TITLE: RAS PATHWAY REGULATES GENES INVOLVED IN DNA DAMAGE RESPONSE DURING OXIDATIVE STRESS IN *Paracoccidioides brasiliensis*

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ABSTRACT:

Paracoccidioidomycosis is a granulomatous disease caused by fungi of the genus *Paracoccidioides* and widely distributed in Latin America. The disease has high disabling potential and yet is still absolutely neglected. These fungi undergo cell differentiation induced by changes in temperature and this event is critical to the ability to cause disease. The knowledge of which molecular patterns of the fungus may be true virulence factors is still limited by the difficulties inherent to genetic manipulation of these fungi. To overcome these barriers, we need to understand the mechanisms of DNA repair used by the fungus. This is because the establishment of a knockout strain depends on the occurrence of homologous recombination events. The present communication showed that *P. brasiliensis* has a conserved repair machinery to respond to DNA double strand breaks and that this machinery is responsive to oxidative stress. These double-strand breaks repair pathways communicate with the Ras GTPase and Hog1 Map kinase pathways. The genes *PbRAD51* and *PbRAD52* are active elements during DNA repair and participate in the response to oxidative stress in *P. brasiliensis*. In addition, inhibition of the non-homologous end joining (NHEJ) mediated repair pathway has been observed to provide a protective response to *P. brasiliensis* yeasts during a normally lethal oxidative stress condition for the fungus. Perhaps, this event is related to the greater efficiency of the repair by homologous recombination induced by the inhibition of NHEJ. Finally, it was observed that the combination of the damping of the NHEJ pathway, through the inhibition of DNL4 (DNA ligase 4) or GSK3, together with the induced oxidative stress do increase the efficiency of transformation processes in yeasts of *P. brasiliensis*. Indeed, inhibitors of NHEJ pathway caused a protective effect on yeast cells challenged with high concentration of H2O2. This effect correlates with an increase in expression of scavenger genes like catalase and superoxide dismutase. Altogether, these findings broaden the repertoire of possibilities to achieve the establishment of a knockout strain in *P. brasiliensis*.

Keywords: *Paracoccidioides brasiliensis,* DNA repair, RAS-GTPase, Hog-1 MAP Kinase, oxidative stress.

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