controls. Transmission Eletronic Microscopy analysis was performed for the same conditions and differences on cell wall morphogenesis was observed. Further information in necessary in order to understand the mechanisms involved in cell wall morphogenesis and *PbDRK1* potential signaling pathway related to it. Drk1 characterization and its signaling pathway will help the understanding of mechanisms involved with dimorphism, virulence and possibly PCM development.

Keywords: *Paracoccidioides brasiliensis*; paracoccidioidomycosis; histidine kinase; dimorphism; cell wall

Financial Support: FAPESP, CNPq