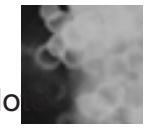

Comparison of a randomly amplified polymorphic DNA (RAPD) analysis and ATB ID 32C system for identification of clinical isolates of different *Candida* species

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Summary The objective of this work was to compare the usefulness of a randomly amplified polymorphic DNA (RAPD) assay to that of the ATB ID32C kit (bioMérieux, France) for identification of different species of *Candida* isolated from clinical specimens. The RAPD-PCR patterns obtained with OPE-18 primer for identification of clinical isolates were consistent, and the different independent assays revealed reproduction of the band patterns. RAPD with the OPE-18 primer is a very specific and sensitive method for identification of *Candida glabrata, Candida guilliermondii, Candida tropicalis, Candida pelliculosa, Candida albicans, Candida krusei,* and *Candida lusitaniae*.

Key words *Candida*, RAPD, ATB ID32C, Identification, Clinical isolates

Comparación de un método de amplificación aleatoria del ADN polimorfo (RAPD) y el sistema ATB ID32C para la identificación de aislamientos clínicos de *Candida*

Resumen El objetivo de este trabajo ha sido comparar la utilidad de un método de amplificación aleatoria del ADN polimorfo (RAPD) y la del método ATB ID32C (bioMérieux, Francia) para identificar aislamientos clínicos de diferentes especies de *Candida*. Los patrones de RAPD obtenidos con el cebador OPE-18 fueron estables y consistentes en los diferentes ensayos independientes y mostraron una buena reproducibilidad. La RAPD con el cebador OPE-18 es un método sensible y específico para la identificación de los aislamientos de *Candida glabrata, Candida guilliermondii, Candida tropicalis, Candida pelliculosa, Candida albicans, Candida krusei,* and *Candida lusitaniae*.

Palabras clave *Candida*, RAPD, ATB ID32C, Identificación, Aislamientos clínicos

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Candida species have become an important cause of nosocomial infection [2]. *Candida albicans* remains the most common cause of candidiasis, but other species are not uncommon [5]. Thus, early and accurate diagnosis of an invasive fungal infection is critical for timely and appropriate treatment [1,13]. Numerous methods have been developed for identifying clinical isolates, such as the API 20C or ATB ID 32C systems [4,12,14,16], but these can require several days [18]. Molecular methods have been used for typing *Candida* isolates for epidemiologic purposes [5,15]. Nonetheless, several studies have suggested randomly amplified polymorphic DNA analysis (RAPD) as an easy and reliable tool for the identification of several pathogenic fungi, including different species of *Candida* [3], *Penicillium* [7] and *Saccharomyces* [11]. Distinctive RAPD patterns have been described for *Candida* [3,9,15,16] and RAPD methods are particularly promising because of their simplicity, specificity and sensitivity [16].

The objective of this work was to compare the usefulness of an RAPD assay to that of the ATB ID32C kit (bioMérieux, France) for identification of different species of *Candida* isolated from clinical specimens.

Candida isolates were obtained from four microbiological laboratories situated in Mexico City, and in Guadalajara, Monterrey, and Guanajuato, Mexico. A total of 92 clinical isolates, including 17 *Candida albicans*, 14 *Candida glabrata*, 15 *Candida tropicalis*, 11 *Candida lusitaniae*, 10 *Candida guilliermondii*, five *Candida parapsilosis*, seven *Candida krusei*, one *Candida pelliculosa*, one *Candida colliculosa*, two *Candida dubliniensis*, two *Candida rugosa,* and seven C*andida kefyr* were analyzed; 10 culture collection strains were included as quality controls. All yeasts were identified by the germ tube test, while chlamydospore production and morphology were identified on cornmeal agar (Difco, USA) and ATB ID 32C test.

For DNA extraction, yeasts were grown on Sabouraud dextrose agar plates (Difco) at 37 °C for 24-48 h. A single colony was cultured overnight on YPD broth (1% yeast extract, 2% peptone, 2% dextrose) at 37 °C and 200 rpm agitation. DNA was extracted using the DNeasy Isolation Kit (Qiagen, USA), this kit facilitating the rapid recovery of sufficient DNA for PCR amplification and allowing for multiple samples to be extracted in parallel. DNA concentrations and A260/A280 ratios were determined using a spectrophotometer Lambda 1A (Perkin-Elmer, USA). An A260/A280 ratio of 1.8-2.1 was considered acceptable.

RAPD profiles were obtained with primer OPE-18 (5'-GGACTGCAGA-3') (Gibco BRL, USA). RAPD analysis was performed according to a previously described method [9] with minor modifications. The reaction mixture contained 1 µl genomic DNA (10 ng/µl), 1 µl OPE-18 primer, 2 µl of a deoxynucleotide triphosphate mixture (0.5 µl each of dATP, dCTP, dGPT, and dTTP), 1 µl $MgCL_2$ (2 mM), and 0.24 µl Taq DNA polymerase (1.2 U) in the PCR buffer provided by the manufacturer (Gibco BRL). Amplification consisted of 38 1-min cycles at 94 °C, 1 min at 36 °C, and 2 min at 72 °C. A 5-10 µl sample of each PCR product was analyzed by electrophoresis on 1.2% (wt/vol) agarose (Gibco BRL) gel slabs (14 cm 10 cm 6 mm) with tris-acetate buffer (1xTAE; 0.04 M tris-acetate pH 8.4, 1 mM EDTA) at 80 V for 2-3 h. Gels were stained with 0.5 µg of ethidium bromide per ml of deionized water for 20 min, this followed by a 30-min wash in deionized water. DNA bands confirming a positive PCR were visualized with a UV transiluminator and photographed and documented with an Eagle Eye System (Strategene, USA). The RAPD patterns obtained were analyzed by the Sigma Gel version 1.0 (JandelScientific, USA). The ability to differentiate between different species of *Candida* based on RAPD patterns was compared and analyzed in terms of sensitivity, specificity, positive predictive value, and negative predictive value.

Table 1. RAPD-band monomorphic applied diagnoses.

Species	Sizes (bp) of bands
C. albicans	2,219; 1,391; 994
C. glabrata	1,049; 949
C. guillermondii	2,864; 1,638; 935; 835; 585
C. kefyr	1,638; 1,508; 917; 667
C. krusei	3,052; 1,505; 961
C. lusitaniae	2,101; 1,982; 1,055; 836
C. parapsilosis	1,232; 759
C. rugosa	1,330; 1,036; 775; 498
C. tropicalis	1,839; 1,638; 810; 706; 602

Table 2. Comparison of the identification methods ATB ID 32C, RAPD, germ tube, and chlamydospore production.

Species	Clinical isolates (n)	Positive results (n)			
		ATB ID 32C	RAPD	Germ tube	Clamydospores
C. albicans	17	17	20	15	13
C. dubliniensis	2	2	0	0	2
C. glabrata	14	14	14	0	0
C. guilliermondii	10	10	10	0	0
C. kefyr	8	8	7	0	0
C. krusei	6	6	7	0	0
C. lusitaniae	9	9	11	0	0
C. parapsilosis	8	8	5	0	0
C. tropicalis	15	15	15	7	2
C. rugosa	1	1	2	0	0
C. pelicullosa	1	1	1	0	0
C. colicullosa	1	1	0	0	0
Total	92	92	92	22	17

This report revealed that use of RAPD with the primer OPE-18, previously described by Lockhart et al. [9], is highly discriminatory for identifying the most frequently encountered species of yeast in clinical specimens. Consistent RAPD patterns (Figure 1) were obtained using OPE-18 oligonucleotide and allowed rapid, accurate, reliable, and simple identification of *Candida* isolates [16]. However, significant differences in sizes (bp) of bands were reported previously by Bautista-Muñoz et al. [3] with the same primer (OPE-18) and other primers. Table 1 depicts the molecular weights of the monomorphic RAPD bands considered for identification of the different *Candida* species to the 1 kb DNA ladder. In addition, use of RAPD fingerprints can aid in the study of nosocomial fungal-infection epidemiology by possessing the ability to delineate discriminate among strains of the various *Candida* species evaluated [16]. PCR assays with several other target sequences have been recently reported [6]; nevertheless, species identification usually involves further manipulation of the amplified products utilizing restriction enzyme digestion [17], radioactive or enzyme-labeled probes [10], or DNA sequencing [1,15]. In the present study, RAPD sensitivity for total isolates was 91% (84 of 92 isolates were correctly identified), reinforcing the previously described RAPD procedures for *Candida* species identification [3].

Table 2 depicts the results and differences obtained in species identification. This study confirmed previous reports [9,12,16], which demonstrates that RAPD methods

Table 3. Sensitivity and specificity values (%) for RAPD identification of clinical isolates of different species of *Candida*.

Species	No.	RAPD/PCR Primer OPE-18				Germ tube				Chlamydospores			
		Sen	Spe	PPV	NPV	Sen	Spe	PPV	NPV	Sen	Spe	PPV	NPV
C. albicans	17	100	96	84	99	88	91	67	97	76	97	86	95
C. dubliniensis	2	0	0	NA	NA								
C. glabrata	14	100	100	NA	NA								
C. guillermondii	10	100	100	NA	NA								
C. kefyr	8	87	100	100	99								
C. krusei	6	100	98	99	100								
C. lusitaniae	9	100	97	99	100								
C. parapsilosis	8	62	100	100	96								
C. tropicalis	15	100	100	NA	NA								
C. rugosa	1	100	50	NA	NA								
C. pelicullosa	1	100	100	NA	NA								
C. colicullosa	1	0	0	NA	NA								

Sen = sensitivity, Spe = specificity, PPV= positive predictive value, NPV = negative predictive value, NA = not applicable.

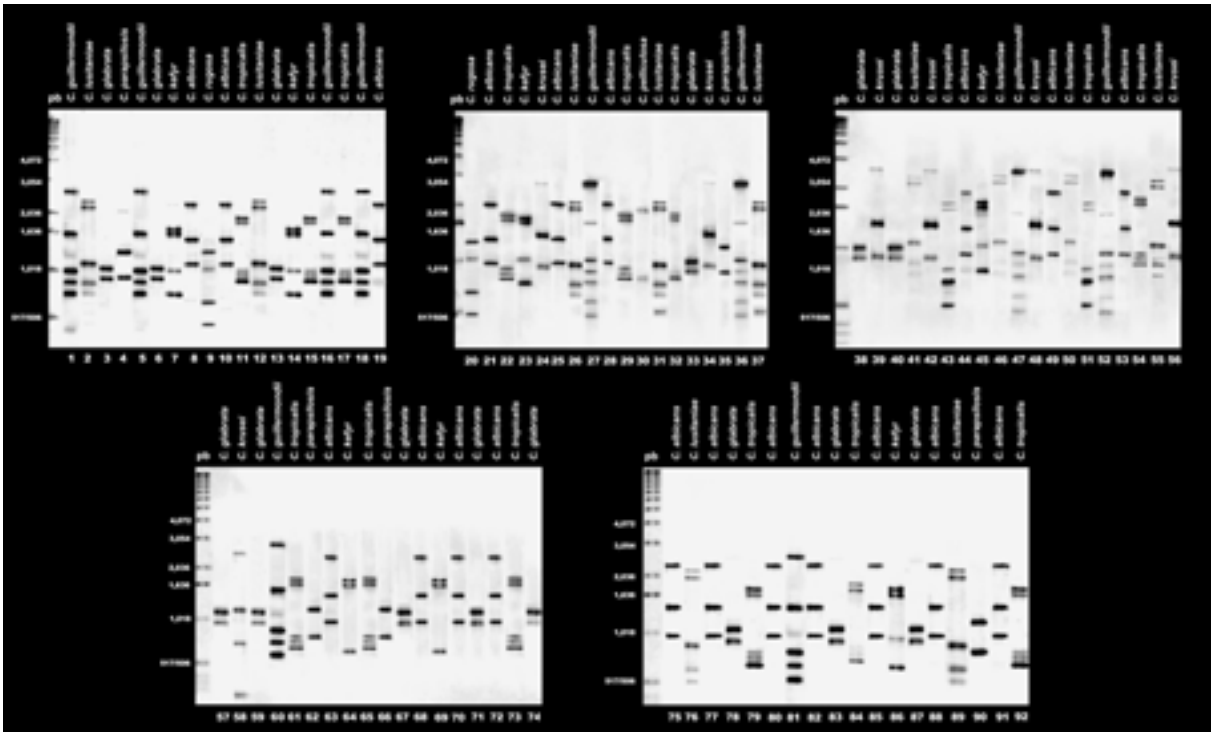

Figure 1. RAPD (primer OPE-18) performed with genomic DNA from individual clinical isolates of different *Candida* species.

performed with different oligonucleotides basically generated consistent patterns, with several species-unique fragments. RAPD-PCR depicted 100% sensitivity and specificity for identification of *C. glabrata*, *C. guilliermondii*, C. *tropicalis*, and *C. pelliculosa* isolates. For *C. krusei*, *C. lusitaniae*, and *C. albicans*, sensitivity was 100%, but specificity decreased to 98, 97, and 96%, respectively. In contrast, for *C. kefyr* and *C. parapsilosis*, specificity was 100%, but sensitivity was 87 and 62%, respectively. These diagnostic values were lower for the biological tests than for RAPD in *C. albicans* identification (Table 3).

RAPD profiles are highly consistent due to the low degree of diversity and primary clonal nature in populations of several pathogenic yeasts, including various species of *Candida* [9,17]. Notwithstanding this, few reports [3] have described intraspecific diversity and reproductive capabilities in *Candida*. All these data suggest that the major monomorphic bands obtained by RAPD analysis are useful for differentiation of pathogenic *Candida* species. RAPD fingerprints are species-specific and sufficiently simple to obtain identification without computer-assisted analysis [16]. Nevertheless, assay cost increases substantially if several RAPD primers are required for species identification [8].

In summary, a RAPD assay with the OPE-18 primer is very specific and sensitive for identification of important pathogenic *Candida* species such as *C. glabrata, C. guilliermondii, C. tropicalis, C. pelliculosa, C. albicans, C. krusei,* and *C. lusitaniae*.

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