

**TITLE:** S-NITROSO-PROTEOME OF *PARACOCCIDIOIDES BRASILIENSIS* IN RESPONSE TO ACUTE NITROSATIVE STRESS.

**AUTORS:** Navarro, M.V.<sup>1</sup>, Castilho, D.G.<sup>1</sup>, Chaves, A.F.A.<sup>1</sup>, Calado, J.C.P.<sup>1</sup>, Conceição, P.M.<sup>2</sup>, Iwai, L.K.<sup>3</sup>, de Castro, B.F.<sup>2</sup>, Casula, I.<sup>2</sup>, Batista, W.L.<sup>1,2</sup>.

**INSTITUTION:**<sup>1</sup> Department of Microbiology, Immunology and Parasitology, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil. <sup>2</sup> Department of Pharmaceutical Sciences, Universidade Federal de São Paulo, Diadema, São Paulo, Brazil, <sup>3</sup> Laboratory of Applied Toxinology, Center of Toxins, Immune-response and Cell Signaling, Instituto Butantan, São Paulo, SP, Brazil

## **ABSTRACT**

*Paracoccidioides brasiliensis* is the fungus causative of paracoccidioidomycosis (PCM), a systemic mycosis endemic in Latin America. After infection, the survival of the fungus depends on its adaptability to host conditions, such as nitrosative stress caused by nitric oxide (NO) produced by the immune cells. Currently, NO is viewed as a remarkably important signaling molecule, involved in regulating stress responses. Protein S-nitrosylation, characterized by covalent attachment of nitroso group to cysteine thiol, has emerged as a central mechanism of NO-dependent cellular regulation. We have previously reported that low NO concentrations lead to Ras S-nitrosylation and induce cell proliferation in *P. brasiliensis*. However, others targets of NO in this fungus are unknown. In present study, using a combination of the biotin switch assay and label-free LC-MS/MS analysis, we revealed the S-nitroso-proteome of the *P. brasiliensis* under different NO concentration. Using a mass spectrometry-based approach, we mapped 474 S-nitrosylated proteins, where 25 and 30 were exclusively found on the groups treated with 0.25 and 10  $\mu\text{M}$  of  $\text{NO}_2$  respectively. Immunoblotting assay was performed to assess protein S-nitrosylated profiles. Distinct band patterns among samples treated with different NO concentrations were observed. S-nitrosylation levels increased when cells were treated with 0.25  $\mu\text{M}$  NO, suggesting the participation of this post-translational modifications in the fungus growth induced by low concentrations of RNS. Changes in the fungus redox state were also observed. It has been demonstrated that the distinct redox-dependent events (cell proliferation or death) were promoted by different NO concentrations.

**Key words:** Nitrosative stress; *Paracoccidioides brasiliensis*; Proteomics; S-nitrosylation

**Development Agencies:** CNPq and FAPESP