THE IMPACT OF PARACOCCIN GENE SILENCING ON *Paracoccidioides brasiliensis* VIRULENCE

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Among the endemic deep mycoses in Latin America, paracoccidioidomycosis (PCM), caused by thermodimorphic fungi of the *Paracoccidioides* genus, is a major cause of morbidity. About 80 % of the reported cases occur in Brazil, with an estimated 3,360 new cases per year. Several recent studies have employed the methodology of gene modulation in *P. brasiliensis* (Pb18) using antisense RNA (AsRNA) and *Agrobacterium tumefaciens*-mediated transformation (ATMT) to identify proteins that influence the fungus virulence. Through this technology, we generated yeasts that were silenced for paracoccin (PCN). Our previous studies suggested that paracoccin (PCN), a multidomain fungal protein with both lectin and enzymatic activities, may be a *P. brasiliensis* virulence factor. These biological activities may potentially explain the role of PCN in fungal cell growth. To explore this, we used AsRNA and ATMT methodology to obtain three independent PCN-silenced *P. brasiliensis* strains (As*PCN1*, As*PCN2*, and As*PCN3*), which were characterized regarding its involvement in *P. brasiliensis* biology and pathogenicity. As*PCN1*, As*PCN2*, and As*PCN3* showed relative PCN expression levels that were 60 %, 40 %, and 60 % of the WT strain, respectively. PCN-silencing led to the aggregation of fungal cells, blocked the morphological yeast to mycelium transition, and rendered the yeast less resistant to macrophage fungicidal activity. In addition, mice infected with PCN-silenced yeasts showed fungal burden reduction by 96 % in comparison to animals infected with the WT strain, which also displayed an extensive destruction of lung tissue. Finally, mice infected with the PCN-silenced yeast strains had lower mortality than those infected with the WT strain. These data demonstrate that PCN acts as a *P. brasiliensis* (Pb18) contributory virulence factor directly affecting fungal pathogenesis.

Keywords: Fungal virulence, Paracoccin, Paracoccin-silencing, Paracoccidioidomycosis, *Paracoccidioides brasiliensis*

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