TITLE: MOLECULAR IDENTIFICATION OF PARACOCCIDIOIDOMYCOSIS AGENTS

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ABSTRACT:
Paracoccidioidomycosis (PCM) is a systemic fungal infection caused by etiologic agents belonging to the *Paracoccidioides* genus. The disease is acquired following inhalation of *Paracoccidioides* propagules present in the soil, leading to infections varying between acute to chronic PCM. The disease has a wide distribution in Latin America, being Brazil responsible for ~80% of the cases. Laboratory diagnosis of PCM is traditionally made by combining direct exam, culture, and serological assays. However, these techniques are laborious and time-consuming. To overcome this problem, we propose a PCR-based assay to, not only identify *Paracoccidioides*, but also differentiate between the biological species *P. brasiliensis* complex and *P. lutzii*, thus far contributing to achieving rapid and accurate diagnosis of PCM. The 43,000-Da glycoprotein (gp43/ exon 2) sequences from *P. brasiliensis* and *P. lutzii* were retrieved from GenBank and used for *in silico* screening to identify parsimony informative regions (that were divergent interspecifically and conserved intra-specifically), which could be used for primer design. Two species-specific pairs of primers were designed, one for each *Paracoccidioides.* Primers candidates were validated *in silico* using Primer-BLAST software and no amplicon was obtained from other pathogenic or non-pathogenic fungi (Fungi taxid4751), but *Paracoccidioides*. These pairs of primers were tested *in vitro* as singleplex PCRs using 48 *P. brasiliensis* and 13 *P. lutzii* isolates, with high specificity (100%) and sensitivity (100%). Judging from the different amplicon sizes (308 bp for *P. brasiliensis* and 142 bp for *P. lutzii*), we optimized a duplex PCR-assay combining both pairs of primers, to identify *Paracoccidioides* agents in a single round reaction. All samples evaluated were positive for each species-specific pairs of primers using a duplex-PCR assay, which matched the results obtained by the DNA barcoding ITS1/2+5.8s region amplification and sequencing (very good agreement; *Kappa* value = 1.0). Our duplex-PCR assay showed the applicability of this method, which exhibited an absolute specificity (100%) for the two species and a high sensitivity (100%) even down to low DNA concentrations (1 pg) in a single round reaction. We demonstrated that our PCR-assay has the potential to aid the diagnostic of PCM agents with high accuracy and efficiency, being an inexpensive and fast molecular tool.

Keywords: *Paracoccidioides brasiliensis*, *Paracoccidioides lutzii*, paracoccidioidomycosis, molecular diagnosis, duplex PCR-assay

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