

Title: IDENTIFICATION AND DIFFERENTIATION OF *Candida parapsilosis* COMPLEX SPECIES BY PCR-RFLP OF THE ITS REGION of rDNA

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Abstract:

Nowadays, it has been accepted that there are three species formerly grouped under *Candida parapsilosis*: *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis*, and in fact, the antifungal susceptibility profiles and distinct virulence attributes, demonstrate the difference among these species. Accurate, faster and economical fungal species identification has been the main goal in mycology, especially if a species complexes are involved. Therefore, the aim of this study was the development of a new molecular method to differentiate the *C. parapsilosis* complex species. The reference strains of *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis* sequences available in GenBank database was searched in order to identify complete sequence for ITS1-5.8S-ITS2 region, comprising the complete forward and reverse primers ITS1 and ITS4. The primers and the sequences selected were used *in silico* PCR amplification on the FastPCR version 6.0 software to determine the length of the products, and then this product were subjected RFLP with the restriction enzymes in the database of pDRAW32 DNA analysis software version 1.1.125. *HhaI* and *Sau96I* were selected for RFLP analysis based on the presence of at least one cleavage site and on generation of three fragments that should be distinguished on a conventional agarose gel electrophoresis. The gel electrophoresis produced 117, 178, and 225 bp fragments for ATCC 22019 (*C. parapsilosis*); 102, 183, and 225 bp fragments for ATCC 96141 (*C. orthopsilosis*); and 114, 187, and 228 bp fragments for ATCC 96143 (*C. metapsilosis*). Ninety-eight clinical *C. parapsilosis sensu lato* strains isolated from the bloodstream (blood and catheter) of critically ill patients were included in this work. *C. parapsilosis* (ATCC 22019), *C. orthopsilosis* (ATCC 96141), and *C. metapsilosis* (ATCC 96143) were used as reference strains. The molecular identification by PCR-RFLP characterized all 98 clinical isolates, 59 isolates were identified as *C. parapsilosis* sensu stricto, 37 as *C. orthopsilosis*, and two as *C. metapsilosis*, which is in agreement with the results of DNA sequencing of the D1/D2 region of the 28S rDNA gene previously described. This methodology is appropriated for early identification of this yeast complex, mainly for hospitalized patients allowing the early diagnosis and antifungal therapy.

Keywords: *Candida parapsilosis* complex, bloodstream infections, PCR-RFLP

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