TITLE**: Multiplex PCR as improvement to detect *Histoplasma capsulatum* mating types**

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ABSTRACT

Histoplasma capsulatum is a dimorphic fungus with worldwide distribution, which in environmental sites or when cultivated in laboratory at 25°C grows in its filamentous phase. When cultivated at 37°C in specific culture media or when in parasitism, it grows in yeast form. Histoplasmosis, the systemic mycosis caused by *H. capsulatum*, is initiated when humans or other mammals inhale the propagules of the filamentous phase that reach the pulmonary alveoli; rapidly these structures convert to yeast form. *H. capsulatum* is a heterothallic ascomycete which has a sexual and asexual stage. The asexual stage has two compatibility types (mating types), which are codified by the MAT1 locus, with two idiomorphic regions: MAT 1-1 and MAT 1-2, or positive and negative (+ and –, respectively). It has been suggested that the strains of each mating type are not equally represented among clinical isolates, with frequently a ratio of 7:1 (−/+); however, environmental samples exhibit a 1:1 ratio. Molecular methods are used to discovery *H. capsulatum* mating types, using specific primers to the idiomorphic regions MAT 1-1 or MAT 1-2, which has been carried out separately. The aim of this study was to perform a multiplex PCR to detect both mating types at once. For the experiments, it was used two reference strains, a MAT 1-1 G217B (accession number EF433757) and a MAT 1-2 G186AR (accession number EF433756), both with mating type sequences deposited in GenBank. An *in silico* assay was performed using FastPCR software, which displayed no primers annealing to non-specific sequences. After this analysis, an *in vitro* assay was performed to evaluate the possibility of intercurrences, using four primers in a single multiplex reaction. At the end of the *in vitro* step, no intercurrences was observed in the reaction, and thus, the reference strains displayed just specific fragment amplification and absence of double bands. Thus, the use of a two-pair primers in a multiplex reaction decreases the expense of reagents, and has given reproducible results. Using a single reaction, there is an improvement in the time spent for results analysis, which has been reduced by 50%. Thus, in conclusion, this initiative consisted in a successful, less laborious methodology for mating types analysis, besides saving reagents reflecting in economy on laboratorial costs.

Keywords: *Histoplasma capsulatum*, mating type, Multiplex-PCR

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