JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1996, p. 615–621 Vol. 34, No. 3
0095-1137/96/$04.00+0

Rapid Identification of *Candida* Species by DNA Fingerprinting with PCR

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Received 7 August 1995/Returned for modification 10 October 1995/Accepted 7 December 1995

DNA polymorphisms in different species and strains of the genus *Candida* were assessed by amplifying genomic DNA with single nonspecific primers. This PCR method employed an arbitrary primer (the 10-mer AP3), a primer derived from the intergenic spacer regions (T3B), and the microsatellite primers $(GTG)_5$ and $(AC)_{10}$. Distinctive and reproducible sets of amplification products were observed for 26 different *Candida* and 8 other fungal species. The numbers and sizes of the amplification products were characteristic for each species. All yeast species tested could be clearly distinguished by their amplification patterns. With all primers, PCR fingerprints also displayed intraspecies variability. However, PCR profiles obtained from different strains of the same species were far more similar than those derived from different *Candida* species. By comparing species-specific PCR fingerprints of clinical isolates with those of reference strains, clinical isolates could be identified to the species level even if they could not be identified by routine biochemical methods.

The anamorphic yeast genus *Candida* includes many pathogenic species of yeasts that cause a variety of clinical syndromes in humans, ranging from superficial infection to invasive disease in immunocompromised patients. For compelling prognostic, epidemiological, and therapeutic reasons, it is essential to identify accurately the etiological species of a clinical isolate of *Candida*. Routine procedures for species identification involve the examination of colony and microscopic morphologies and the assessment of various biochemical reactions (50). Commercial carbohydrate assimilation systems are widely used to identify species of *Candida* or other genera of medically important yeasts. However, some of the biochemical reactions ascribed to certain species vary among different strains of the same species, and considerable strain variation may lead to problems in identification (53).

DNA-based methods have been reported to recognize *Candida* species in culture or in clinical material. Isolates of *Candida* have been identified by such methods as karyotyping by pulsed-field gel electrophoresis, the analysis of restriction fragment length polymorphisms, and Southern hybridization with appropriate DNA probes (16, 22, 23, 25, 38, 39, 41, 42, 46, 48). However, the characterization of *Candida* isolates by these techniques is laborious and time-consuming. To detect *Candida* species in clinical material, PCR assays have been based on the amplification of genes that encode actin (17), heat shock protein 90 (6), cytochrome P-450 lanosterol-a-demethylase (4, 5), and both nuclear (14, 15, 24, 35, 45) and mitochondrial (33, 34) ribosomal DNAs. Few of these assays are able to detect *Candida* species other than *C. albicans* (5, 24, 33, 34).

In the absence of specific nucleotide sequence information, species-specific DNA polymorphisms can be detected by PCR with single arbitrary primers differing in length and nucleotide composition. This technique of randomly amplified polymorphic DNA was originally described by Welsh and McClelland (51) and Williams et al. (54). We have successfully applied a similar method of PCR fingerprinting to biotype and analyze the epidemiological patterns of clinical strains of *C. albicans* (40) and other fungi (30–32). The PCR profiles of these isolates consisted of various species-specific and subspecies-specific amplification products. Lehmann et al. (21) and Niesters et al. (35) amplified species- and subspecies-specific polymorphic DNA fragments in an investigation of five and eight species of *Candida*, respectively. In this report, we describe species-specific PCR fingerprints that were produced by using different single primers to amplify genomic DNAs of 26 species of *Candida* and 8 other fungal species. By comparing PCR profiles derived from clinical isolates with those of reference strains, different species of *Candida* could be identified, even if they could not be identified with biochemical methods.

MATERIALS AND METHODS

Yeast isolates. The yeast strains used and their sources are listed in Tables 1 and 2. Yeasts were grown on Sabouraud glucose agar supplemented with chloramphenicol for 48 h at 37°C. The identification of isolates of *C. albicans* was confirmed by the production of germ tubes and by the formation of chlamydospores (50). Other species of yeasts were identified by their carbohydrate assimilation patterns on ID 32C strips (bioMerieux SA, Marcy-l'Etoile, France). The test strips were incubated for up to 48 h and evaluated both manually with the analytical profile index and spectrophotometrically with the ATB reader and corresponding identification software. Yeasts were stored on slants of Sabouraud glucose agar at 4°C or at −20°C.

DNA extraction. Genomic DNA was extracted by three different methods. To obtain large amounts of DNA, the method of Gruber (12) was used as described previously (40). Because only small amounts of DNA are needed for PCR fingerprinting, two minipreparation methods were used to isolate DNA from yeasts. First, by the method of Lee (20), isolates were inoculated from agar plates or slants into 5 to 7 ml of YPD medium (20 g of glucose per liter, 10 g of yeast extract per liter, and 10 g of pancreatic peptone per liter), and the tubes were rotated at 200 rpm overnight at 37°C. The cells were harvested by centrifugation (4,000 rpm for 5 min, Eppendorf centrifuge) at room temperature and resuspended in 500 μl of 1 M sorbitol solution containing 25 μg Lyticase (Sigma Chemical Co., St. Louis, Mo.). The mixture was incubated for 30 min at 37°C and centrifuged for 1 min, and the supernatant was discarded. The spheroblasts were resuspended in 0.5 ml of 3× TE buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA),

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TABLE 1. Strains used in this study[a]

Species	Strain[b]	Comment
Candida albicans	CBS 562^{NT}	Serotype A
	CBS 5983	Serotype B
	CBS 1905^{NT}	*C. stellatoidea* (synonym) (identical to ATCC 11006)
	ATCC 14053	
	ATCC 76615	
Candida castelli	CBS 4332^{T}	
Candida ciferrii	CBS 4856^{T}	
Candida famata	CBS 1795^{T}	*Torulopsis candida* (alternative)
Candida glabrata	NCYC 350	*Torulopsis glabrata* (alternative)
	MB 1010	
Candida guilliermondii	ATCC 6260^{T}	Identical to CBS 566^{T}
Candida intermedia	CBS 572^{T}	
Candida kefyr	Y 601	
	ATCC 4135^{T}	*C. pseudotropicalis* (synonym) (identical to CBS 607^{T})
Candida krusei	CBS 573^{T}	
Candida lambica	CBS 1876^{T}	
Candida lipolytica	CBS 599^{T}	
Candida lusitaniae	CBS 4413^{T}	
Candida norvegica	CBS 4238^{T}	
Candida norvegensis	CBS 1922^{T}	
Candida parapsilosis	CBS 604^{T}	
Candida pulcherrima	CBS 610^{T}	
Candida rugosa	CBS 613^{T}	
Candida sake	CBS 159^{T}	
Candida sphaerica	CBS 141^{T}	
Candida tropicalis	CBS 94^{T}	
Candida utilis	CBS 621^{T}	
Candida valida	CBS 638^{T}	
Candida viswanathii	CBS 4024^{T}	
Candida zeylanoides	CBS 619^{T}	
Clavispora opuntiae	CBS 7068^{T}	
Cryptococcus curvatus	CBS 570^{T}	Formerly *Candida curvata*
Cryptococcus humicolus	CBS 2041^{T}	Formerly *Candida humicola*
Cryptococcus neoformans	ATCC 3544	
Issatchenkia orientalis	MB 16	
Pichia carsonii	CBS 2285^{T}	
Pichia etchellsii	CBS 2011^{T}	
Saccharomyces cerevisiae	CBS 1171^{T}	
Saccharomyces kluyveri	CBS 3082^{T}	
Saccharomycopsis capsularis	CBS 2519^{NT}	
Trichosporon cutaneum	CBS 2466^{NT}	Formerly *Geotrichum cutaneum*, *Mycoderma cutaneum*

[a] Strains were obtained from the Centraal bureau voor Schimmelcultures (CBS), Baarn, The Netherlands, the American Type Culture Collection (ATCC), Rockville, Md., or the National Collection of Yeast cultures, (NCYC), Norwich, United Kingdom, except *C.* (*T.*) *glabrata* MB 1010, *C. kefyr* Y 601, and *I. orientalis* MB16, which were supplied by Pfizer Laboratories, Illertissen, Germany.

[b] NT, neotype culture; T, type culture.

25 μl of 20% sodium dodecyl sulfate solution was added, and the specimens were heated for 20 min at 65°C. After the addition of 400 μl of potassium acetate (5 M, pH 5.2), the lysates were stored for 30 min on ice and then centrifuged for 5 min. Seven hundred fifty microliters of the supernatant was mixed with the same volume of isopropanol for at least 30 min at room temperature. The pellet obtained by centrifugation for 5 min at room temperature was resuspended in 300 μl of TE buffer. The DNA was then precipitated by adding 40 μl of 3 M sodium acetate (pH 5.2) and 200 μl of isopropanol. After a quick centrifugation, the pellet was washed with 70% ethanol and air dried.

The second method of DNA extraction was the cetyltrimethylammonium bromide minipreparation method of Gardes and Bruns (10). A few yeast colonies were taken from an agar plate or slant and resuspended in 300 μl of extraction buffer (consisting of 100 mM Tris-HCl [pH 8.0], 1.4 M NaCl, 20 mM of EDTA, 2% cetyltrimethylammonium bromide, and 2% β-mercaptoethanol). The cells were weakened by three rounds of freezing and thawing and crushed with a micropestle. One volume of chloroform was added, and the mixture was vortexed and centrifuged at 13,000 × *g* for 15 min. The upper phase was removed, and the DNA was precipitated with ca. 600 μl of cold isopropanol. After centrifugation for 10 min, the pellet was washed with 70% ethanol and air dried. The DNA isolated by either method was dissolved in 1× TE buffer and stored at 4°C. The DNA concentration was estimated by measuring the optical densities at 260 and 280 nm.

PCR fingerprinting. The following oligonucleotides were used as single primers in the PCR experiments: the 10-mer oligonucleotide AP3 (5′-TCA CGA TGC A) (54), the simple repeat sequences $(GTG)_5$ (5′-GTG GTG GTG GTG GTG) (1) and $(AC)_{10}$ (5′-ACA CAC ACA CAC ACA CAC AC) (35), and T3B (5′-AGG TCG CGG GTT CGA ATC C) (26), which was derived from tRNA intergenic spacers. Amplification reactions were performed in volumes of 50 μl containing 1 to 25 ng of template DNA, reaction buffer (10 mM Tris-HCl [pH 8.0], 50 mM KCl, 1.5 mM $MgCl_2$, 3 mM magnesium acetate), 200 mM each dATP, dCTP, dGTP, and dTTP (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.), and 1.5 U of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Primer AP3, $(AC)_{10}$, or T3B was added at a final concentration of 25 pmol/50-μl assay mixture, and $(GTG)_5$ was used at 5 pmol/50-μl assay mixture. Samples were overlaid with sterile, light mineral oil (Sigma), and amplified in a thermocycler (Perkin-Elmer 9600) as follows: initial denaturation for 5 min at 95°C; denaturation for 15 s at 95°C; annealing for 30 s at 36°C for the AP3 primer, 50°C for the $(GTG)_5$ primer, 52°C for the T3B primer, or 54°C for the $(AC)_{10}$ primer; and extension for 1.20 min at 72°C. This was followed by a final extension cycle of 6 min at 72°C. Reaction tubes were kept at 4°C prior to analysis. The number of PCR cycles varied: 45 cycles were used with the AP3 primer, and 32 cycles were used with all other primers. Samples were concentrated to a volume of approximately 20 μl (Speed Vac; Savant, Hicksville, N.Y.) and electrophoresed in 1.2% agarose gels (5 mm by 25 cm by 20 cm) for 5 h at 3 V cm^{-1} in 0.5× TBE buffer (0.045 M Tris-borate [pH 8.3], 1 mM EDTA). The gels were stained with ethidium bromide and photographed, and the DNA fragments were sized and compared by the use of scanner-associated computer hardware and software (RFLPscan, version 2.01; Scanalytics CSP Inc., Billerica, Mass.). Similarity indices, representing the ratio of shared bands to total bands within two lanes being compared during a matching operation, were estimated for the different yeast species and for isolates of the same species.

RESULTS

Generation of species-specific fingerprints by using single nonspecific primers. To determine whether the PCR fingerprint technique could be employed for species identification, each sample of genomic DNA from 26 species of *Candida* and 8 other fungal species was amplified separately with four primers, the 10-mer AP3, the simple repeats $(GTG)_5$ and $(AC)_{10}$, and T3B, which was derived from the tRNA spacer. The PCR protocols used with different primers were optimized with regard to primer concentration, amount of template DNA, annealing temperature, and cycling program, as previously described (11). Each of the four primers generated different sets of amplification products, which varied in band positions and intensities. With all primers, different strains yielded up to 26 bands ranging from 0.2 to 4.0 kb in length. Figure 1 shows the PCR profiles of 26 different *Candida* species and 8 other species obtained with primers T3B and AP3. The number and sizes of the amplification products were characteristic for each species. Substantial interspecies variation was observed within the genus *Candida*, and all species could be clearly distinguished by their PCR fingerprints. When different *Candida* species were compared, similarity indices varied between 0.100 and 0.500.

To assess the stability of the PCR fingerprints, three strains, *C. albicans* CBS 5983, *C. albicans* ATCC 14053, and *C. famata* (*Torulopsis candida*) CBS 1795, were subcultured six times. The PCR fingerprints revealed identical amplification patterns. Figure 2 illustrates the PCR profiles of *C. famata* (*T. candida*) CBS 1795 after the first, third, and sixth passages. In addition, the method of DNA extraction had no influence on the PCR

TABLE 2. Clinical isolates used in this study[a]

Species	Strain	Site of isolation	Underlying disease	Geographic origin
C. albicans	Ca3	Oral cavity	AIDS	Berlin, Germany
	Ca4	Stool	AIDS	Berlin, Germany
	Ca5	Sputum	AIDS	Berlin, Germany
C. guilliermondii	Cgu1	Skin	Superficial candidiasis	Berlin, Germany
C. glabrata	Cg1	Oral cavity	Oral candidosis	Berlin, Germany
	Cg2	Blood	Sepsis	Berlin, Germany
	Cg3	Cerebrospinal fluid	Sepsis	Berlin, Germany
	Cg4	Oral cavity	Oral candidosis	Berlin, Germany
	Cg5	Vagina	Genital candidosis	Luanda, Angola
	Cg6	Vagina	Genital candidosis	Luanda, Angola
	Cg7	Vagina	Genital candidosis	Madagascar
	Cg8	Vagina	Genital candidosis	Madagascar
C. krusei	Ck4[b]	Vagina	Mucocutaneous candidosis	Freiburg, Germany
C. parapsilosis	Cp1[c]	Skin	Psoriasis	Frankfurt, Germany
C. tropicalis	Ct1	Endocardium	Endocarditis	Berlin, Germany
	Ct2[b]	Oral cavity	None	Freiburg, Germany
	Ct3	Vagina	Genital candidosis	Luanda, Angola
	Ct4	Vagina	Genital candidosis	Luanda, Angola
	Ct5	Vagina	Genital candidosis	Madagascar

[a] Except as indicated, all isolates were from the strain collection of the Department of Dermatology, Charité Hospital, Humboldt-University Berlin.
[b] Given to us by H. J. Grundmann, Institute of Environmental Medicine and Hospital Hygiene, University Freiburg/Br.
[c] Obtained from I. Menzel, Department of Dermatology, Hospital of the Johann-Wolfgang-Goethe-University of Frankfurt/M.

profiles (Fig. 2). Since it was quicker and simpler than the two other methods, the cetyltrimethylammonium bromide miniprep was used to extract DNAs from subsequent yeast isolates.

Variability of PCR fingerprints among strains of a given species. With all primers, the PCR fingerprints of different isolates of the same species showed different degrees of intraspecies variation. Different strains of *C. albicans* (CBS 562, CBS 5983, ATCC 14053, and CBS 1905) produced similar but not identical amplification patterns (Fig. 1A and C, lanes 2 to 5, respectively). When the PCR fingerprints of these strains of *C. albicans* were compared, the similarity indices varied between 0.884 and 0.955 with primer T3B, between 0.800 and 0.880 with primer AP3 (with the exception of CBS 1905, as shown below), and between 0.920 and 1.000 with primer $(GTG)_5$. Similar results were observed with additional clinical isolates of *C. albicans*, *C. krusei* (data not shown), and *C. tropicalis* and *Candida* (*Turolopsis*) *glabrata* (Fig. 3). Only minor differences in the PCR profiles of isolates from different geographical regions (Table 2) were found. With primer T3B, comparisons of the PCR fingerprints of clinical isolates of *C. albicans*, *C.* (*T.*) *glabrata*, and *C. tropicalis* yielded similarity indices that ranged from 0.837 to 1.000, from 0.760 to 0.982, and from 0.875 and 0.980, respectively. No intraspecies variation was found among the PCR fingerprints of seven strains of *C. parapsilosis*, although they were isolated from patients in different geographical areas.

PCR fingerprinting was helpful in establishing the identification of isolates in several yeast taxa. Similar but not identical PCR fingerprints were produced by all *C. albicans* strains, including the CBS 1905 strain (formerly *C. stellatoidea*), with primer T3B, which supports the classification of *C. stellatoidea* as a subspecies of *C. albicans* (Fig. 1A) (19). However, primer AP3 generated PCR fingerprints of *C. albicans* CBS 1905 (formerly *C. stellatoidea*) that exhibited more variation (Fig. 1C), and similarity indices of about 0.600 were calculated when the fingerprints were compared with those of all other *C. albicans* strains tested. This result with AP3 supports the observation that the genotype of *C. albicans* subsp. *stellatoidea* is somewhat different, as suggested by electrophoretic karyotyping (16, 19, 42, 53). The PCR fingerprints of *C. kefyr* Y 601 and ATCC 4135 (formerly *C. pseudotropicalis*) were highly similar (Fig. 4). With all four primers, a similarity index of 0.990 was calculated for these two isolates, which are considered to be synonymous (9, 29). A high degree of uniformity was also observed between the DNA of the anamorph *C. krusei* (CBS 573) and its teleomorph, *Issatchenkia orientalis* (MB 16) (Fig. 4). Among the four primers, the similarity indices for these strains were 0.830 to 0.980.

Identification of problematical clinical isolates of *Candida* by PCR fingerprinting. The production of species-specific DNA polymorphisms within the genus *Candida* by PCR fingerprinting prompted us to investigate whether this technique could be used to identify species of unknown clinical yeast isolates. Three *Candida* isolates (Table 2) were obtained from a patient with AIDS who received antimycotic therapy for 8 months. These strains grew very slowly and were resistant to fluconazole. They failed to produce chlamydospores on rice agar but formed germ tubes. With the ID 32C system, all three isolates generated a profile number (7367 1400) that did not correspond to any species-specific carbohydrate assimilation pattern. PCR fingerprints indicated that all three isolates were a single strain of *C. albicans* (Fig. 5, left).

Another *Candida* isolate was derived from a patient with superficial candidasis (Table 2). This strain was identified by the ID 32C system either as *C. famata* (*T. candida*), which is not known to cause cutaneous infections in humans, or *C. guilliermondii*, with a low discrimination value. PCR fingerprinting identified this isolate as *C. guilliermondii* (Fig. 5, left), which has been implicated as a cause of skin infections.

Another problematic strain of *Candida* was isolated from the skin of a patient with psoriasis (Table 2). This isolate could not be identified from its macroscopic and microscopic appearance. The carbohydrate assimilation profile produced by the ID 32C system suggested that the isolate was either *C. parap-*

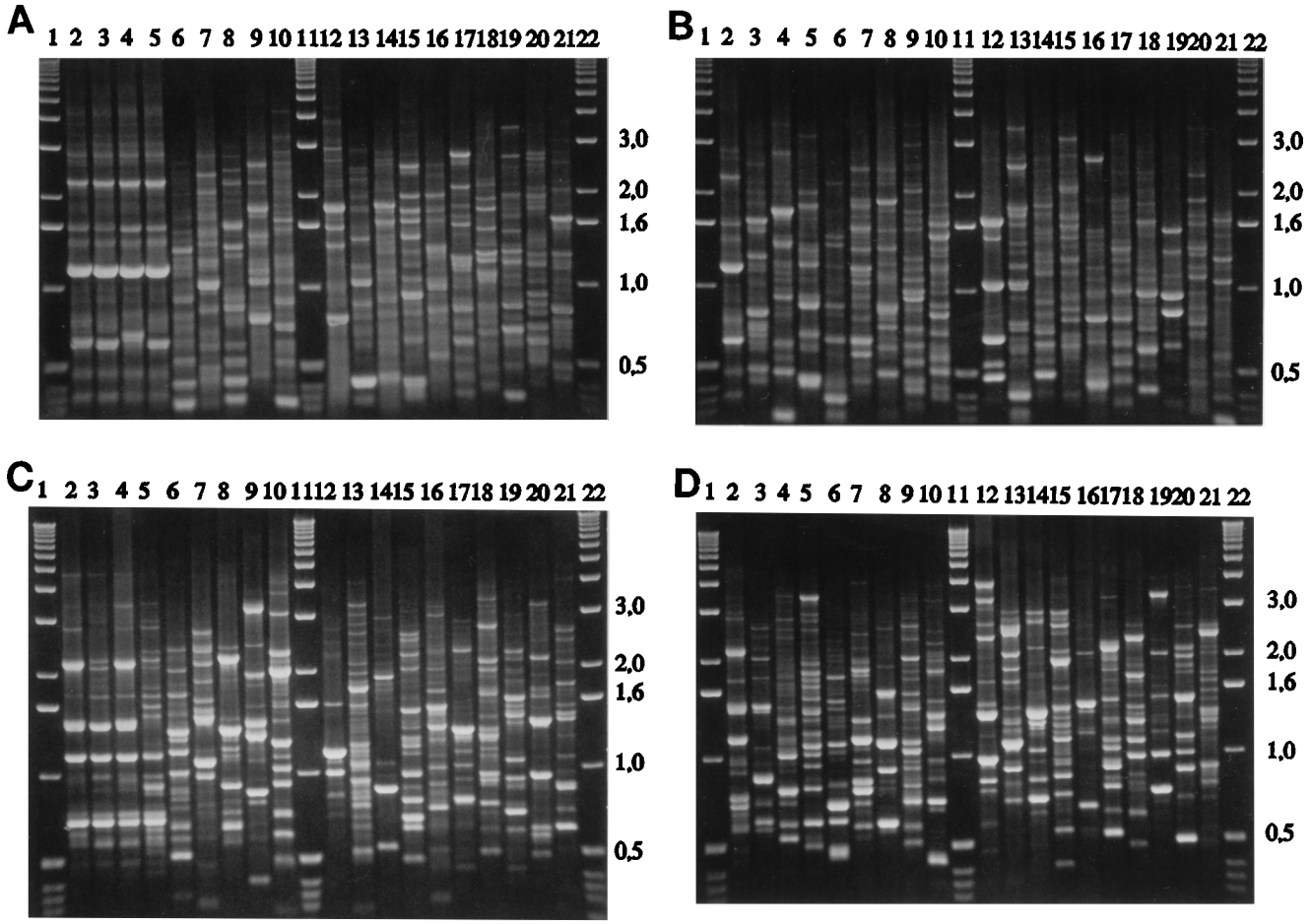

FIG. 1. PCR fingerprints of different *Candida* species obtained with primers T3B (A and B) and AP3 (C and D). (A and C) Lanes 2 to 5, *C. albicans* (ATCC 14053, CBS 562, CBS 5983, and CBS 1905, respectively); lane 6, *C.* (*T.*) *glabrata* NCYC 350; lane 7, *C. kefyr* Y 601; lane 8, *C. krusei* CBS 573; lane 9, *C. guilliermondii* ATCC 6260; lane 10, *C. parapsilosis* CBS 604; lane 12, *Cryptococcus humicolus* CBS 2041; lane 13, *C. ciferrii* CBS 4856; lane 14, *Cryptococcus curvatus* CBS 570; lane 15, *C. famata* (*T. candida*) CBS 1795; lane 16, *C. lipolytica* CBS 599; lane 17, *C. lusitaniae* CBS 4413; lane 18, *C. pulcherrima* CBS 610; lane 19, *C. rugosa* CBS 613; lane 20, *C. tropicalis* CBS 94; lane 21, *C. norvegensis* CBS 1922; lanes 1, 11, and 22, molecular size markers in kilobases. (B and D) Lane 2, *C. albicans* ATCC 14053; lane 3, *C. zeylanoides* CBS 619; lane 4, *C. intermedia* CBS 572; lane 5, *C. norvegica* CBS 4238; lane 6, *C. sake* CBS 159; lane 7, *C. sphaerica* CBS 141; lane 8, *C. utilis* CBS 621; lane 9, *C. valida* CBS 638; lane 10, *C. viswanathii* CBS 4024; lane 12, *C. castelli* CBS 4332; lane 13, *C. lambica* CBS 1876; lane 14, *Pichia etchellsii* CBS 2011; lane 15, *Pichia carsonii* CBS 2285; lane 16, *Trichosporon* (*Geotrichum*, *Mycoderma*) *cutaneum* CBS 2466; lane 17, *Saccharomyces cerevisiae* CBS 1171; lane 18, *Saccharomyces kluyveri* CBS 3082; lane 19, *Saccharomycopis capsularis* CBS 2519; lane 20, *Clavispora opuntiae* CBS 7068; lane 21, *Cryptococcus neoformans* ATCC 3544; lanes 1, 11, and 22, molecular size markers in kilobases.

silosis or *C. sake*. Comparison of the PCR fingerprints of this unknown isolate with those of the reference strains of *C. parapsilosis* and *C. sake* clearly indicated that the isolate was *C. parapsilosis* (Fig. 5, right).

DISCUSSION

Species of *Candida* continue to be a frequent source of hospital-acquired infections. Although *C. albicans* remains the most common agent of fungal infections, an increasing number of mycoses are caused by other species, including *C.* (*T.*) *glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. lusitaniae* (3, 18, 27, 28, 44, 47). Several *Candida* species, such as *C. lusitaniae*, *C. tropicalis*, *C. krusei*, *C. guilliermondii*, and *C.* (*T.*) *glabrata*, have also been shown to develop resistance during antifungal therapy or to possess intrinsic resistance to the antifungal agents (7, 8, 13, 27, 35, 36, 49, 55). Since pathogenicity and antifungal susceptibility often vary among species, a rapid and accurate identification of the disease-causing species of *Candida* is crucial for clinical treatment and epidemiological studies.

This report describes the assessment of DNA polymorphisms in different species and strains within the genus *Candida* by amplifying the genomic DNA with single nonspecific primers. This PCR method employed an arbitrary primer (the 10-mer AP3) (54), a primer derived from intergenic spacers (T3B) (26), and two microsatellite primers, $(GTG)_5$ (1) and $(AC)_{10}$ (35). With these four primers, distinctive and reproducible sets of amplification products were observed for all *Candida* species tested. All 26 *Candida* species, as well as 8 other fungal species, could be easily distinguished by their PCR fingerprint patterns. The discriminating powers of the four primers used in this study were nearly the same. However, more prominent and significant differences were observed among the profiles generated by primers T3B and AP3, which therefore might be better for the identification of *Candida* species than $(GTG)_5$ and $(AC)_{10}$. With all primers, the PCR fingerprints revealed variability among isolates of a single spe-

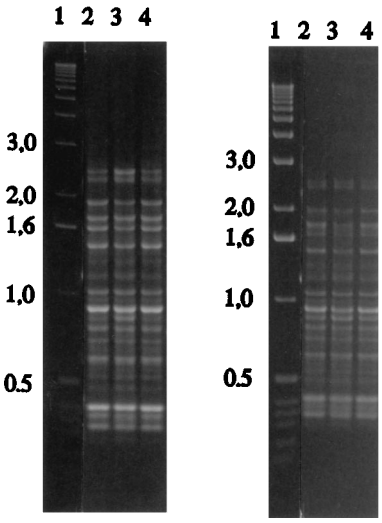

FIG. 2. To evaluate the stability of the PCR fingerprinting, DNA of *C. famata* (*T. candida*) CBS 1795 was passed through six generations in vitro. Lanes 2 to 4 (left), PCR profiles obtained after first, third, and sixth passages, respectively. The DNA of the same strain extracted by three different methods (see Material and Methods) yielded identical PCR patterns (lanes 2 to 4, right). Primer T3B was used in both experiments. Lanes 1, molecular size markers in kilobases.

cies. However, the variation among profiles obtained from different strains of the same species was far less than the variation observed among different species of *Candida*.

Comparison of PCR fingerprints of clinical isolates with those of reference strains enabled the identification of different *Candida* species, even if they could not be typed by biochemical methods. A fluconazole-resistant *Candida* isolate, which produced germ tubes but not chlamydospores and which could not be identified by the carbohydrate assimilation system ID 32C, yielded a PCR fingerprint characteristic for *C. albicans*. In two other cases, in which the ID 32C system was unable to differentiate between *C. famata* (*T. candida*) and *C. guilliermondii* and between *C. parapsilosis* and *C. sake*, the correct identification of *C. guilliermondii* and of *C. parapsilosis*, respectively, was determined from the PCR fingerprints. Recently, we described vaginal *C. albicans* isolates from Africa that were not able to utilize the aminosugars glucosamine and *N*-acetylglucosamine and that were therefore misidentified as *C. sake* by the ID 32C technique. The correct species identification was established only by PCR fingerprinting (43). A great advantage of the PCR technique is that it permits the species identification of clinical isolates with altered morphology, growth characteristics, and biochemical properties.

Very similar amplification patterns were produced by the anamorph yeast *C. krusei* and its teleomorph, *I. orientalis*. Whether this PCR assay could be used for identification of anamorph and teleomorph yeast pairs will require further investigation.

Our results agree with those of Lehmann et al. (21) and Niesters et al. (35), who used similar PCR fingerprint techniques to detect species-specific DNA polymorphisms within the genus *Candida*. Using a different set of single nonspecific primers [with the exception of primer $(AC)_{10}$, which was de-

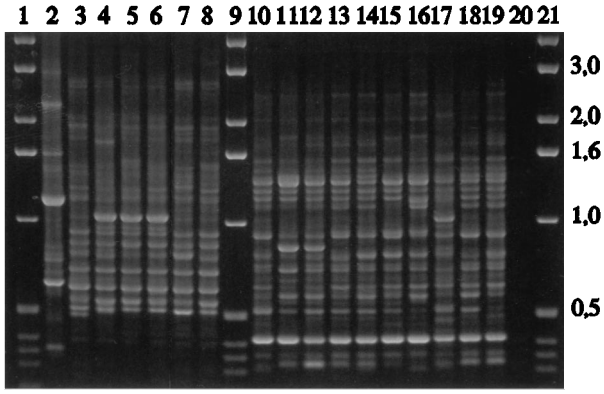

FIG. 3. PCR profiles of different *C. tropicalis* and *C.* (*T.*) *glabrata* strains amplified with primer T3B. Lane 2, *C. albicans* reference strain ATCC 14053; lane 3, *C. tropicalis* reference strain CBS 94; lanes 4 to 8, *C. tropicalis* wild-type strains (Ct1, Ct2, Ct3, Ct4, and Ct5, respectively), lanes 10 and 11, *C.* (*T.*) *glabrata* reference strains NCYC 350 and MB 1010, respectively; lanes 12 to 19, *C.* (*T.*) *glabrata* wild-type strains (Cg1, Cg2, Cg3, Cg4, Cg5, Cg6, Cg7, and Cg8, respectively); lane 20, control sample without DNA; lanes 1, 9, and 21, molecular size markers in kilobases.

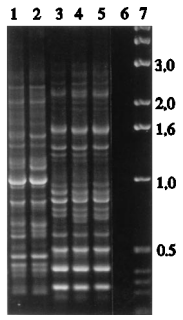

FIG. 4. Comparison of T3B-primed PCR fingerprints of the synonymous isolates *C. kefyr* Y 601 and ATCC 4135 (formerly *C. pseudotropicalis*) as well of the anamorphic yeast *C. krusei* and its teleomorph *I. orientalis*. Lane 1, *C. kefyr* Y 601; lane 2, *C. kefyr* ATCC 4135; lane 3, *C. krusei* CBS 573; lane 4, *C. krusei* clinical isolate Ck4; lane 5, *I. orientalis* MB 16; lane 6, control sample without DNA; lane 7, molecular size markers in kilobases.

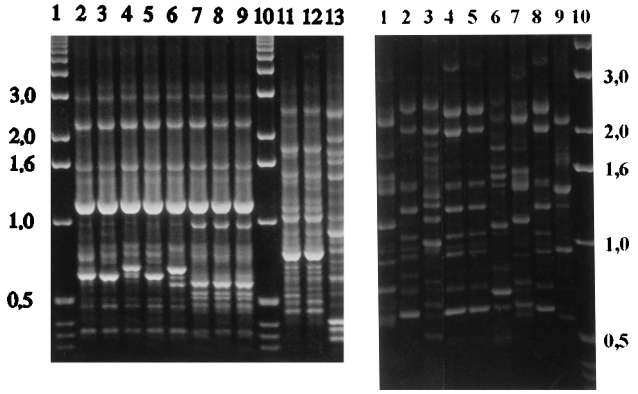

FIG. 5. Identification of clinical isolates by comparing their PCR profiles with those obtained from reference strains. (Left) Primer T3B. Lanes 2 to 6, *C. albicans* reference strains ATCC 14053, CBS 562, CBS 5983, CBS 1905, and ATCC 76615, respectively; lanes 7 to 9, fluconazole-resistant isolates derived from an AIDS patient (Ca3, Ca4, and Ca5, respectively); lane 11, *C. guilliermondii* reference strain ATCC 6260; lane 12, strain isolated from a patient with superficial candidiasis (Cgu1); lane 13, *C. famata* (*T. candida*) reference strain CBS 1795; lanes 1 and 10, molecular size markers in kilobases. (Right) Primer AP3, lanes 2, 5, and 8, isolate obtained from a patient suffering from psoriasis (Cp1); lane 1, *C. albicans* ATCC 14053; lane 3, *C.* (*T.*) *glabrata* NCYC 350; lane 4, *C. parapsilosis* CBS 604; lane 6, *C. sake* CBS 159; lane 7, *Cryptococcus neoformans* ATCC 3544; lane 9, *C. tropicalis* CBS 94; lane 10, molecular size marker in kilobases.

scribed first by Niesters et al. (35)], these groups reported species-specific amplification patterns for five and eight different *Candida* species, respectively. Extending the investigation to 26 different *Candida* species and 8 other fungal species, the present study confirms their suggestion that PCR fingerprinting with single primers could be successfully applied to species identification within the genus *Candida*. PCR fingerprinting is faster and simpler to perform than most other methods of genotypic analysis for species differentiation and has the advantage that a great variety of species can be identified by using the same methodology.

It has been suggested that PCR fingerprints might be accurate indicators of genetic distances because PCR fingerprinting randomly samples sequence polymorphisms distributed throughout the genome (52). Relatedness is deduced from the number of amplified fragments that two strains or species have in common. Importantly, these fragments are usually nonallelic, being scored simply as present or absent (2). However, as with restriction fragment length polymorphisms, the probabilities of losing and regaining a band are unknown, and length polymorphisms are usually indistinguishable from nucleotide substitution polymorphisms, so that some of the characters are not truly independent. We are currently comparing data obtained by the PCR fingerprint method with those derived from sequencing of the ribosomal DNA to determine whether PCR fingerprints may be applied to phylogenetic studies within the genus *Candida.*

ACKNOWLEDGMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft to G. Schönian and H. J. Tietz (Scho 448/3-1) and, in part, by grants from the Deutsche Forschungsgemeinschaft to W. Meyer (Me 1393/1-1) and from the National Institutes of Health to T. G. Mitchell (AI 28836).

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