

GENE OVEREXPRESSION IN *Paracoccidioides brasiliensis* (Pb18) PROVIDES NOVEL EVIDENCE FOR THE PARACOCCIN ROLE AS A VIRULENCE FACTOR

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The fungi of the genus *Paracoccidioides spp* are etiological agents of paracoccidioidomycosis (PCM), a severe deep mycosis endemic in Latin America. These thermodimorphic fungi develop as filamentous at 25 °C to 30 °C and as yeasts at 35 °C to 37 °C. Few *Paracoccidioides spp* proteins have been fully characterized, Gp43, Pb27, Sod3, Pb40, Hsp60, SconC, Cdc42, PbHAD32 and Pb14-3-3. Our group identified and characterized a *P. brasiliensis* (Pb18) protein, denoted Paracoccin (PCN), a multidomain fungal protein with both lectin and enzymatic activities. It is more expressed in hyphae than yeasts. The system based on *Agrobacterium tumefaciens*-mediated transformation (ATMT) has been used for the genetic manipulation of *P. brasiliensis*. In this work, we used ATMT methodology to obtain PCN-overexpressing *P. brasiliensis* yeasts and took advantage of these transformed organisms to investigate the role of Paracoccin in the fungal biology and relationship with host immune cells. We noticed that: (i) the PCN-overexpressing yeasts exhibit a differential expression of the PCN mRNA compared to WT *P. brasiliensis*; (ii) the increased PCN-expression did not affect the growth nor the viability of yeasts; (iii) the yeast cells size showed differences among the transformants and WT. The size of the mother and daughter yeast cells was not similar in the cultures of PCN-overexpressing or WT yeasts (iv) the PCN-overexpressing clones transitioned more quickly than the WT strains and empty vector transformants; (v) PCN-overexpression is associated with increased susceptibility to phagocytosis by macrophages, whereas the fungicidal activity of these cells was not affected; and (vi) the pulmonary fungal burden was significantly higher in mice that were infected with the PCN-overexpressing yeasts than in mice infected with WT yeasts. The obtained results consists additional evidence of the PCN role as a fungal virulence factor.

Keywords: Paracoccin, *Paracoccidioides brasiliensis*, Paracoccidioidomycosis, PCN-overexpression yeasts

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