IDENTIFICATION OF ENVIRONMENTAL *CRYPTOCOCCUS LAURENTII* ISOLATES BY ITS BARCODING

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Abstract

Currently, Cryptococcosis is the leading invasive fungal infection around the world and affects several hosts. Only *Cryptococcus neoformans* and *Cryptococcus gattii* are recognized as pathogenic species, however, other saprophytes species, such as *Cryptococcus laurentii*, have been associated to infections, especially in immunocompromised patients. Although few studies have been carried out to identify *C. laurentii*, it was already demonstrated high intraspecific variability which has stimulated the use of molecular biology techniques aiming its best identification. The aim of this study was to evaluate environmental isolates by sequencing of the Internal Transcribed Spacer (ITS) region. One hundred environmental isolates phenotypically identified as *C. laurentii* from different countries were evaluated by sequencing of the ITS region. Four different species were identified whereas eight isolates remained characterized only at the genus level. Of 100 isolates, 75% were confirmed as *C. laurentii*, 10% as *Cryptococcus terrestris*, 6% as *Cryptococcus flavescens* and 1% as *Cryptococcus rajasthanensis*. The *C. laurentii* isolates were separated into four major groups with intraspecific variability of up to 2.3%, and *C. rajastahnensis* was the phylogenetically closest species. The *C. flavescens* isolates were separated into three groups and presented an interspecific variability of up to 2.6% when compared with its closest species, *C. terrestris*. The highest interspecific divergence of 17.9% among isolates of group I of *C. flavescens* and *Cryptococcus* sp. was evidenced. The mean interspecific divergence was 12.1%, while the mean intraspecific variability was 0.35%. The growing number of immunocompromised individuals during the last decades has led to an increase of fungal opportunistic infections worldwide. *Cryptococcus laurentii* share phenotypic characteristics with cryptic species and has been associated to 25 cases of cryptococcosis. The intraspecific variability observed among 100 isolates of *C. laurentii* is consistent with the first ITS Barcode study of this species. Furthermore, we demonstrated the existence of different cryptic species among isolates phenotypically identified as *C. laurentii*, which were differentiated by ITS sequencing, confirming its applicability as a DNA Barcode for Fungi.

Keywords: *Cryptococcus laurentii*, ITS Barcode, genetic variability

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