JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2003, p. 414–420 Vol. 41, No. 1
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.1.414–420.2003

Identification of *Candida* spp. by Randomly Amplified Polymorphic DNA Analysis and Differentiation between *Candida albicans* and *Candida dubliniensis* by Direct PCR Methods

Consuelo Bautista-Muñoz, Xavier M. Boldo, Lourdes Villa-Tanaca, and César Hernández-Rodríguez*

Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, I.P.N., Mexico D.F., Mexico

Received 26 February 2002/Returned for modification 8 May 2002/Accepted 19 October 2002

Because *Candida* species have innately highly variable antifungal susceptibilities, the availability of a fast and reliable species identification test is very important so that suitable and effective therapeutic measures may be taken. Using three oligonucleotide primers, we established a randomly amplified polymorphic DNA (RAPD) analysis method that enabled direct identification of the most common opportunistic pathogenic *Candida* species. RAPD analysis revealed a characteristic molecular fingerprint for each *Candida* species. Differences between the profiles for *Candida albicans* and *C. dubliniensis* were evident. RAPD analysis is a relatively easy, reproducible, and reliable technique that can be useful in providing genetic fingerprints for the identification of strains. In addition, a collection of different *C. albicans* strains was identified by a specific PCR based on multiple secreted aspartic proteinase (*SAP*) genes and the dipeptidyl aminopeptidase (*DAP2*) gene. Our findings demonstrate that PCR based upon the *SAP* and *DAP2* sequences is a simple, rapid, clear, and direct technique for the identification and differentiation of *C. albicans* and *C. dubliniensis*.

The incidence of candidiasis caused by *Candida* species continues to increase in proportion to the growing number of immunocompromised, cancer, and postoperative patients. Traditional means of identification of pure cultures of *Candida* spp. include laborious and slow morphological and assimilation tests that can take several days to identify the isolates in a culture (36), and clinical yeast isolates are sometimes misidentified when automated biochemical systems are used (7). Several PCR methods for the differentiation of some *Candida* species have been reported (2, 9, 10, 14, 15, 27, 28). Also, many PCR-based methods that use several unique or multicopy molecular targets for the highly sensitive detection of *Candida albicans* in culture or biological samples have been developed. Only a few methods for the detection and identification of several species by a single and direct PCR (15) or multiplex PCR (3, 12) have been proposed. Unfortunately, those studies did not include a significant number of clinical isolates of each species.

Several studies have proposed that randomly amplified polymorphic DNA (RAPD) analysis could be directly used as an easy and secure tool for the identification of several pathogenic and food-borne microbial species, including *Saccharomyces* spp. (25), *Penicillium* spp. (8), and *Candida* spp. (1, 19, 20, 23, 29, 33, 35). This report describes a stable and repeatable RAPD PCR assay for the direct, easy, and relatively rapid identification of nine commonly isolated pathogenic *Candida* species of clinical relevance. *C. albicans* strain identification was confirmed by a very specific PCR based on the secreted aspartic protease (SAP) multigene family and the dipeptidyl aminopeptidase (*DAP2*) gene that permits the clear differentiation between the phylogenetically closely related species *C. albicans* and *C. dubliniensis*.

Candida sp. strains were obtained from four microbiological laboratories from the cities of Mexico City, Guadalajara, Monterrey, and Guanajuato in Mexico. A collection of 36 *C. albicans*, 47 *C. glabrata*, 83 *C. tropicalis*, 38 *C. lusitaniae*, 12 *C. guilliermondii*, 2 *C. dubliniensis*, 12 *C. parapsilosis,* 6 *C. krusei*, and 11 *C. kefyr* strains obtained from different clinical sites were analyzed. In addition, two strains obtained from culture collections (*C. albicans* ATCC 10231 and ATCC 24433) and donated strains (*C. albicans* 132A; *C. dubliniensis* CD36 and CD92; and *C. glabrata* J931010, 1480.41, LA817, and RA732) were included.

The strains were identified by germ tube formation in serum, formation of chlamydospores on cornmeal agar (Gibco BRL, Gaithersburg, Md.), and the assimilation profiles determined with the ID 32 C kit (BioMerieux SA, Marcy l'Etoile, France).

For DNA extraction, the yeasts were routinely grown on Sabouraud dextrose agar plates at 37°C for 24 to 48 h. A single colony was then subcultured overnight on YPD broth (1% yeast extract, 2% peptone, 2% dextrose) at 37°C with shaking at 200 rpm. DNA was extracted from this culture by an existing protocol (19). DNA concentrations and A_{260}/A_{280} ratios were determined spectrophotometrically with a spectrophotometer (Lambda 1A; Perkin-Elmer). An A_{260}/A_{280} ratio of 1.8 to 2.1 was considered acceptable.

The RAPD profiles were obtained with primers (10-mers) OPE-18 (5′-GGACTGCAGA-3′), OPE-04 (5′-GTGACATG CC-3′), and OPA-18 (5′-AGCTGACCGT-3′) (Gibco BRL) (21). RAPD analysis was performed by a previously described method (21) with minor modifications. Briefly, every reaction mixture for RAPD analysis contained 10 ng of genomic DNA; the appropriate primer at 0.4 μM; dATP, dCTP, dGTP, and

* Corresponding author. Mailing address: Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, I.P.N., Apartado Postal CON 174, México, D.F. CP 06400, Mexico. Phone and fax: (52 55) 57 29 62 09. E-mail: chdez38@hotmail.com.

dTTP each at a concentration of 200 μM; 2 mM $MgCl_2$; and 1.2 U of *Taq* DNA polymerase in the PCR buffer provided by the manufacturer (Gibco BRL). Amplification consisted of 38 cycles, each of 1 min at 94°C, 1 min at 36°C, and 2 min at 72°C. Five strains of each species except *C. dubliniensis* were tested on different days starting from the DNA extraction step to confirm the reproducibility of the method.

Three PCR procedures based on *C. albicans* sequences were designed: a multiplex PCR for amplification of the *SAP1-SAP2-SAP3* and the *SAP4-SAP5-SAP6* genes; a specific PCR for independent amplification of the *SAP1*, *SAP2*, and *SAP3* genes; and a specific PCR for amplification of the *DAP2* gene. Sequence data for *C. albicans* were obtained from GenBank and the Stanford Genome Technology Center website (http://www.sequence.stanford.edu/group/candida). Clustal X (version 1.81) software was used for gene alignment (34), and DNAMAN software (version 3, 1994 to 1997; Lynnon BioSoft) was used for primer design and estimations of the secondary structures and melting temperatures.

To design the multiplex PCR for the *SAP1-SAP2-SAP3* and the *SAP4-SAP5-SAP6* genes, alignment was performed by using the sequences of the following genes (the GenBank accession numbers are given in parentheses): *SAP1* (L12449, L12450, L12451, L12452, and X56867), *SAP2* (M83663), *SAP3* (L22358), *SAP4* (L25338), *SAP*5 (Z30191), *SAP6* (Z30192), *SAP7* (Z30193), *SAP8* (AF043330), *SAP9* (AF043331), and *Apr1* (U36754). Two forward primers, SAP123 (5′-CTGATT TATGGGTTCCTGAT-3′; positions 385 to 405 of the *C. albicans SAP1* gene) and SAP456 (5′-AAAAGATCACCTTTAT TTTTAGA-3′; positions 282 to 304 of the *C. albicans SAP4* gene), were chosen for specific amplification of the *SAP1-SAP2-SAP3* and *SAP4-SAP5-SAP6* DNA fragments, respectively. Only one reverse universal primer, SAP123456 (5′-TC AAAAAGTTATCACCAAGAAT-3′; positions 1188 to 1209 of the *C. albicans SAP4* gene), was designed for amplification of *SAP1-SAP2-SAP3-SAP4-SAP5-SAP6*. By using primers SAP123, SAP456, and SAP123456 in the same reaction, two expected bands of approximately 820 and 1,015 bp were observed on 1.2% agarose electrophoresis gels when *C. albicans* DNA was used.

By using the alignments of the SAP genes (see above), three PCRs for the independent amplification of the *SAP1*, *SAP2*, and *SAP3* genes were designed. The forward primer used for amplification of the *SAP1*, *SAP2*, and *SAP3* genes was SAP123, as indicated above. Specific reverse primers Antisense 1 (5′-T GGCAGCATTGGGAGAGTTG-3′; positions 385 to 404 of the *C. albicans SAP1* gene), Antisense 2 (5′-GTTGATCAAT TGAAGTAGAATC-3′; positions 400 to 421 of the *C. albicans SAP2* gene), and Antisense 3 (5′-CATGTCCCTTGTGAAGT AGT-3′; positions 510 to 529 of the *C. albicans SAP3* gene) were chosen for specific amplification of the *SAP1*, *SAP2*, and *SAP3* genes, respectively. The expected sizes of the fragments amplified from the *C. albicans SAP1*, *SAP2*, and *SAP3* genes were 390, 258, and 172 bp, respectively.

A specific PCR for the *C. albicans DAP2* gene was designed by analyzing the *DAP2* sequence. Forward primer DAP2 sense (5′-TCTTTACAACACGATGAGATTG-3′; positions 17,085 to 17,106 of *C. albicans* contig6-2225) and DAP2 antisense (5′-TCTAAATTGATTTTTTCTGATTTC-3′; positions 16,783 to 16,760 of *C. albicans* contig6-2225) were chosen for the specific amplification of a 347-bp fragment from *C. albicans* DNA.

All *SAP* and *DAP2* gene-based PCRs were performed in a buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 200 μM, each primer at a concentration of 0.4 μM, 10 ng of genomic DNA, and 2 U of *Taq* polymerase (Gibco BRL). Basically, the PCR conditions except the annealing temperature were the same as those described above. Amplification conditions included a denaturation step for 5 min at 94°C, followed by 35 amplification cycles consisting of 1 min at 94°C, 1 min at 55°C (*SAP1-SAP2-SAP3*, *SAP4-SAP5-SAP6*, and *DAP2*) or 59°C (*SAP1*, *SAP2*, and *SAP3*), and 2 min at 72°C. A final extension step was performed for 5 min at 72°C.

For all PCR-based procedures, amplification was done with a DNA thermal cycler (9600; Perkin-Elmer), the final volume of the reaction mixture was 25 μl, and samples were overlaid with 20 μl of mineral oil (U.S. Biochemicals, Cleveland, Ohio) prior to PCR amplification. A sample (5 to 10 μl) of each PCR product was analyzed by electrophoresis in 1.2% (wt/vol) agarose (Gibco BRL) gel slabs (14 cm by 10 cm by 6 mm) with Tris-acetate buffer (1× TAE; 0.04 M Tris-acetate [pH 8.4], 1 mM EDTA) at 80 V for 2 to 3 h. When products of similar sizes were obtained, electrophoresis was performed with high-performance agarose (Agarose 1000; Boehringer Mannheim Corp., Indianapolis, Ind.). The gels were soaked in ethidium bromide solution (0.5 μg/ml), and the DNA was visualized in a transilluminator under UV light. The DNA molecular size marker was derived from bacteriophage lambda DNA digested with *Eco*RI and *Hin*dIII (Sigma Chemical Co., St. Louis, Mo.). Also, a 100-bp DNA ladder (Gibco BRL) was used as a molecular size marker.

The RAPD patterns obtained for the different strains of *Candida* species with oligonucleotides OPE-18 and OPA-18 were species specific (Fig. 1). *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. guilliermondii* had profiles with limited polymorphisms. The RAPD patterns for the *C. albicans* strain collection were obtained by using oligonucleotides OPE-18 (Fig. 2), OPE-04, and OPA-18 independently. With oligonucleotide OPE-18, the RAPD patterns of the collection of *Candida* sp. strains showed the principal monomorphic bands considered for identification (Fig. 2 and 3). Basically, the same pattern was observed within species; and species-specific monomorphic bands were obtained for *C. albicans, C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr*, *C. lusitaniae*, and *C. guilliermondii* (Table 1). Three repetitions of RAPD analysis showed the same fingerprints, and nonconsistent bands were considered.

To be able to document more precisely the differences between *C. albicans* and *C. dubiniensis* found by RAPD analysis, we performed PCR procedures by amplifying the genes that encode some specific and nonspecific proteases of these phylogenetically related yeasts, which for a long time were not recognized as two different species. The PCR procedures based on the *SAP* genes and the *DAP2* gene designed in this study were able to amplify DNA only from *C. albicans* and *C. dubliniensis*. By using multiplex PCRs for the *SAP1-SAP2-SAP3* and the *SAP4-SAP5-SAP6* genes and a specific *SAP1* gene-based protocol, we were able to amplify PCR products from the DNAs extracted from a collection of *C. albicans*

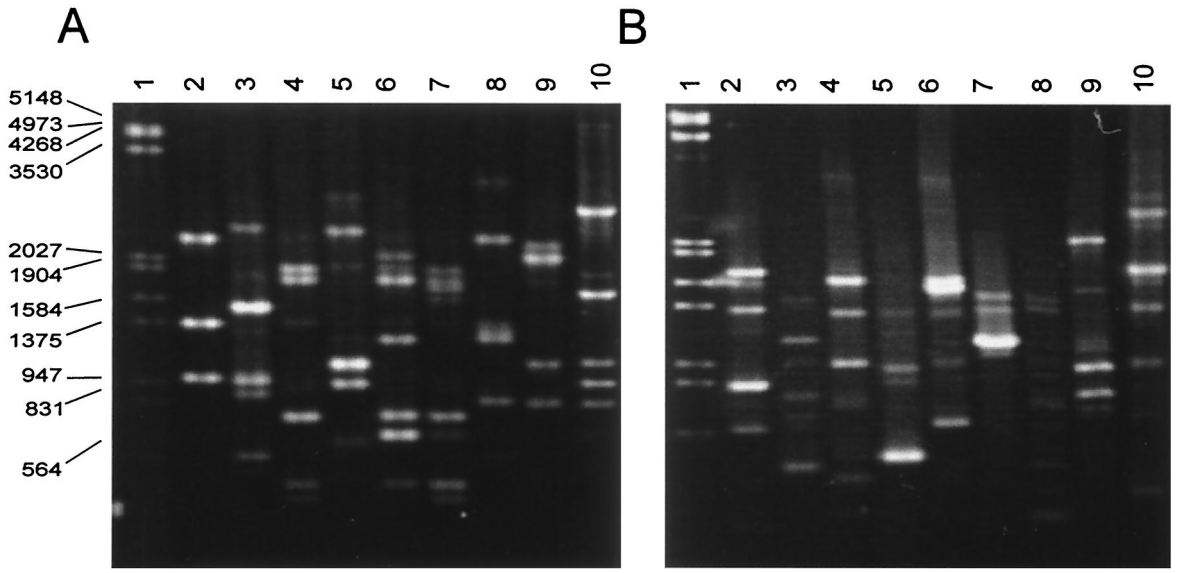

FIG. 1. RAPD patterns for *Candida* species obtained with primers OPE-18 (A) and OPA-18 (B). Lane 1, molecular size marker (in base pairs); lanes 2 to 10, *C. albicans* ATCC 10231, *C. dubliniensis* CD36, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. kefyr*, *C. krusei*, *C. lusitaniae*, and *C. guilliermondii*, respectively.

strains and *C. dubliniensis* (Fig. 4 and 5A and B). The *SAP2*- and *DAP2*-based PCR procedures amplified from *C. albicans* DNA products of 258 bp (Fig. 5C) and 347 bp (Fig. 5E), respectively, but no DNA fragment could be obtained from *C. dubliniensis*. When the *SAP3*-based PCR was performed, products of 172 and approximately 134 bp from *C. albicans* and *C. dubliniensis*, respectively, could be distinguished by using high-performance agarose (Fig. 5D). In all PCR procedures a minimum of 100 yeast cells suspended in water and blood was required for a positive reaction (data not shown).

The data presented here describe the species-specific RAPD patterns of *Candida* spp. obtained with three primers. The *SAP* multigene family-based PCR procedures and the *DAP2* gene-based PCR procedure were also performed to distinguish between *C. albicans* and *C. dubliniensis* and to confirm the identities of *C. albicans* isolates.

The "gold standard" for definitive yeast identification requires assimilation and fermentation tests, which can take several days for identification (36). Several PCR methods for the differentiation among some *Candida* species have been reported. The drawbacks to these approaches are the additional experimental procedures required, including hybridization with species-specific probes (9), restriction fragment length polymorphism analysis (37), enzyme immunoassay (EIA) (10, 11, 28), 5′ exonuclease assay with fluorescent DNA probes (13, 27), and sequencing or a second PCR with species-specific primers (2, 17), to ensure the identity of each species after DNA amplification. The PCR-EIA method is more expensive

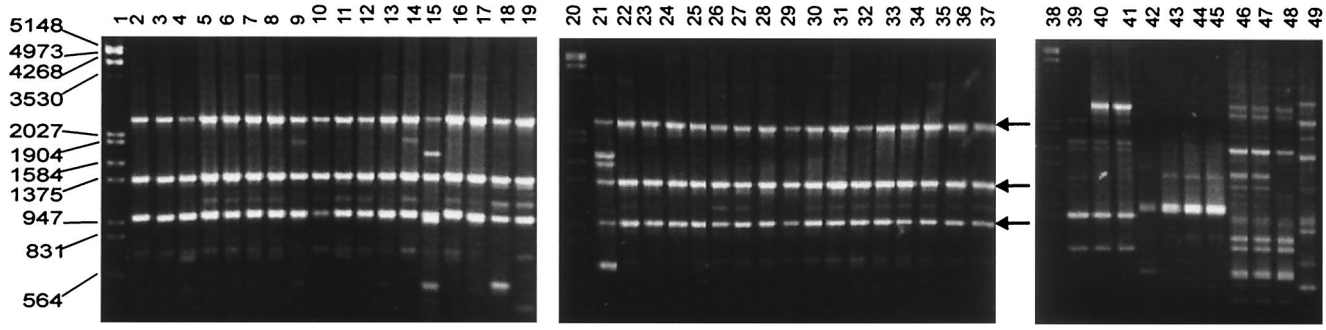

FIG. 2. RAPD patterns for *C. albicans* strains obtained with primer OPE-18. Arrows indicate the monomorphic bands useful for identification. Lanes 1, 20, and 38, molecular size markers (in base pairs); lanes 2 to 37, *C. albicans* ATCC 10231, CAL19, CAL20, CAL21, CAL22, CAL23, CAL24, CAL25, CAL26, CAL27, CAL28, CAL30, CAL31, CAL32, CAL33, CAL34, CAL35, CAL36, CAL37, CAL38, CAL39, CAL40, CAL41, CAL43, CAL1, CAL3, CAL7, CAL8, CAL9, CAL10, CAL11, CAL12, CAL13, CAL15, and CAL16, respectively; lane 39, *Saccharomyces cerevisiae* DBY; lane 40, *Saccharomyces pasturianus*; lane 41, *Saccharomyces carlsbergensis*, lane 42; *Schizosaccharomyces pombe* 972 h^-; lane 43, *S. pombe* 975; lane 44, *S. pombe* LV7 h^-; lane 45, *S. pombe* 4D; lane 46, *Kluyveromyces lactis* wm27; lane 47, *Kluyveromyces lactis* wm37; lane 48, *Kluyveromyces lactis* 2/1; lane 49, *Yarrowia lipolytica* SA-1.

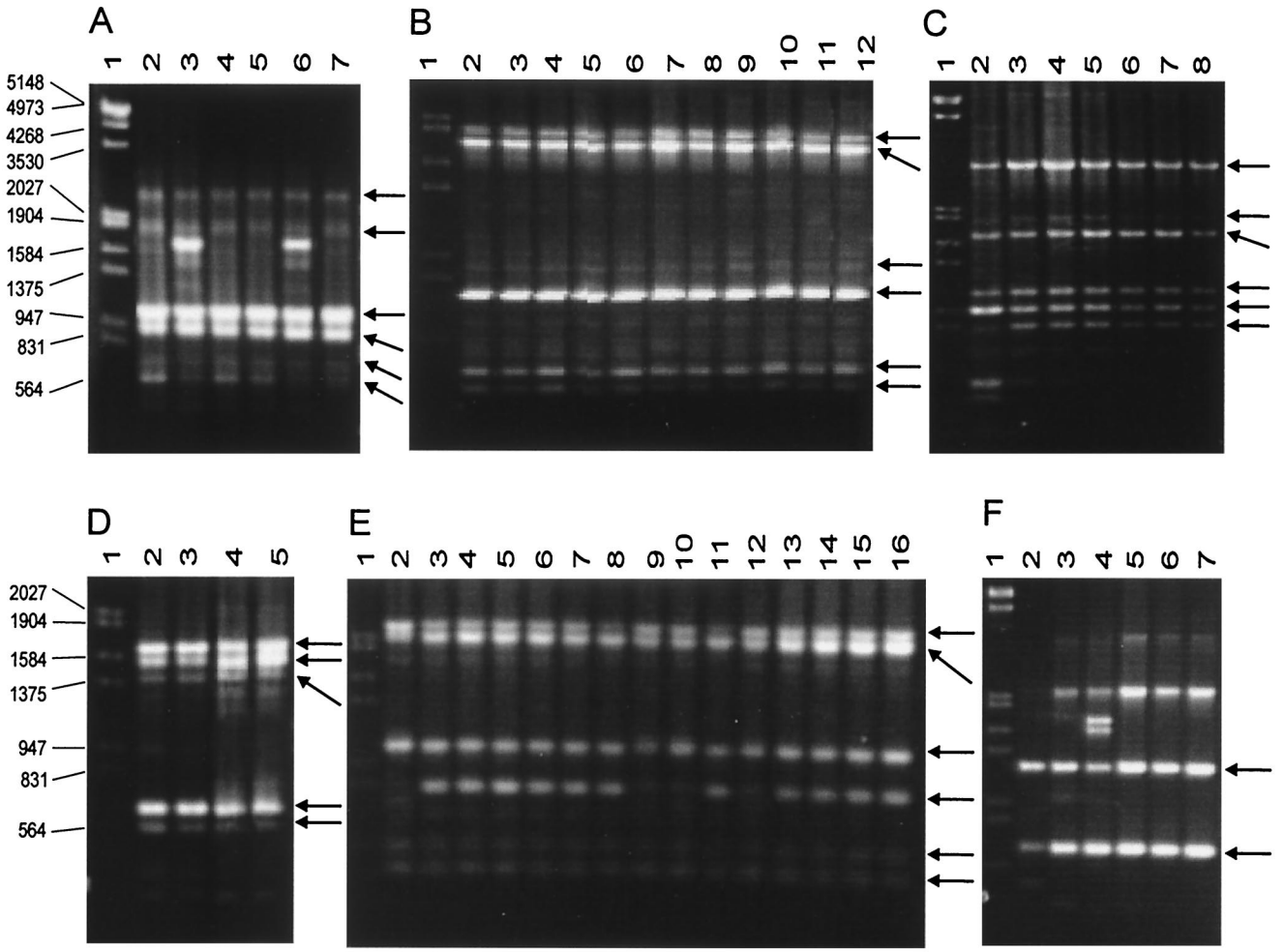

FIG. 3. RAPD patterns for *C. glabrata*, *C. tropicalis*, *C. guilliermondii*, *C. kefyr*, *C. lusitaniae*, and *C. parapsilosis* strains obtained with primer OPE-18. Arrows indicate the monomorphic bands for each species. Lanes 1, molecular size markers (in base pairs). (A) Lanes 2 to 7, *C. glabrata* CGL29, CGL1, CGL3, CGL2, CGL47, and CGL42, respectively; (B) lanes 2 to 12, *C. tropicalis* CTR64, CTR65, CTR66, CTR67, CTR68, CTR69, CTR70, CTR71, CTR72, CTR73, and CTR74, respectively; (C) lanes 2 to 8, *C. guilliermondii* CGU6, CGU7, CGU8, CGU9, CGU10, CGU11, and CGU12, respectively; (D) lanes 2 to 5, *C. kefyr* CKE10, CKE11, CKE1, and CKE2, respectively; (E) lanes 2 to 16, *C. lusitaniae* CLU8, CLU25, CLU26, CLU27, CLU28, CLU29, CLU30, CLU31, CLU32, CLU33, CLU34, CLU35, CLU36, CLU37, and CLU38, respectively; (F) lanes 2 to 7, *C. parapsilosis* CPA4, CPA8, CPA9, CPA10, CPA11, and CPA12, respectively.

and complex than RAPD analysis. Although it may have a better potential for automation, better reproducibility, and a better sensitivity of detection, precise identification of fungi by the PCR-EIA method requires a battery of genus- and species-specific probes to identify species (10, 11, 28), whereas species identification by RAPD analysis requires only PCR with a single primer, agarose electrophoresis, UV transillumination, and band comparison analysis.

A few PCR procedures for the direct identification of *Candida* species have been reported. These approaches only require electrophoresis on an agarose gel and ethidium bromide staining to detect PCR products. However, some of these studies are limited because of the small number of medically important species included (2, 15, 22), the small number of strains or isolates of each species probed (15, 22), the need for the use of several species-specific primers (2, 14, 15, 16, 22), or the requirement of a multiplex PCR (3, 12, 15, 16, 22) or because they did not assay whether the methods are able to distinguish between *C. albicans* and *C. dubliniensis* (2, 3, 15).

Two similar studies proposed a direct multiplex PCR based on amplification of fungal fragments of different sizes (internal transcribed spacers ITS1 and ITS2 and the 5.8S ribosomal DNA region) to identify several fungal and *Candida* species (3, 12). However, those studies were not blinded, did not include strain collections to assess intraspecific variations, and did not distinguish between *C. dubliniensis* and *C. albicans*. Another recently published paper describes a topoisomerase II gene-based PCR that can identify several medically important *Candida* species including *C. dubliniensis*; however, this approach must be performed with several mixtures of primers or by a nested PCR approach (16).

A simpler procedure for the identification of nine *Candida* spp. could be performed by the RAPD fingerprinting assay described here. The procedure required only electrophoresis

TABLE 1. RAPD diagnostic monomorphic bands for identification of *Candida* spp.

Primer	Sizes (bp) of RAPD monomorphic bands								
	C. albicans	*C. dubliniensis*	*C. tropicalis*	*C. glabrata*	*C. parapsilosis*	*C. kefyr*	*C. krusei*	*C. lusitaniae*	*C. guilliermondii*
OPE-18	2,418, 1,374, 970	1,811, 1,504, 948, 882, 508	1,986, 1,846, 1,702, 815, 500, 406	2,394, 1,835, 1,046, 917, 686, 555	1,232, 659	1,731, 1,615, 1,492, 722, 610	3,591, 2,403, 1,258, 830	2,295, 2,081, 1,104, 800, 594, 484	3,061, 1,859, 1,671, 1,107, 939, 822
OPE-04	2,861, 1,504, 1,284, 815	2,529, 1,504, 1,195, 1,083	2,974, 1,530, 1,322, 1,220, 1,013, 917, 608, 508	1,874, 1,439, 946, 881	1,373, 1,240, 1,001, 696, 545	2,127, 1,937, 1,796, 1,445, 1,225, 929	1,811, 1,268, 1,134, 1,028, 878, 774, 679, 450	1,878, 1,692, 1,559, 1,301, 1,038	3,805, 3,105, 2,685, 2,239, 1,865, 1,679, 1,312, 1,155, 946, 655, 581
OPA-18	1,677, 1,329, 801	2,473, 2,029, 1,772, 1,504, 1,400, 1,307, 1,083, 882	3,399, 2,615, 1,581, 1,302, 1,066	2,002, 1,340, 945, 467	1,620, 1,512, 1,306, 969, 600	2,180, 1,901, 1,372, 1,108	1,443, 1,330, 969, 889, 734, 470	2,028, 1,053, 946	3,595, 3,165, 2,805, 2,505, 2,325, 1,737, 1,603, 1,375, 985

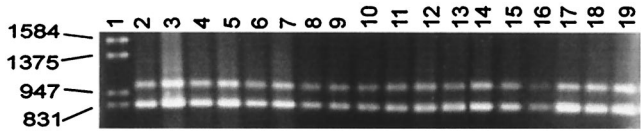

FIG. 4. Expected amplification products of approximately 820 and 1,015 bp from DNA extracted from *C. albicans* strains for identification of *C. albicans* strains by multiplex PCR for the *SAP1-SAP2-SAP3* and the *SAP4-SAP5-SAP6* genes. Lane 1, molecular size marker (in base pairs); lanes 2 to 19, *C. albicans* ATCC 10231, 132A, CAL19, CAL20, CAL21, CAL22, CAL23, CAL24, CAL25, CAL26, CAL27, CAL28, CAL30, CAL31, CAL32, CAL33, CAL34, and CAL35, respectively.

on an agarose gel and ethidium bromide staining to detect PCR products. Highly consistent, clear, and repetitive RAPD profiles were obtained with each of three different primers and collections of clinical strains isolated in different cities and on different dates. The limiting factor of this technology is that RAPD analysis cannot be adapted to the identification of *Candida* spp. in clinical samples like other PCR methods designed to amplify specific genes can. Moreover, in order to maintain the reproducibility of the characteristic RAPD patterns, constant amounts of DNA must be used, e.g., 10 ng in this study, whereas other PCR methods can be optimized to directly detect smaller amounts of the target DNA present in clinical samples.

The results of this study reinforce the value of previously described RAPD analysis procedures for the identification of *Candida* spp. (19, 20, 23, 29, 33, 35). Our study and those of others reported previously (17, 19, 21, 23, 24, 29, 33) have shown that RAPD methods performed with different oligonucleotides basically generated consistent patterns, with several shared fragments unique to each species. RAPD profiles are highly consistent due to the low degree of diversity and primary clonal nature of populations of several pathogenic yeasts, including *C. albicans* (32) and *C. glabrata* (21). Unfortunately, a few reports have described the intraspecific diversity and reproductive capabilities of other *Candida* spp. All these data suggest that the major monomorphic bands obtained by RAPD analysis are useful for the differentiation of pathogenic *Candida* species.

Several of the PCR procedures reported previously were unable to distinguish between *C. albicans* and *C. dubliniensis* (12, 14). For example, in our laboratory, a PCR based on the previously described *CHS1* gene (15) produced no discernible differences in the sizes of the PCR products between these two species (data not shown). Recently, several reports described relatively simple phenotypic (4, 10, 13, 18, 26), genotypic, or PCR-based methods that can be used to distinguish between these species by PCR fingerprinting (5, 24), multiplex PCR (22), or other direct PCR procedures (6, 16, 18). In the present study, three additional PCR procedures based on the *SAP* and *DAP2* genes have been proposed as additional strategies for the specific identification of *C. albicans* and as a means of distinguishing *C. albicans* from *C. dubliniensis*. The RAPD analysis procedure with three independent primers described here emphasizes the evident differences in RAPD profiles between *C. albicans* and *C. dubliniensis* described previously (30, 31).

Many studies have described molecular diagnostic and iden-

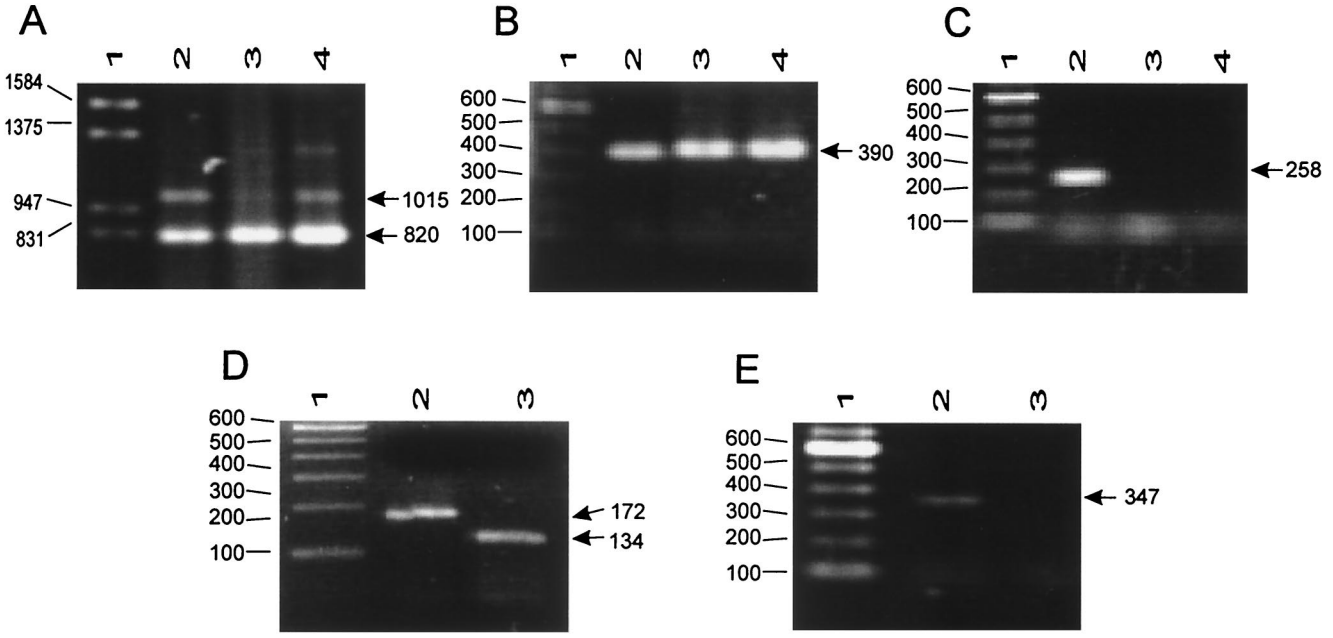

FIG. 5. Identification and differentiation of *C. albicans* and *C. dubliniensis* by PCR-based protocols. (A) Multiplex PCR for the *SAP1-SAP2-SAP3* and the *SAP4-SAP5-SAP6* genes; (B) PCR for the *SAP1* gene; (C) PCR for the *SAP2* gene; (D) PCR for the *SAP3* gene; (E) PCR for the *DAP2* gene. Arrows show the expected amplification products. Lanes 1, molecular size marker (in base pairs); lanes 2, *C. albicans* ATCC 10231; lanes 3, *C. dubliniensis* CD36; lanes 4, *C. dubliniensis* CD92.

tification procedures for *Candida* spp. of medical importance, but an extensive comparative study to evaluate the sensitivities, reproducibilities, and costs of several methods with isolates from various origins does not exist. The assays described here allow the relatively rapid identification of *Candida* species and offer alternatives to conventional morphologically and physiologically based identification procedures and their associated problems.

We are grateful to the following individuals for donating strains: Derek J. Sullivan, University of Dublin; David R. Soll, University of Iowa; Luis Rivera, Universidad de Guanajuato; Fernando Velarde, Hospital Civil de Guadalajara; and Octavio Novoa, Hospital Durango de México.

C.B.-M. is a fellow from CONACyT and PIFI, I.P.N. L.V.-T. and C.H.-R. received support from COFAA and EDD, I.P.N. Sequence data for *Candida albicans* were obtained from the Stanford Genome Technology Center website (http://www.sequence.standford.edu/group/candida). Sequencing of *C. albicans* was accomplished with the support of NIDR and the Burroughs Wellcome Fund. This work was supported by grants from CONACyT (grants 26437-N and CGPI200430), I.P.N., Mexico City, Mexico.

REFERENCES

1. **Andrighetto, C., E. Psomas, N. Tzanetakis, G. Suzzi, and A. Lombardi.** 2000. Randomly amplified polymorphic DNA (RAPD) PCR for the identification of yeasts isolated from dairy products. Lett. Appl. Microbiol. **30:**5–9.
2. **Burgener-Kairuz, P., J.-P. Zuber, P. Jaunin, T. G. Buchman, J. Bille, and M. Rossier.** 1994. Rapid detection and identification of *Candida albicans* and *Torulopsis* (*Candida*) *glabrata* in clinical specimens by species-specific nested PCR amplification of a cytochrome P- 450 lanosterol-α-demethylase (L1A1) gene fragment. J. Clin. Microbiol. **32**:1902–1907.
3. **Chang, H. C., S. N. Leaw, A. H. Huang, T. L. Wu, and T. C. Chang.** 2001. Rapid identification of yeasts in positive blood cultures by a multiplex PCR method. J. Clin. Microbiol. **39:**3466–3471.
4. **Coleman, D., D. Sullivan, B. Harrington, K. Haynes, M. Henman, D. Shanley, D. Bennett, G. Moran, C. McCreary, and L. O'Neill.** 1997. Molecular and phenotypic analysis of *C. dubliniensis*: a recently identified species linked with oral candidosis in HIV-infected and AIDS patients. Oral Dis. **3**(Suppl. 1)**:**S96–S101.
5. **Diaz-Guerra, T. M., E. Mellado, M. Cuenca-Estrella, F. Laguna, and J. L. Rodríguez Tudela.** 1999. Molecular characterization by PCR-fingerprinting of *C. dubliniensis* strains isolated from two HIV-positive patients in Spain. Diagn. Microbiol. Infect. Dis. **35:**113–119.
6. **Donnelly, S. M., D. J. Sullivan, D. B. Shanley, and D. C. Coleman.** 1999. Phylogenetic analysis and rapid identification of *Candida dubliniensis* based on analysis of *ACT1* intron and exon sequences. Microbiology **145:**1871–1882.
7. **Dooley, D. P., M. L. Beckius, and B. S. Jeffrey.** 1994. Misidentification of clinical yeast isolates by using the updated Vitek Yeast Biochemical Card. J. Clin. Microbiol. **32:**2889–2892.
8. **Dupont, J., S. Magnin, A. Marti, and M. Brousse.** 1999. Molecular tools for identification of *Penicillium* starter cultures used in the food industry. Int. J. Food Microbiol. **49:**109–118.
9. **Einsele, H., H. Hebart, G. Roller, J. Löffler, I. Rothenhöfer, C. A. Müller, R. A. Bowden, J. van Burik, D. Engelhard, L. Kanz, and U. Schumacher.** 1997. Detection and identification of fungal pathogens in blood by using molecular probes. J. Clin. Microbiol. **35:**1353–1360.
10. **Elie, C. M., T. J. Lott, E. Reiss, and C. J. Morrison.** 1998. Rapid identification of *Candida* species with species-specific DNA probes. J. Clin. Microbiol. **36:**3260–3265.
11. **Fujita, S., B. A. Lasker, T. J. Lott, E. Reiss, and C. J. Morrison.** 1995. Microtitration plate enzyme immunoassay to detect PCR-amplified DNA from *Candida* species in blood. J. Clin. Microbiol. **33:**962–967.
12. **Fujita, S.-I., Y. Senda, S. Nakaguchi, and T. Hashimoto.** 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeasts strains. J. Clin. Microbiol. **39:**3617–3622.
13. **Guiver, M., K. Levi, and B. A. Oppenheim.** 2001. Rapid identification of *Candida* species by TaqMan PCR. J. Clin. Pathol. **54:**362–366.
14. **Haynes, K. A., and T. J. Westerneng.** 1996. Rapid identification of *Candida albicans*, *C. glabrata*, *C. parapsilosis* and *C. krusei* by species-specific PCR of large subunit ribosomal DNA. J. Med. Microbiol. **44:**390–396.
15. **Jordan, J. A.** 1994. PCR identification of four medically important *Candida* species by using a single primer pair. J. Clin. Microbiol. **32:**2962–2967.
16. **Kanbe, T., T. Horii, T. Arishima, M. Ozeki, and A. Kikuchi.** 2002. PCR-based identification of pathogenic *Candida* species using primer mixes specific to *Candida* DNA topoisomerase II genes. Yeast **19:**973–989.
17. **King, D., J. Rhine-Chalberg, M. A. Pfaller, S. A. Moser, and W. G. Merz.** 1995. Comparison of four DNA-based methods for strain delineation of *Candida lusitaniae*. J. Clin. Microbiol. **33:**1467–1470.
18. **Kurzai, O., W. J. Heinz. D. J. Sullivan, D. C. Coleman, M. Frosch, and F. A. Muhlschlegel.** 1999. Rapid PCR test for discriminating between *Candida*

albicans and *Candida dubliniensis* isolates using primers derived from the pH-regulated PHR1 and PHR2 genes of *C. albicans*. J. Clin. Microbiol. **37:**1587–1590.
19. **Lehmann, P. F., D. Lin, and B. A. Lasker.** 1992. Genotypic identification and characterization of species and strains within the genus *Candida* by using random amplified polymorphic DNA. J. Clin. Microbiol. **30:**3249–3254.
20. **Liu, D., S. Coloe, S. L. Jones, R. Baird, and J. Pedersen.** 1996. Genetic speciation of *Candida* isolates by arbitrary primed polymerase chain reaction. FEMS Microbiol. Lett. **145:**23–26.
21. **Lockhart, S. R., S. Joly, C. Pujol, J. D. Sobel, M. A. Pfaller, and D. R. Soll.** 1997. Development and verification of fingerprinting probes for *Candida glabrata*. Microbiology **143:**3733–3746.
22. **Mannarelli, B. M., and C. P. Kurtzman.** 1998. Rapid identification of *Candida albicans* and other human pathogenic yeasts by using short oligonucleotides in a PCR. J. Clin. Microbiol. **36:**1634–1641.
23. **Melo, A. S., L. P. de Almeida, A. L. Colombo, and M. R. Briones.** 1998. Evolutionary distances and identification of *Candida* species in clinical isolates by randomly amplified polymorphic DNA (RAPD). Mycopathologia **142:**57–66.
24. **Meyer, W., K. Maszewska, and T. C. Sorrell.** 2001. PCR fingerprint: a convenient molecular tool to distinguish between *C. dubliniensis* and *C. albicans*. Med. Mycol. **39:**185–193.
25. **Molnár, O., R. Messner, H. Prillinger, U. Stahl, and E. Slavikova.** 1995. Genotypic identification of *Saccharomyces* species using random amplified polymorphic DNA analysis. Syst. Appl. Microbiol. **18:**136–145.
26. **Park, S., M. Wong, S. A. Marras, E. W. Cross, T. E. Kiehn, V. Tyagi, and D. S. Perlin.** 2000. Rapid identification of *C. dubliniensis* using a species-specific molecular beacon. J. Clin. Microbiol. **38:**2829–2836.
27. **Shin, J. H., F. S. Nolte, B. P. Holloway, and C. J. Morrison.** 1999. Rapid identification of up to three *Candida* species in a single reaction tube by a 5′ exonuclease assay using fluorescent DNA probes. J. Clin. Microbiol. **37:**165–170.
28. **Shin, J. H., F. S. Nolte, and C. J. Morrison.** 1997. Rapid identification of *Candida* species in blood cultures by a clinically useful PCR method. J. Clin. Microbiol. **35:**1454–1459.
29. **Steffan, P., J. A. Vázquez, D. Biokov, C. Xu, J. D. Sobell, and R. A. Akins.** 1997. Identification of *Candida* species by randomly amplified polymorphic DNA fingerprinting of colony lysates. J. Clin. Microbiol. **35:**2031–2039.
30. **Sullivan, D. J., T. J. Westerneng, K. A. Haynes, D. E. Bennet, and D. C. Coleman.** 1995. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. Microbiology **141:**1507–1521.
31. **Tamura, M., K. Watanabe, T. Imai, Y. Mikami, and K. Nishimura.** 2000. New PCR primer pairs specific for *Candida dubliniensis* and detection of the fungi from the *Candida albicans* clinical isolates in Japan. Clin. Lab. **46:**33–40.
32. **Taylor, J. W., D. M. Geiser, A. Burt, and V. Koufopanou.** 1999. The evolutionary biology and population genetics underlying fungal strains typing. Clin. Microbiol. Rev. **12:**126–146.
33. **Thanos, M., G. Schönian, W. Meyer, C. Schweynoch, Y. Gräser, T. G. Mitchell, W. Presber, and H.-J. Tietz.** 1996. Rapid identification of *Candida* species by DNA fingerprinting with PCR. J. Clin. Microbiol. **34:**615–621.
34. **Thomson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24:**4876–4882.
35. **Tietz, H.-J., A. Küssner, M. Thanos, M. Pinto de Andrade, W. Presber, and G. Schönian.** 1995. Phenotypic and genotypic characterization of unusual vaginal isolates of *Candida albicans* from Africa. J. Clin. Microbiol. **33:**2462–2465.
36. **Warren, N. G., and K. C. Hazen.** 1999. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 1184–1199. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, P. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology,7th ed. American Society for Microbiology, Washington, D. C.
37. **Williams, D. W., M. J. Wilson, M. A. O. Lewis, and A. J. C. Potts.** 1995. Identification of *Candida* species by PCR and restriction fragment length polymorphic analysis of intergenic spacer regions of ribosomal DNA. J. Clin. Microbiol. **33:**2476–2479.