Title: Phylogenetic analysis and haplotype network of the rDNA ITS regions from *Candida* species clinical isolates

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ABSTRACT

Due to some *Candida* species present few morphological variations, the use of molecular biology techniques can be an alternative to overcome the limitations of phenotypic identification methods. Molecular systematics of fungi is based mainly on the analysis of ITS regions 1 and 2 (Internal Transcribed Spacer) of the ribosomal DNA, which is also widely used in order to establish the evolutionary relationships, contributing in epidemiological research. In this sense, the objective was to establish the phylogenetic relationship among clinical isolates of *Candida* spp through the amplification of the rDNA ITS region to contribute to the understanding and epidemiological research. PCR amplification was performed for ITS regions. Forward and reverse sequencing of the fragments was performed with an ABI 3500 automated DNA sequencer using the original PCR primers. The quality of each electropherogram was evaluated using BioEdit and consensus sequences were obtained using CAP3 software. The assembled sequences were compared with the sequences deposited in GenBank and thereafter they were aligned in ClustalW2. The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap (MEGA 6.0). Also haplotype networks were constructed using the median-joining method to analyze the relationship between haplotypes generated by the Network 4.1.1.2. The phylogenetic tree constructed presented bootstrap values ≥ 70% in most of the nodes. Different clausters were found for species *C. tropicalis, C. albicans, C. guilliermondii, C. parapsilosis, C. lusitaniae, C. kefyr, C. krusei* and *C. glabrata*. With the pair estimated track it could be noted that the number of nucleotide substitutions occurring in each of the sequences for the ITS region, since the divergence of the sequence thar originated the remaining sequences compared, 40% of the residues changed on average demonstrating that this region has conserved sites, but some of them develop through the accumulation of substitutions and the number of differences between them may indicate the time that they diverged from a common ancestor. The same can be observed in the analyzed haplotype networks, which showed different patterns of *Candida* spp rDNA. The study suggested the utility of this region for identification of different haplotypes, which may contribute to understanding the evolutionary history of this gene, and reinforces the application of ITS region as a bar code to clinically relevant fungi.

Keywords: yeast, ITS, evolutionary history, DNA barcoding, molecular identification

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