

Title Proteomic analysis of *Paracoccidioides brasiliensis* under nitrosative stress

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Abstract

Paracoccidioides brasiliensis and *P. lutzii* are the causing agents of paracoccidioidomycosis (PCM), a systemic mycosis endemic in Latin America, which is established through conidia inhaling. This fungus is considered a facultative intracellular pathogen, able to survive and replicate inside macrophages. The survival of *P. brasiliensis* in the host depends on fungus adaptation in several, conditions, such as oxidative/nitrosative stress produced by the host immune cells. Currently, there is few knowledge about the *P. brasiliensis* signaling pathways involved in the fungus evasion mechanism of the host defense response. However, it is know that some of these pathways are triggered by reactive oxygen and nitrogen species (ROS/RNS) produced by host cells. This work investigated the modulation profile of redox post-translational modifications (S-nitrosylation and nitration) and the fungus proteomic profile before and after *in vitro* treatment with NO (nitric oxide) donor. It has been demonstrated that low concentrations of GSNO stimulated *P. brasiliensis* proliferation, when compared with no treatment control. On the other hand, high concentrations leads to fungus death. To assess S-nytrosilation and nitration profiles after treatment with different NO concentrations, protein was measured by immunoblotting using a specific anti-nitroso-Cys or anti-Nitro-Tyr antibody. With this approach, we observed distinct band patterns among samples treated with different NO concentrations. S-nytrosilation and nitration levels increased when cells were treated with 0,25 μ M NO, suggesting participation of this post-translational modifications in the fungus growth induced by low concentrations of RNS. Besides, using mass spectrometry, 117 proteins were identified in control group, 206 and 207 in groups treated with 0,25 μ M and 10 μ M de NO, respectively. Proteins found exclusively in treated groups showed distinct profiles. On the group treated with 0,25 μ M of NO were found proteins related with proliferation, transcription and cellular grow events, whereas on the group treated with 10 μ M of NO were found proteins related with virulence and oxidative stress response. Therefore, different concentrations of NO induce distinct redox environment-dependent events.

Key words: *Paracoccidioides brasiliensis*. Nitrosative stess. Proteomics. Nitration. S-nitrosylation .

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