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MOLECULAR PROFILE OF ORAL *CANDIDA ALBICANS* ISOLATES FROM HIV-INFECTED PATIENTS AND HEALTHY PERSONS

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ABSTRACT

Candida species are part of the normal microbiota of most people, but can also be responsible for infections, acting mainly as opportunistic microorganisms. The typing of *Candida* isolates is important for hospital epidemiology studies. The aim of this study was to analyze the molecular profile of *Candida albicans* isolates from the oral cavity of individuals with and without HIV and verify genetic relationships between them using the RAPD-PCR methodology. Two different groups, containing a total of 116 *C. albicans* isolates, were analyzed. Group 1 contained 75 isolates from saliva samples from HIV patients with no clinical signs of oral candidiasis, and Group 2 contained 41 isolates from healthy volunteers without HIV. Every isolate was typed by RAPD-PCR using the random primers OPA9 and OPB11. The genotypic analysis showed 33 different genotypes defined by OPA9 and 34 by OPB11. The primer OPA9 defined 26 genotypes with the isolates from Group 1, and 21 genotypes from Group 2. This study reveals that the genetic diversity of *C. albicans* isolates from HIV-positive individuals may be more complex than in seronegative individuals.

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INTRODUCTION

Some species of *Candida* are part of the normal microbiota of more than half the healthy human population, where they colonize the digestive and genitourinary tracts (Kam and Xu, 2002). However, these species are responsible for infections in immunocompromised individuals (Sant`Ana *et al.*, 2002).

The colonization of the oral cavity occurs in approximately 40–60% of healthy individuals; however, it is a major indicator of the development of oral candidiasis or systemic infection in patients with Acquired Immunodeficiency Syndrome (AIDS) (Campisi *et al.*, 2002). Among HIV-positive individuals, 45% to 88% present oral colonization by

Candida species (Sant'Ana *et al.,* 2002; Delgado *et al.,* 2009; Berberi *et al.,* 2014). Isolates of *Candida* spp. exhibit high phenotypic and genotypic variation, and this feature enables the application of different methods in order to characterize and differentiate strains of diverse origins. The study of phenotypic and/or genotypic variation is generally used in epidemiological studies, for example, to confirm an infectious outbreak and characterize the responsible species of *Candida*, thereby assisting in the adoption of measures aimed to eliminate and prevent the spread of the infectious agent (Bonfim-Mendonça *et al.,* 2013; Marcos-Zambrano *et al.,* 2014). However, the physiological and biochemical methods are less efficient when compared to molecular methods, which are generally more sensitive, effective and often faster (Candido *et al.,* 2000; Bonfim-Mendonça *et al.,* 2013; Marcos-Zambrano *et al.,* 2014; Soll, 2000).

The molecular methods used to characterize the clinical isolates of *Candida* spp. have variable sensitivities. The most commonly used methods are pulsed field gel electrophoresis (PFGE), DNA fingerprinting, multilocus enzyme electrophoresis (MLEE), electrophoretic karyotyping (EK), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) with and without hybridization, restriction enzyme analysis (REA), microsatellites or simple sequence repeats (SSR) and multilocus sequence typing (MLST) (Bonfim-Mendonça *et al.,* 2013; Saghrouni *et al.,* 2013; Katiraee *et al.,* 2014; Li *et al.,* 2015). Each one of these techniques has specific characteristics that should be considered depending on the aim of a particular study. The RAPD-PCR methodology uses random primers for amplification, and does not depend on knowledge of specific genomic sequences of the species studied (Valerio *et al.,* 2006). This technique has been used to investigate the genetic relationship between strains of *Candida* spp. obtained from various anatomical sites in an attempt to relate them with pathogenesis and epidemiological characteristics of the infection, when further phenotypic differences may not be evident (Costa *et al.,* 2010).

The purpose of this study was to analyze the molecular profile of *Candida albicans* isolates from the oral cavity of individuals with and without HIV infection using the RAPD-PCR methodology.

MATERIALS AND METHODS

Isolates

One hundred and sixteen isolates of *C. albicans* were included in the study. Seventy five of these were obtained from saliva samples from HIV-positive patients with no clinical signs of oral candidiasis (Group 1), which were attended at the Clinical Hospital of the Federal University of Uberlandia, Uberlandia, Minas Gerais, Brazil. The other 41 were isolated from healthy volunteers without HIV (Group 2). The identification of *C. albicans* isolates was confirmed by polymerase chain reaction (PCR) using specific primers, according to Estrada-Barraza et al. (2011) and Luo and Mitchell (2002). The isolate of *C. albicans* ATCC 90028 was included in the tests as a control.

The study was approved by the Ethics Committee on Human Research of the Federal University of Uberlandia (protocol n. 368/11).

Genomic DNA extraction and genotyping by RAPD-PCR

The extraction of genomic DNA from each isolate was taken from colonies grown on Sabouraud Dextrose agar, incubated for 24 hours at 35°C according to Bolano et al. (2001).

RAPD-PCR tests were performed using the primers OPA9 (5' GGGTAACGCC 3') and OPB11 (5' GTAGACCCGT 3') (Ludwig Biotec, Alvorada, RS, Brazil). Reactions were performed in a final volume of 25 uL containing 50 ng of template DNA, 0.25 mM of each deoxynucleotide (dATP, dCTP, dGTP and dTTP; Ludwig Biotec, Brazil), 1U of Taq polymerase Platinum™ (Invitrogen, Carlsbad, California, USA) with the appropriate reaction buffer, added to 3.5 mM of $MgCl_2$ and 40 pmol of primers. The PCR reaction included an initial amplification cycle of 3 minutes at 94ºC, followed by 40 cycles of: 94ºC for 45 seconds, 36ºC for 45 seconds and 72ºC for 1 minute and 30 seconds, plus 10 min at 72°C and maintained at 4ºC. The amplified products were submitted to agarose gel electrophoresis (1.5%) containing 5µL/ml of the fluorescent dye EasyView (EasyPath, São Paulo, SP, Brasil). The gels were observed under ultraviolet light (L-Pix HE, Loccus Brazil, Cotia, SP, Brazil) and images were captured using the photodocumentation system (Image Lab-1D Loccus Brazil, Cotia, SP, Brazil). The experiments were carried out in duplicate.

The size of each band formed after RAPD-PCR gel electrophoresis was compared to the molecular standards (100 bp, Ludwig Biotec, Alvorada, RS, Brazil). The profile of each sample (fingerprinting) was visually examined, and the bands were encoded as present (1) or absent (0). RAPD profiles obtained for each isolate (strain) with the respective primer were used for the construction of dendrograms. These data allowed the construction of binary matrices that were analyzed using the Multi-variate Statistical Package (MVSP version 3.2). The genetic relationships (similarity coefficients) were calculated using the Jaccard coefficient (Sj). The value Sj 1.00 and 0.99 indicates the same genotype, values between 0.80 and 0.99 indicate clonally related isolates (highly similar, but not identical), and values below 0.80 mean different samples. Based on Sj values, the dendrograms were generated using the UPGMA method (unweighted pair-group method with arithmetical averages).

RESULTS

The genotypic analysis of the 116 isolates of *Candida albicans* by RAPD-PCR methodology showed 33 different genotypes with the primer OPA9 and 34 with the primer OPB11 (Table 1). The primer OPA9 formed 26 genotypes with the isolates from Group 1 and 21 genotypes from Group 2. Of all 33 genotypes identified by primer OPA9, 14 genotypes included isolates from both of the two groups of subjects. The primer OPB11, however, formed 22 genotypes with both groups. Of all 34 genotypes identified by primer OPB11, 10 were shared by both groups (Table 1). Figure 1 illustrates the genetic variation of the isolates in Group 1, showing the bands obtained with *C. albicans* isolates of HIV–infected individuals. The dendrograms are showed in Figures 2 and 3. The number of isolates per cluster varied from 1 to 8 in Group 1 and from 1 to 5 in Group 2 for both primers OPA9 and OPB11.

DISCUSSION

The genetic variability of *C. albicans* has been shown through the application of various molecular tools, including RAPD-PCR (Pinto *et al.,* 2004; Saghrouni *et al.*, 2013). The present study analyzed isolates of *C. albicans* from the oral cavity of individuals with HIV (Group 1) and non-carriers of HIV (Group 2), using two random primers.

Table 1. Frequency of clusters formed by *C. albicans* isolates with primers OPA9 and OPB11

Primer	Number of Genotypes	Group	Genotypes		
			Per cluster	Shared between groups	Exclusive for each group
OPA9	33	G1	26	14	12
		G2	21		7
OPB11	34	G1	22	10	12
		G2	22		12

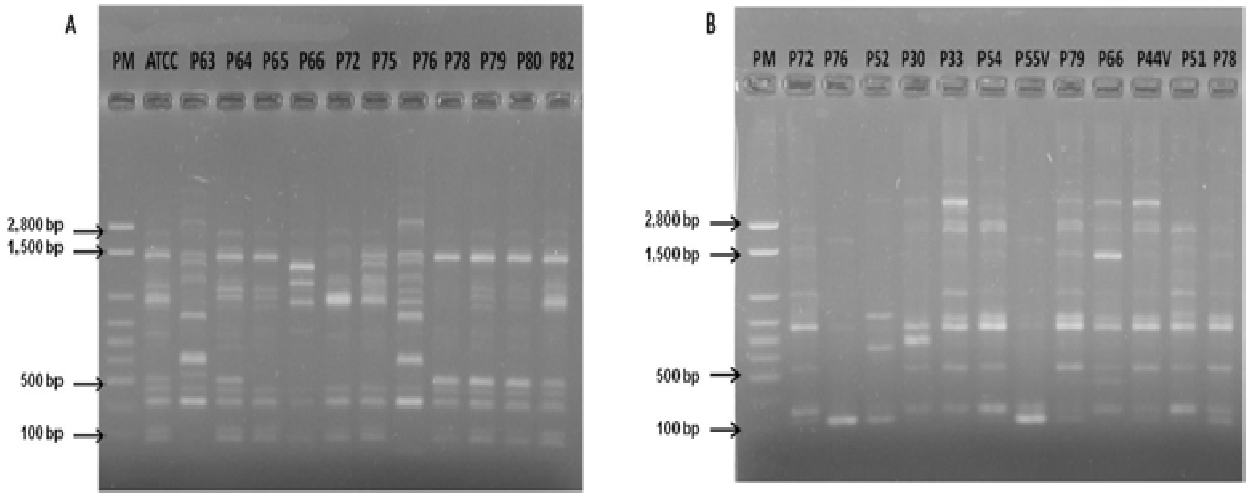

Figure 1. Genetic variation within C. albicans isolates from HIV-positive patients, as determined by RAPD-PCR analysis using random primers OPA9 (A) and OPB11 (B). Column 1 - PM: molecular size standard 100 bp; column 2 - ATCC: strain 90028; in the other columns: strains of the patients

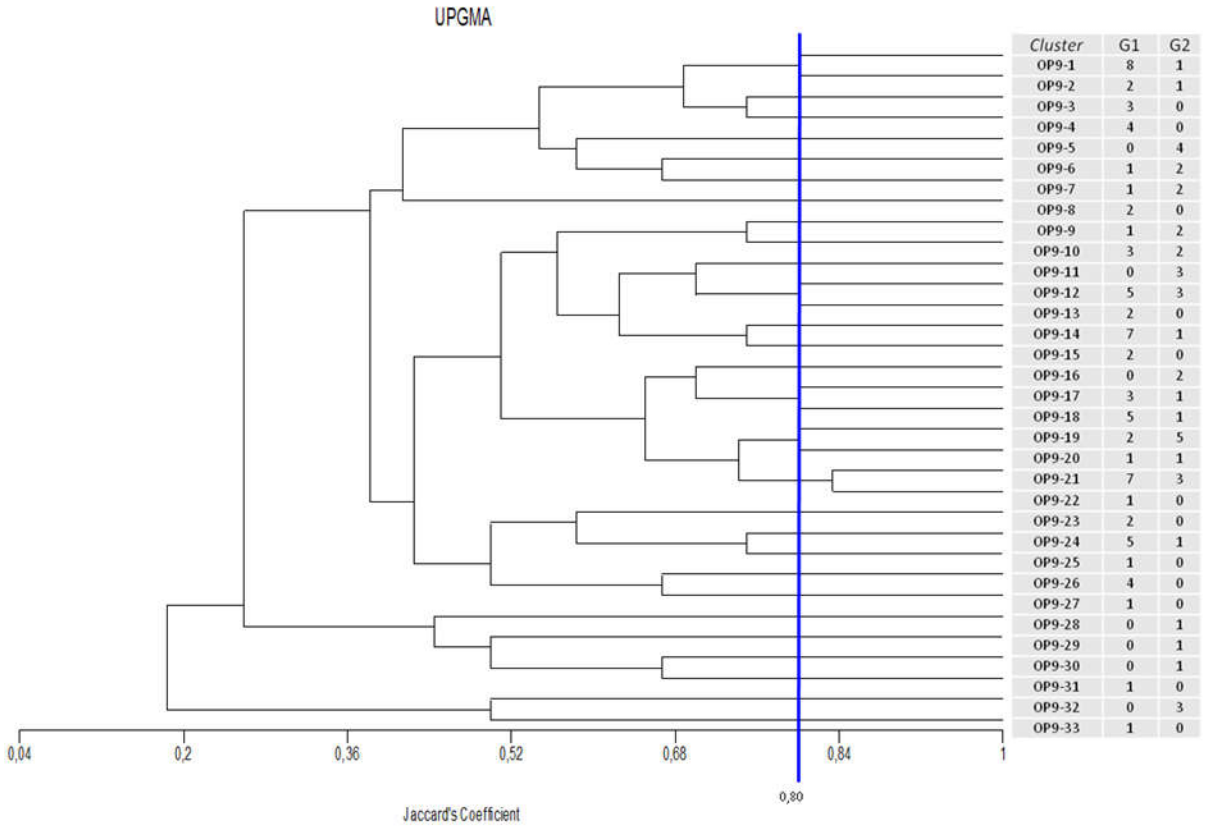

Figure 2. Dendrogram of *C. albicans* isolates from HIV-positive patients and individuals without HIV, using primer OPA9. The genetic similarity coefficients are identified in the horizontal line. *Cluster*: refers to isolates with 100% genetic similarity; G1 and G2: refers to the number of isolates belonging to the respective *cluster*; G1: Group 1 (HIV-positive individuals); G2: Group 2 (HIV-negative individuals)

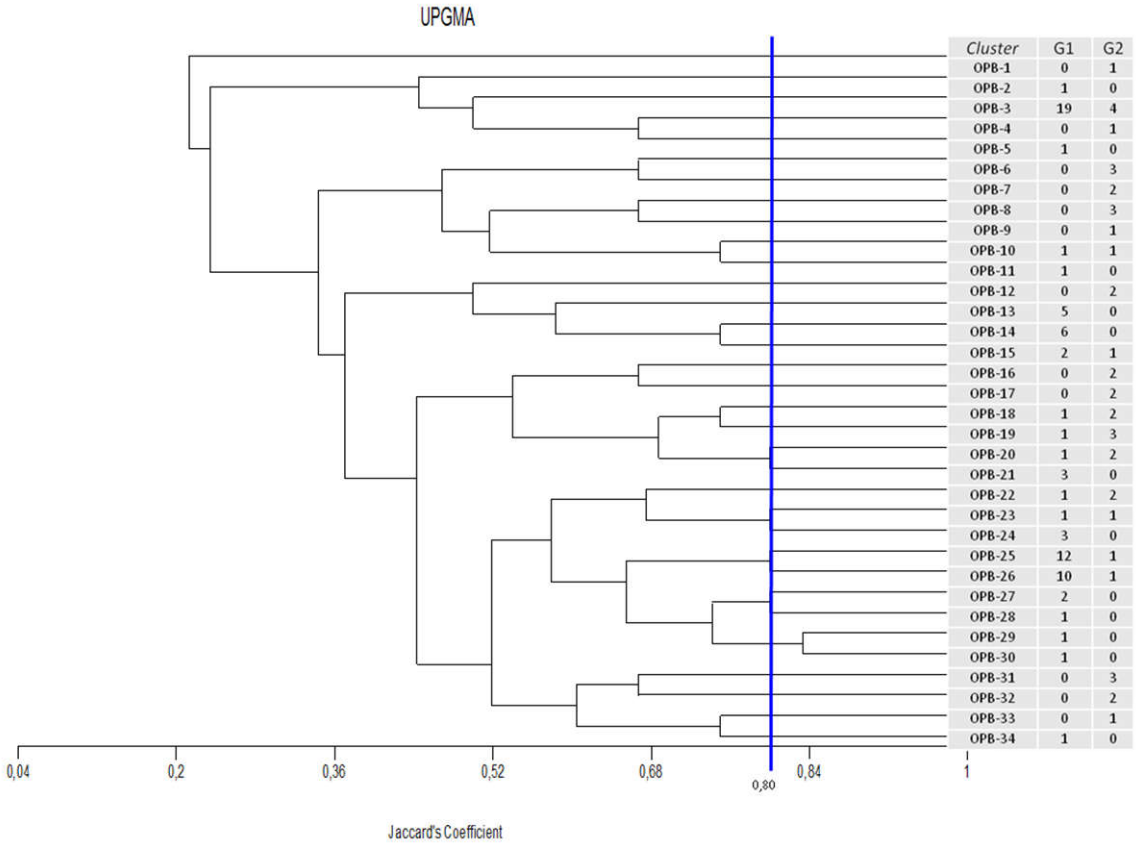

Figure 3. Dendrogram of *C. albicans* isolates from HIV-positive patients and individuals without HIV using primer OPB11. The genetic similarity coefficients are identified in the horizontal line. *Cluster*: refers to isolates with 100% genetic similarity; G1 and G2: refers to the number of isolates belonging to the respective *cluster*; G1: Group 1 (HIV-positive individuals); G2: Group 2 (HIV-negative individuals)

According to Saghrouni et al. (2013) nosocomial candidiasis can be investigated by genotyping *C. albicans* strains with the aim of identifying the strains related to outbreaks, identifying the origin of an infection, determining routes of acquisition and transmission, and monitoring the emergence of strains resistant to antifungal drugs. The main advantages of RAPD analysis are related to the need for little materials, speed of execution, relatively low cost after implantation of the methodology in the laboratory, and versatility in epidemiological studies (Valerio *et al.,* 2006; Giolo and Svidzinski, 2010). The possible variations of the parameters used are among the disadvantages of the method, as the concentration of primers, differences in temperature cycles of PCR, and concentration of magnesium in the reaction may affect the interpretation and comparison of results between different studies (Boriollo *et al.,* 2005). However, the technical expertise of laboratory technicians and standardization of internal reaction conditions and reagents allow good reproducibility in the laboratory.

The choice of primers is crucial to discriminate or differentiate genotypes of different isolates (Bacelo *et al.,* 2010). The analysis of 116 isolates of *C. albicans* in this study showed great genetic variability among them (33 genotypes were evidenced with the primer OPA9 and 34 with OPB11). In our study, isolates of Group 1 showed a higher number of genotypes (26 genotypes) with the primer OPA9 than Group 2 (21 genotypes). This suggests that HIV-positive individuals can be colonized by a greater diversity of *C. albicans* strains, but this needs to be confirmed by other studies, including other primers and more individuals.

These types of studies still have the opportunity to associate the different genotypes with virulence factors or resistance to antifungal drugs (Barros *et al*., 2008). The interpretation of the RAPD-PCR results can be difficult and complex depending on the aims of the study, and becomes more complex as the number of isolates studied increases, and the largest number of primers included. However, RAPD-PCR may be an important tool in studies that aim to type Candida isolates. Future research will open opportunities to investigate isolates grouped under the same molecular profiles for antifungal resistance and will aid in the search for new drugs without the need to include large numbers of isolates. The results of this study contribute to the understanding of the molecular epidemiology of C. albicans circulating among HIV-positive individuals, revealing that the genetic diversity of isolates from these patients may be more complex than in HIV-negative individuals.

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