TITLE: ESTABLISHMENT OF A MUTANT STRAIN FOR *PbKU70* IN THE DIMORPHIC FUNGUS *Paracoccidioides brasiliensis* USING THE *Agrobacterium tumefaciens*-MEDIATED TRANSFORMATION SYSTEM

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ABSTRACT:

The thermo dimorphic fungi of the genus *Paracoccidioides* are the cause of paracoccidioidomycosis (PCM), a disease that mainly affects workers in rural areas, with high prevalence in South America. PCM is a disease with great work-disabling potential and specific virulence factors of the etiological agent are still poorly understood. Recent works have uncovered fungal virulence factors of the genus through antisense RNA technology. Although of great value, the silencing of a gene does not assure the causal role of the gene on the observed phenotype. To establish a causal relationship between the gene and its respective phenotype, it is usually necessary to completely eliminate the gene product and observe the acquired phenotype. Unfortunately, conventional gene knockout strategies still have little effectiveness in fungi of the genus *Paracoccidioides*. One strategy that has proven useful in solving similar problems in other microorganisms is the induction of homologous integration by reducing the expression of the *KU70* gene involved in the non-homologous DNA junction pathway. The protein Ku70 is necessary for the reckoning of DNA double-strand and subsequent progression of non-homologous-end-joint repair pathway. Mutants with high rates of homologous integration have been acquired through this procedure. The genome database *P. brasiliensis* has annotation for the *PbKU70* gene, which makes it a potential target for the strategy. In the present communication, we have established a protocol in order to knockdown the *PbKU70* gene of *P. brasiliensis* using *Agrobacterium tumefaciens* mediated transformation system. We had a success rate of 58% to 74% gene expression reduction. Finally, this mutant was submitted to the mitotic stability test, performed by successive passages in selective medium, and remained growing. This strategy established a mutant lineage, which could serves as a genetic background for knockout studies of other genes. With the possibility of performing knockouts in *P. brasiliensis* genes the functional studies of genes responsible for encoding virulence factors and resistance to the different types of stress inflicted by host cells become more feasible.

Keywords: *Paracoccidioides brasiliensis, Agrobacterium tumefasciens*, *PbKU70*, NHEJ

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