ARTIGO ORIGINAL

RAPD PROFILE AMONG *Candida albicans* ISOLATES BY USING DIFFERENT PRIMERS

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ABSTRACT

By using RAPD method, a total of 28 primers were screened for discrimination among *Candida albicans* isolates. Seven of these primers were selected because they presented reproducible DNA banding patterns for all the strains of *C. albicans*. The same genetic profile was obtained by RAPD method using OPG 14 (5'-GGATGAGACC-3') while the OPG 17 (5'- ACGACCGACA-3') demonstrated capacity to differentiate the isolates of *C. albicans* in 4 genotypes. According to the results obtained, we concluded that by using these primers, the RAPD analysis may be useful in providing genotypic characteristics for *C. albicans* in epidemiological investigation.

KEY WORDS: *Candida albicans.* Profile. RAPD.

INTRODUCTION

The incidence of *Candida* infections has markedly increased over the last decades, particularly among hospitalized patients and immunocompromised hosts (10, 12) and the most common etiological agent is *Candida albicans* (16, 18). Many different methods have been utilized to differentiate *C. albicans* isolates to assess a variety of epidemiological questions regarding this organism. Advances in molecular biology have allowed the use of fingerprinting methods such as karyotyping using pulsed field gel electrophoresis (PFGE), restriction fragment

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Received for publication in: 28/9/2009. Revised form in : 4/1/2010. Accepted in: 10/2/2010.

length polymorphism (RFLP), randomly amplified polymorphic DNA analysis (RAPD), and Southern hybridization with moderately repetitive DNA probes for detection and typing of *Candida* strains and for differentiation of the species (3, 7, 8, 15, 17, 22). The RAPD method is less time consuming, easy to apply and it has been used by several investigators to detect inter-strains variation in pathogenic yeasts and for *Candida* species identification (19, 20, 24).

In this work we investigated whether 28 different primers were able to amplify and to characterize *C. albicans* isolates using the RAPD method.

MATERIALS AND METHODS

Patients and *C. albicans* isolates

A total of 14 *C. albicans* isolates designated as Ca 21, 24, 35, 38, 46, 47, 58, 60, 69, 71, 72, 75, 83, and 91 were recovered from oral mucosa of 14 AIDS patients, under care at the Hospital for Tropical Diseases (HDT) in Goiânia, Goiás state, Brazil. The subjects were diagnosed as group IV according to the criteria defined by the CDC (Center for Disease Control and Prevention, Atlanta, USA). All the participants agreed with the research and the study was approved by the Bioethics Committee of HDT. Material from lesions of mouth was cultured on Sabouraud dextrose agar and the yeast isolates were identified as *C. albicans* by the production of characteristic germ tubes, development of chlamydospores in corn meal agar containing Tween 80, and assimilation and fermentation of carbon sources, as recommended by Kurtzman & Fell (11). Identification was confirmed by API 20C Aux System for yeasts (BioMerieux, Marcy L' Etoile, France). *C. albicans* ATCC 10231 and *C. parapsilosis* 22019 were included as controls. Additional tests as cultivation on CHROMagar Candida medium (CHROMagar, Paris, France), growth at 45°C on agar Sabouraud dextrose and in hypertonic Sabouraud broth, and assimilation tests using xylose and α-methyl-D-glucoside (6, 9) were used to help us to discriminate between *C. albicans* and *Candida dubliniensis*.

DNA extraction

DNA was extracted as described by Sherer & Stevens (21). Briefly, yeasts cells were cultured on YEPD broth (1% yeast extract, 2% peptone, 2% dextrose) and incubated overnight at 37°C with shaking (240 rpm). The yeast cells were collected by centrifugation, suspended in 1 ml of 1M sorbitol-50mM phosphate buffer (pH 7.5) containing 2% of β-mercaptoethanol (Sigma Chemical Co, St Louis, MO) and 2 mg of yeast lytic enzyme (lyticase, Sigma). After 1h of incubation at 37°C, the suspension was centrifuged and the pellet was suspended in 0.5 ml of 50 mM EDTA (pH 8.0)-0.2% sodium dodecyl sulfate, and incubated at 70°C for 30 minutes. After the addition of 5 M potassium acetate, the suspension was left at 0°C for 30 minutes

and then centrifuged. The supernatant was treated with RNase and the DNA was extracted with an equal volume of chloroform isoamyl alcohol and precipitated with cold ethanol. Finally, the DNA was dissolved in 100 μL of TE buffer.

Randomly Amplified Polymorphic DNA analysis

Twenty eight random primers from Operon Technologies (Biotechnologies), OPG 01 (5'-CTACGGAGGA-3'), OPG-02 (5'-GGCACTGAGG-3'), OPG-03 (5'-GGCACTGAGG-3'), OPG-04 (5'-AGCGTGTCTG-3'), OPG-05 (5'-CTGAGACGGA-3'), OPG-06, (5'-GTGCCTAACC-3'), OPG-07 (5'-GAACCTGCGG-3'), OPG-08 (5'-TCACGTCCAC-3'), OPG-09 (5'-CTGACGTCAC-3'), OPG-10 (5'-AGGGCCGTCT-3'), OPG-11 (5'-TGCCCGTCGT-3'), OPG-12 (5'-CAGCTCACGA-3'), OPG-14 (5'-GGATGAGACC-3'), OPG-15 (5'-ACTGGGACTC-3'), OPG-16 (5'- AGCGTCCTCC-3'), OPG-17 (5'-ACGACCGACA-3'), OPG-18 (5'-GGCTCATGTG-3'), OPG-19 (5'-ACGACCGACA-3'), OPG-20 (5'-TCTCCCTCAG-3'), OPO-01 (5'-GGCACGTAAG-3'), OPO-02 (5'-ACGTAGCGTC-3'), OPO-03 (5'-CTGTAGCGTC-3'), OPO-04 (5'-AAGTCCGCTC-3'), OPO-05 (5'-CCCAGTCACT-3'), OPO-06 (5'-CCACGGGAAG-3'), OPO-08 (5'-CCTCCAGTGT-3'), OPO-13 (5'-GTCAGAGTCC-3'), OPC-01 (5'-TTCGAGCCAG-3') were used in RAPD analysis for 14 *C. albicans* isolates. The isolate of *C. albicans* ATCC 10231 was included in this study.

Amplification reactions were performed in a mini cycler (Perkin-Elmer Cetus). The RAPD was performed as follows: a reaction volume of 25 μL contained each 0.2 mM deoxynucleoside triphosphate, 2.5 mM magnesium chloride, 1 μM of primer, *Taq* buffer, 0.5 U of *Taq* polymerase (Invitrogen), and 50 ng of candidal DNA as template. The first five cycles included 2 minutes of denaturation at 98ºC, 2 minutes of annealing at 50ºC and 1 min of primer extension at 35ºC, followed by 35 cycles of 1.5 minutes at 92ºC, 1.0 min at 35ºC, 2.5 min at 92ºC and the final extension step was prolonged at 72ºC for 5 min. The resulting DNA fragments were separated through a 0.8% agarose gel stained with ethidium bromide, and then visualized over a source of UV light and photographed. The sizes of the DNA fragments were determined by comparison with a molecular weight marker (λ/Hind III). All the isolates were typed twice to assess the reproducibility of this technique.

The DNA fragment profiles were analyzed by the presence or absence of amplification product in agarose gels. To do this conversion, DNA fragments were labeled based on their migration in the gel, usually starting from the top to the bottom. The criterion used for genotyping was the difference in banding positions of the isolates in each of the RAPD assays. Bands were included in the analysis, if they were visible, regardless of their intensity.

RESULTS

Among the 28 primers analyzed, seven were selected, OPG-02; OPG-05; OPG-11; OPG-14; OPG-16; OPG-17; and OPG-18, because they presented reproducible DNA banding patterns for all the strains of *C. albicans*. The same genetic profiles was obtained by RAPD method using OPG 14 (5'-GGATGAGACC-3'), while the OPG 17 (5'- ACGACCGACA-3') produced multiple banding patterns with fairly equal intensities eliciting 4 different genotypes. The RAPD profiles obtained with the primers OPG 14 and OPG 17 are showed in Figures 1 and 2. OPG 02, OPG 05, OPG 11, OPG 16, and OPG 18 showed only two genotypes among all the *C. albicans* isolates.

The seven primers yielded RAPD profiles that ranged from 100 to 1800 base pairs (bp) for 14 clinical *C. albicans* isolates. Interestingly, we observed that in all isolates bands of approximately 600 bp and 450 bp were present (conserved) for all primers analyzed. The strain *C. albicans* ATCC 10231 showed profile similar to OPG 14 (data not shown).

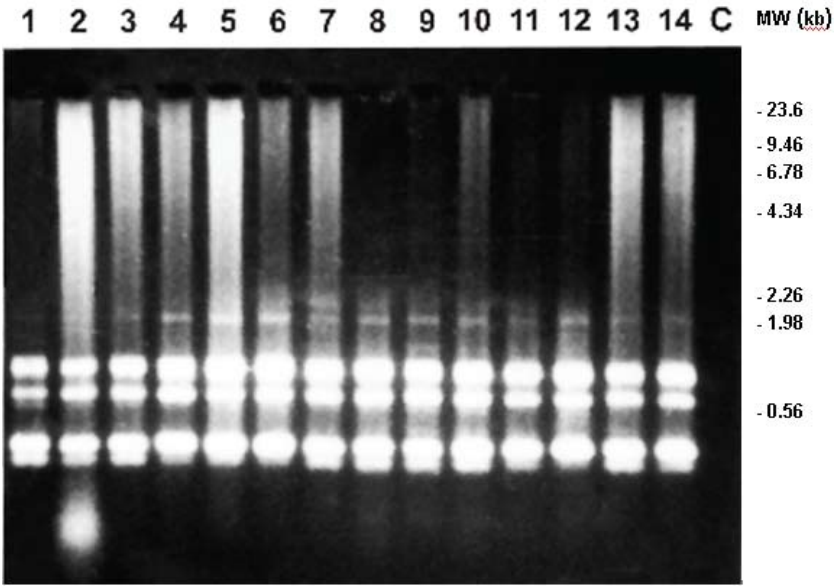

Figure 1. Random amplified polymorphic DNA patterns obtained with the primer OPG 14 for *C. albicans* isolates recovered from AIDS patient oral mucosa. Lines 1-14: Isolates Ca 21, 24, 35, 38, 46, 47, 58, 60, 69, 71, 72, 75, 83, 91. Line C: *Hind III Lambda* DNA size marker standards.

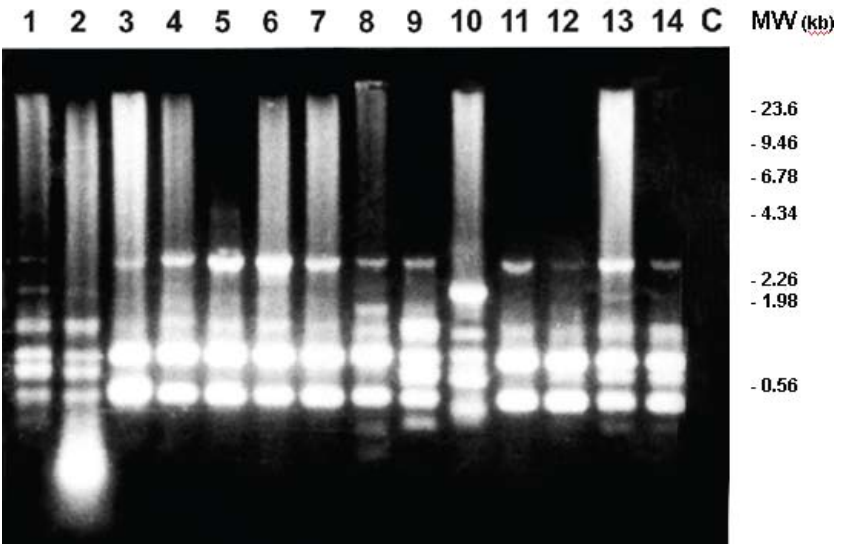

Figure 2. Random amplified polymorphic DNA patterns obtained with the primer OPG 17 for *C. albicans* isolates recovered from AIDS patients oral mucosa. Lines 1-14: Isolates Ca 21, 24, 35, 38, 46, 47, 58, 60, 69, 71, 72, 75, 83, 91. Line C: *Hind III Lambda* DNA size marker standards.

DISCUSSION

C. albicans is a pathogen associated with both systemic and superficial infections, especially in AIDS patients (5, 14). It is unclear whether the pathogenicity of these isolates is due to the genotypic characteristics or to favorable conditions present in hosts. It is probable that the two factors interfere in the pathogenicity of this yeast, thus their characterization is important.

Previous studies showed that the genetic polymorphism of *Candida* species could be used for typing these yeasts (1, 3, 4). RAPD assays has been used to type *Candida* with discriminatory power (25), and in some cases may be important for *Candida* species identification, increasing the capability of the traditional methods as biochemical, physiological, and micromorphological tests used for strains identification (24). This method has several advantages over other methods. It is a fast method and uses only a small amount of DNA (2, 13,15,23). We were particularly interested in determining whether the different primers were able to amplify and to characterize *C. albicans* isolates.

Genotypic differentiation among the isolates was verified by using the OPG 17 that elicited 4 different genotypes in 14 *Candida* isolates, while the OPG 14 showed the same genotype profile for this yeast. The dissimilar band profiles

obtained by OPG 17, imply on high degree of genetic diversity in *C. albicans*. Different genotype profiles with isolates of same species have been detected by other authors (2, 20). In the other hand, the conservation of bands of 600 bp and 450 bp observed for all isolates can be useful for identification and for the epidemiological studies of *C. albicans* by using RAPD method.

In our study we observed that the discriminatory power of RAPD assays rest upon the nature of the primer. According to Dassanayake et al. (4) the resolution of PCR mediated typing of *Candida* strains depends heavily on the choice of primers. In the present study the primer OPG 17 used was able to point out the genomic variability within the *Candida albicans* isolates.

Declaration of interest: The authors report no conflicts of interest. All the work was performed exclusively by authors.

RESUMO

Perfil de amplificação randômica de DNA polimórfico (RAPD) de isolados de *Candida albicans* utilizando diferentes iniciadores

Usando o método de amplificação randômica de DNA polimórfico (Randomly Amplified Polymorphic DNA analysis, RAPD), um total de 28 iniciadores (*primers*) foi estudado para discriminar entre os isolados de *Candida albicans*. Sete destes *primers* foram selecionados porque apresentaram padrões de banda de DNA reprodutíveis para todos os isolados de *C. albicans*. O mesmo perfil genético foi obtido usando OPG 14 (5'-GGATGAGACC-3'), enquanto o OPG 17 (5'-ACGACCGACA-3') demonstrou capacidade de diferenciar os isolados em quatro genótipos. De acordo com os resultados obtidos, nós concluímos que usando estes *primers*, a análise de RAPD pode ser útil em prover características genotípicas em investigações epidemiológicas.

DESCRITORES: *Candida albicans*. Perfil. RAPD.

REFERENCES

1. Bello MD, Gonzalez A, Barnabé C, Larrouy G. First characterization of *Candida albicans* by random amplified polymorphic DNA method in Nicaragua and comparison of the diagnosis methods for vaginal candidiasis in Nicaragua women. *Mem Inst Oswaldo Cruz 97*: 985-989, 2002.
2. Branchini MLM, Geiger DCP, Fishman O, Pignatari AC. Molecular typing of *Candida albicans* strains isolated from nosocomial candidemia. *Rev Inst Med Trop São Paulo 37*: 483-487, 1995.
3. Dassanayake RS, Samaranayake LP. Characterization of the genetic diversity in superficial and systemic human isolates of *Candida parapsilosis* by randomly amplified polymorphic DNA (RAPD). *Acta Pathol Microbiol Scand 108*: 153-160, 2000.
4. Dassanayake RS, Samaranayake YH, Samaranayake LP. Genomic diversity of oral *Candida krusei* isolates as revealed by DNA fingerprinting and electrophoretic karyotyping. *Acta Pathol Microbiol Scand 108*: 697-704, 2000.

5. Delgado W, Aguirre JM. Las micosis orales en la era del SIDA. *Rev Iberoam Micol 14*: 14-22, 1997.
6. Dolapci I, Tekeli A, Gocmen JS, Aysev D, Guriz H. Investigation of *Candida dubliniensis* in *Candida* spp.-positive hemocultures. *Acta Pathol Microbiol Scand 110*: 5391-5395, 2002.
7. Ergon MC, Gulay Z. Molecular epidemiology of *Candida* species isolated from urine at an intensive care unit. *Mycoses 48*: 126-131, 2005.
8. Espinel-Ingroff A, Vazquez JA, Boikov D, Pfaller MA. Evaluation of DNA-based typing procedures for strain categorization of *Candida* spp. *Diagn Microbiol Infect Dis 33*: 231-239, 1999.
9. Faggi E, Pini G. Campisi E, Martinelli C, Difonzo E. Detection of *Candida dubliniensis* in oropharyngeal samples from human immunodeficiency virus infected and non-infected patients and in a yeast from human immunodeficiency virus infected and non-infected patients and in a yeast culture collection. *Mycoses 48*: 211-215, 2005.
10. Jarvis WR. Epidemiology of nosocomial fungal infections with emphasis on *Candida* species. *Clin Infect Dis 20*: 1526-1530, 1995.
11. Kutzman CP, Fell JW. *The Yeasts: a taxonomic study*. 4 ed. New York, Elsevier, 1998.
12. McCullough MJ, Clemons KV, Del Palacio A, Stevens DA. Epidemiology of *Candida albicans* isolates from heroin addicts analyzed by DNA typing. *Med Mycol 36:* 213-217, 1998.
13. Melo ASA, Almeida LP, Colombo AL, Briones MRS. Evolutionary distances and identification of *Candida* species in clinical isolates by randomly amplified polymorphic DNA (RAPD). *Mycopathologia 142*: 57-66, 1998.
14. Melo NR, Taguchi H, Jorge J, Pedro RJ, Almeida OP, Fukushima K, Nishimura K, Miyaji M. Oral *Candida* flora from Brazilian Human Immunodeficiency Virus-infected patients in the highly active antiretroviral therapy era. *Mem Inst Oswaldo Cruz 99*: 425-431, 2004.
15. Muller EMC, Lischewski A, Harmsen D, Kacker J. Standardized molecular typing. *Mycoses 42*: 69-72, 1999.
16. Odds FC, Schmid J, Soll DR. Epidemiology of Candida infections in AIDS. *In* H. Vanden Bossche. *Mycoses in AIDS patient*, New York, 1990. p. 67-74.
17. Pinto PM, Resende MA, Koga-Ito CY, Tendler M. Genetic variability analysis among clinical *Candida* spp. isolates using random amplified polymorphic DNA. *Mem Inst Oswaldo Cruz 99*: 147-152, 2004.
18. Pongsiriwet S, Iamaroon A, Sriburee P, Pattanaporn K, Krisanaprakornkit S. Oral colonization of *Candida* species in perinatally HIV-infected children in Northern Thailand. *J Oral Science 46*: 101-105, 2004.
19. Pujol C, Joly S, Lockhart SR, Noel S, Tibayrenc M, Soll DR. Parity among the randomly amplified polymorphic DNA method, multilocus enzyme electrophoresis, and Southern blot hybridization with the moderately repetitive DNA probe Ca3 for fingerprinting *Candida albicans*. *J Clin Microbiol 35*: 2348-2358, 1997.
20. Robert F, Leberton F, Bougnoux ME, Paugam A, Wassermann D, Schlotterer M, Tourte-Schaefer C, Dupouy-Camet J. Use of random amplified polymorphic DNA as a typing method for *Candida albicans* in epidemiological surveillance of a burn unit. *J Clin Microbiol 33*: 2366-2371, 1995.
21. Scherer S, Stevens DA. Application of DNA typing methods to epidemiology and taxonomy of *Candida* species. *J Clin Microbiol 25*: 675-679, 1987.
22. Soll DR. The Ins and Outs of DNA Fingerprinting the Infectious Fungi. *Clin Microbiol Rev 13*: 332-370, 2000.
23. Tortorano AM, Kibbler C, Peman J, Bernhardt H, Klingspor L, Grillot R. Candidaemia in Europe: epidemiology and resistance. *Int J Antimicrob Agents 27*: 359-366, 2006.
24. Valerio HM, Weikert-Oliveira RCB, Resende MA. Differentiation of *Candida* species obtained from nosocomial candidemia using RAPD-PCR technique. *Rev Soc Bras Med Trop 39*: 174-178, 2006.
25. Vrioni G, Matsiota-Bernard P. Molecular typing of *Candida* isolates from patients hospitalized in an intensive care unit. *J Infect 42*: 50-56, 2001.