

## SPECIFIC PRIMERS FOR THE DETECTION OF PARACOCCIDIOIDES BRASILIENSIS

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Paracoccidioidomycosis is a prevalent systemic mycosis in rural areas of Latin America whose causal agent is *Paracoccidioides brasiliensis* [see review, 1]. The 43 kDa glycoprotein (gp43), a reference *P. brasiliensis* antigen, has been used in serological tests, with highly sensitive results, although cross reactions occasionally happen [2]. RAPD has been used for the molecular characterization of *P. brasiliensis* isolates [3]. When using OPG18 (Operon Biotechnology) as arbitrary primer, two specific DNA fragments (Mw 0.72 and 0.83 Kb), common to and specific for all *P. brasiliensis* samples, are found [3, 4]. We report specific primers derived from the DNA fragments described above, which may be of value for the identification of *P. brasiliensis* strains and of potential use for epidemiological and clinical applications.

DNA from *P. brasiliensis* was extracted [3] and used for PCR assays. They had the following sequences: **MG3 S** (5'- TCT CCA TAC CTG ATG TTG GT-3') and **MG3 AS** (5' – TCG AAG ACA CTC TCC CGT CT – 3') (both originated in the 0.83 kb PCR fragment); **MG2(1)F** (5' – GGG ATT CCC TAG GCA AAC ACT TGT GTG A – 3'); **MG2(1)R** (5' – GTG CAG TTA TCC ACA AGC CAT ATA TTC – 3'); **MG2(2)F** (5' – GGA GAT GAT CTG ACG TTA GTA CGT GAT G – 3') and **MG2(2)R** (5' – ATG CTA ATT TAT GTC ATT CCG CGT CTG – 3') (originated in the 0.72 kb PCR fragment). Southern-blot analysis was performed with a radiolabeled 0.72 kb fragment previously reported [3] as probe.

When using primers MG3 S and MG3 AS in PCR assays, *P. brasiliensis* DNA samples from 33 strains produced a unique 670 bp DNA fragment, in concentrations as low as 1 pg. However, Southern blots produced cross reacting signals with *Histoplasma capsulatum*. Primers MG2(1)F/MG2(1)R and MG2(2)F/MG2(2)R were specific against a panel of fungi, using the 0.72 kb probe [3], although the sensitivity was lower (100 pg and 10 pg for MG2(1)F/MG2(1)R and MG2(2)F/MG2(2)R, respectively). Higher sensitivity was obtained by performing nested PCR with external primers MG2(1)F/MG2(2)R and inner primers MG2(2)F/MG2(1)R, or semi-nested PCR, with a detection limit of 1 pg, although cross-reaction with *H. capsulatum* was also seen. Therefore, specific primers MG2(2)F/MG2(2)R could be of use for the detection of *P. brasiliensis* in a single step PCR, with a detection limit of 10 pg. Its possible use for clinical purposes is currently under study.

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