Title: PROTEOMICS CHARACTERIZATION OF THE *PARACOCCIDIOIDES lutzii* DURING NITROGEN CATABOLITE REPRESSION

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Abstract

The *Paracoccidioides* genus is composed of thermodimorphic fungus that causes paracoccidioidomycosis (PCM), an endemic human systemic mycosis in Latin America. These organisms grow as mycelium in temperatures below 28 °C and as yeast form in temperatures above 37 °C. Nitrogen metabolism is essential for the growth and establishment of pathogenic fungi in host tissues, where this nutrient is scarce. In fact, after internalization by the host, microorganisms are exposed to an extreme nutrient-limited environment. The Nitrogen Catabolic Repression (NCR) is a regulatory mechanism of nitrogen uptake activated when preferred nitrogen sources such as ammonia or glutamine are scarce. In this regard, when non preferred sources are the only source of nitrogen, fungal cells will activate NCR mechanism, regulating and expressing enzymes and permeases that will act in the catabolism of secondary nitrogen sources. In the present study, we characterized *Paracoccidioides lutzii*'s proteome when the NCR is activated. First, transcriptional level expression of general amino acid permease (GAP), a NCR marker, was identified by real time PCR. Briefly, yeast cells from *P. lutzii* were grown in repressive glutamine medium and derepressive prolina medium. Transcriptional analysis by rtPCR demonstrated that after 24 hours, the expression of GAP increase in presence of proline, thereby the proteins was extracted in this point in both conditions and subjected at trypsin digestion and mass spectrometry by Nano UPLC-MS^E. We identified 272 proteins where 45 proteins were down-regulated and 48 proteins up-regulated. Among induced proteins, we identified molecules related to amino acid metabolism; cell rescue, defense and virulence; gluconeogenesis and beta oxidation. Enzymes such as malate synthase and glutamate production were induced. The high levels of α-ketoglutarate and glutamine synthetase are important for the maintenance of flux of nitrogen in the cell. The proteome analysis will elucidate the patterns of interaction with their host and mechanisms used by fungus to capture nutrients, as well as the identification of new molecules potentially related to fungus biology and virulence.

Keywords: Nitrogen starvation, nitrogen uptake, fungi

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