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Detection of *Candida albicans* and Other Yeasts in Blood by PCR

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Primers complementary to the region of genes coding for rRNA in *Candida albicans* were used in PCRs to detect yeast DNA extracted from blood samples containing various *Candida* species. One fragment (105 bp) was amplified from all yeasts tested, whereas a second (684 bp) was only amplified when *C. albicans* DNA was present. The level of sensitivity was 15 ± 5 (mean ± standard error) CFU of *C. albicans* per ml of blood.

Candida species are common human commensals that can cause a wide spectrum of disease; of major concern is a hematogenously disseminated infection which is occurring with increased prevalence in postoperative and immunocompromised patients (5). Identification of candidemia takes a minimum of 2 days with an optimal blood culture system such as the lysis centrifugation method (4, 9), so there is a need for a rapid, sensitive, and specific test to aid in the diagnosis of the disseminated yeast infection. DNA-based diagnostic tests not only are sensitive and specific but also have the potential to decrease the time taken for the laboratory identification of pathogens that are slowly growing or difficult to culture. Such tests may detect nonviable and nonculturable cells as well as viable cells. Drug susceptibilities of *Candida* species vary; for example, most *C. krusei* strains are resistant to fluconazole (14). Therefore, earlier detection and identification of the infecting species in blood or biopsies would facilitate prompt, appropriate treatment.

A number of DNA sequences that are unique to *Candida albicans* and that may be suitable for diagnostic use have been identified (1, 3, 7, 8, 10–12, 16, 17, 19). Sequences encoding cytochrome P-450 lanosterol-14α-demethylase (1), mitochondrial DNA (12), and the secreted aspartyl proteinase (10) have been used in the PCR-based detection of *C. albicans* in blood, urine, or cerebrospinal fluid. Nucleic acid probes that hybridize to repeated sequences of *C. albicans* DNA may provide additional sensitivity, especially when combined with PCR amplification. We recently described two DNA fragments from the rDNA (genes coding for rRNA) repeat unit of *C. albicans* for use as DNA probes to detect *C. albicans* or other yeasts (7). One probe hybridized with all fungal DNAs tested, whereas the second was demonstrated to be a *C. albicans*-specific sequence.

Conserved sequences within the rDNA repeat region are attractive targets for PCR-based detection methods because the genome contains multiple copies, thus increasing the proportion of target DNA. Primers complementary to the conserved rDNA sequences would be suitable for the detection of many fungal species (8, 13, 15). The region also contains species-specific sequences (7, 13, 19), and the juxtaposition of conserved and unique sequences may enable the coamplification of fragments to identify both genus and species in a single PCR. In this paper, we describe a rapid and sensitive method for the detection of *C. albicans* or other yeasts in blood samples, based on PCR amplification of the 5S rDNA and the adjacent nontranscribed spacer (NTS) region in yeast chromosomal DNA. We also demonstrate the coamplification of two fragments containing these sequences from blood spiked with *C. albicans*.

The yeast strains used in this study are listed in Table 1. The identities and serotypes of the *Candida* strains were confirmed with an immune agglutination identification system (Iatron Laboratories, Tokyo, Japan). *C. albicans* A72, MEN, and Ci035 were serotype B; all other *C. albicans* strains were serotype A. Yeast strains were propagated on yeast-peptone-glucose agar, containing, per liter, 5.0 g of yeast extract, 10.0 g of Bacto Peptone (Difco Laboratories, Detroit, Mich.), 20.0 g of glucose, and 15.0 g of agar, and stocks were maintained at −80°C in yeast-peptone-glucose broth containing 15% (vol/vol) glycerol.

A simple DNA extraction method (7) was adapted so that PCR could be performed on yeast cell extracts without DNA precipitation steps. Yeast cells at concentrations of between 10 and 10^5 CFU/ml were suspended in 0.2 ml of 1 M sorbitol containing Zymolyase-100T (0.1 mg/ml) (Seikagaku Kogyo, Tokyo, Japan) and 2-mercaptoethanol (0.5% [vol/vol]), and the suspension was incubated for 2 h at 37°C to produce spheroplasts. Spheroplasts were treated with proteinase K (0.1 mg/ml) in the presence of *Taq* polymerase buffer (Amersham Corp., Arlington Heights, Ill.) for 2 h at 50°C and heated for 10 min at 95°C.

Whole blood was collected in tubes containing blood cell lysis reagents (Isolator 10 tubes; du Pont de Nemours and Co., Wilmington, Del.), and to portions of the blood, yeast cells were added (approximately 10 to 10^3 CFU/ml, as determined by viable cell counts on yeast-peptone-glucose agar). The yeast cells were recovered by a modification of lysis centrifugation (4). A portion of spiked blood (1.0 ml) was centrifuged (7,500 × *g*; 5 min), and the pellet was washed twice with 1.0 ml of 0.85% (wt/vol) NaCl before being layered onto a 0.5-ml Percoll (Pharmacia, Uppsala, Sweden) cushion (75% [vol/vol] in 0.85% [wt/vol] NaCl). Centrifugation (7,500 × *g*; 5 min) through Percoll pelleted the yeast cells yet excluded most of the blood material. This procedure reduced the concentration of blood components that were inhibitory to PCR. The saline and approximately 0.35 ml of the cushion were aspirated; the remaining cushion

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TABLE 1. *Candida* strains used

Species	Strain[a]	Source
C. albicans	ATCC 10261	American Type Culture Collection, Rockville, Md.
	3153	National Collection of Pathogenic Fungi, Central Public Health Laboratory, London, United Kingdom
	A72	A. Cassone (Instituto Superiore di Sanita, Rome, Italy)
	MEN	D. Kerridge (University of Cambridge, Cambridge, United Kingdom)
	Ci002, Ci011, Ci012, Ci035, Ci060, and Ci061	Dunedin Public Hospital and School of Dentistry, Dunedin, New Zealand
C. tropicalis	Ci014	Dunedin Public Hospital and School of Dentistry
	ATCC 13803	American Type Culture Collection
C. krusei	90.147	Communicable Disease Centre, Porirua, New Zealand
C. parapsilosis	90.493	Communicable Disease Centre
	90.454	Communicable Disease Centre
C. kefyr	78.1161	Communicable Disease Centre
C. glabrata[b]	CBS 138	Centraal bureau voor Schimmelcultures, Baarn, The Netherlands

[a] Ci, clinical isolate.
[b] *Torulopsis glabrata*

material, containing the yeast cell pellet, was vortexed, and a portion (0.1 ml) was used for the extraction of yeast cell DNA or for viable cell counts. Less than 5% of the yeast cells added to blood samples were lost during the centrifugation steps. Spheroplasts were formed as described above, and DNA was released by adding Chelex 100 resin (Bio-Rad Laboratories, Richmond, Calif.; 50% [wt/vol] in water; 50 μl) to the spheroplast suspension (50 μl) and heating the mixture for 8 min at 100°C. The addition of Chelex 100 reduced the sample preparation time and further reduced the inhibitory effects of blood components on PCR (18).

PCR amplification was performed directly on 10- or 20-μl portions of yeast cell extracts. The positions of the primers used for PCRs within the *C. albicans* rDNA region are shown in Fig. 1. Synthetic oligonucleotide primers (from DNA Express, Colorado State University) used for amplification of the yeast 5S rDNA fragment were 5'-AGT TTC GCG TAT GGT CTC CC (PCon1) and 5'-GTT GCG GCC ATA TCT AGC AG (PCon2). Those used for amplification of the *C. albicans* NTS fragment were 5'-TAG CGA TGA GGT AGT GCA AGT (PSpA1) and 5'-GCT GCA GCT ACG AAT GTT AG (PSpA2). The reaction mixture (0.1 ml) contained 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris hydrochloride (pH 8.0), 0.1 mg of gelatin per ml, deoxynucleoside triphosphates (125 μM each), primers (1.25 μM each), and 2.5 U of *Taq* DNA polymerase (Amersham). Amplification was carried out in a thermal reactor (Hybaid Ltd., Teddington, United Kingdom) at 95°C for 25 s, 55°C for 40 s, and 72°C for 50 s (32 cycles). *Taq* DNA polymerase was added after the reaction had reached 94°C and before the initiation

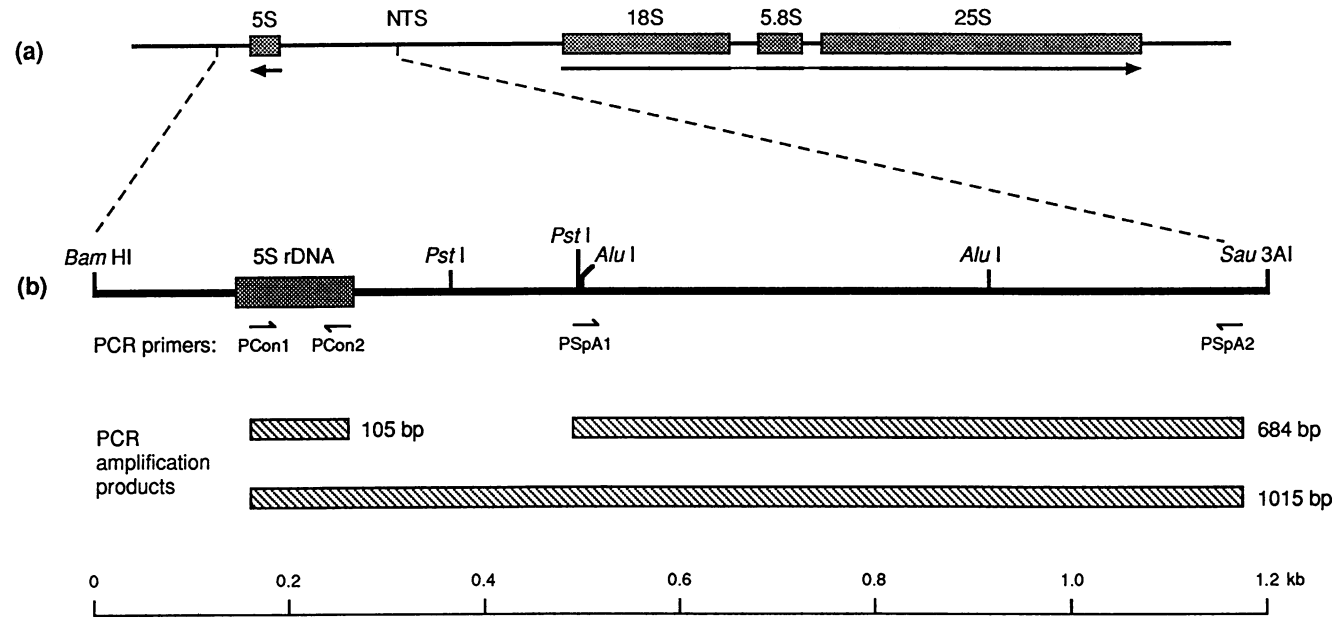

FIG. 1. (a) Physical map of the *C. albicans* rDNA region showing the positions of rRNA genes, the NTS region, and the direction of transcription of rRNAs (from reference 11). (b) Expanded map of the region showing restriction enzyme sites (from reference 2), the positions of primers used in PCR, and the predicted sizes of the amplified products. The 0.38-kb *Bam*HI-*Pst*I fragment containing the *C. albicans* 5S RNA coding region or the 0.41-kb *Alu*I fragment containing the *C. albicans* NTS region was used as a probe to confirm the identities of amplified products (see Fig. 2c and d).

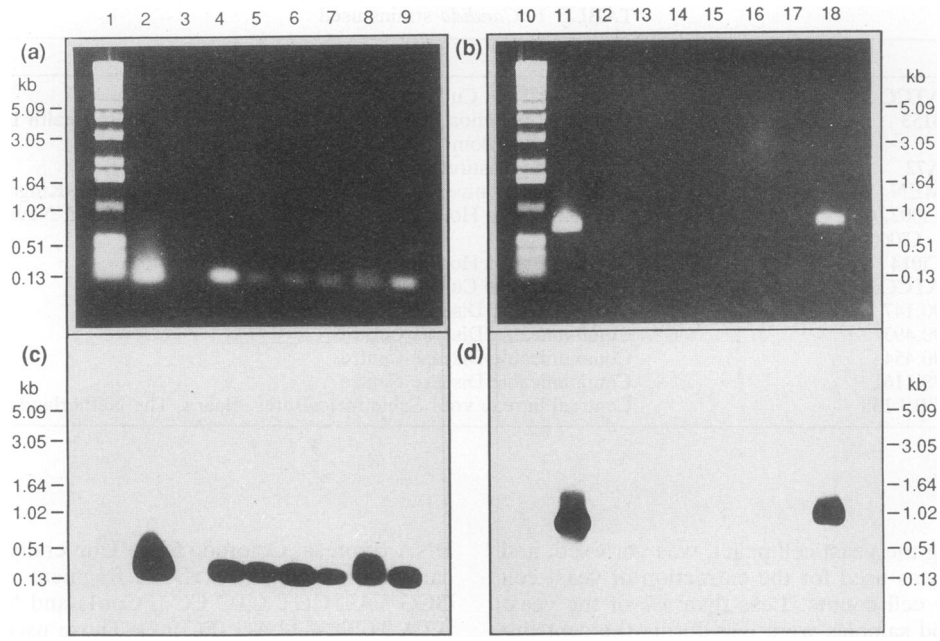

FIG. 2. PCR-amplified products obtained from yeast template DNAs (extracted from 10^5 cells) with primers PCon1 and PCon2 (lanes 2 to 9) or primers PSpA1 and PSpA2 (lanes 11 to 18). (a and b) Ethidium bromide-stained agarose gels. (c and d) Corresponding blots probed with a 0.38-kb *C. albicans* ATCC 10261 5S rDNA fragment (c) or a *C. albicans* ATCC 10261 0.41-kb NTS fragment (d). (Probes are shown in Fig. 1). Lanes contained the following DNA templates: 2 and 11, *C. albicans* ATCC 10261 (serotype A); 3 and 12, human lymphocyte; 4 and 13, *C. krusei* 90.147; 5 and 14, *C. parapsilosis* 90.493; 6 and 15, *C. kefyr* 78.1161; 7 and 16, *C. tropicalis* ATCC 13803; 8 and 17, *C. glabrata* CBS 138; and 9 and 18, *C. albicans* Ci035 (serotype B). Lanes 1 and 10 contained DNA markers (1-kb ladder; Life Technologies Inc., Gaithersburg, Md.).

of temperature cycling. Amplification with primers PCon1 and PCon2 produced a 105-bp fragment from all yeast species tested, including *C. albicans* (Fig. 2a). In contrast, primers PSpA1 and PSpA2 produced a fragment of the predicted size (684 bp; Fig. 1) only with template DNA from *C. albicans* (Fig. 2b). The 684-bp product was also obtained with DNA templates from eight additional strains of *C. albicans* (Table 1). Products of both pairs of primers were detected by ethidium bromide staining of gels when template DNA was prepared from *C. albicans* ATCC 10261 at a cell concentration of 15 ± 5 (mean ± standard error) CFU/ml. Purified human lymphocyte DNA (6) was ineffective as a template with either pair of primers.

To confirm the nature of the PCR products obtained, the amplified fragments were transferred by electroblotting onto a nylon membrane (Amersham Hybond-N+) and reacted with peroxidase-labeled (ECL gene detection system; Amersham) probes from cloned *C. albicans* rDNA. One probe, a 0.38-kb fragment containing the *C. albicans* 5S rDNA sequence (Fig. 1), hybridized only with products obtained with primers PCon1 and PCon2 (Fig. 2c). The second probe, a 0.41-kb fragment of the *C. albicans* NTS sequence (Fig. 1), hybridized only with products amplified with primers PSpA1 and PSpA2 (Fig. 2d).

The primers were then used in PCR to detect yeast cells added to human blood. Amplified products of the predicted size (approximately 100 bp) were obtained with primers PCon1 and PCon2 from extracts of blood spiked with *C. albicans* or with the other yeast species listed in Table 1. With primers PSpA1 and PSpA2, an amplified product of 684 bp was obtained only from extracts of blood spiked with *C. albicans*. Amplification products were not detected when blood without added yeast cells was extracted and used as a template in PCR. Figure 3 shows a representative ethidium bromide-stained agarose gel of products obtained from 10^3 or 15 ± 5 CFU of *C. albicans* (with PSpA1 and PSpA2 or PCon1 and PCon2) or 64 ± 8 CFU of *C. krusei* (with PCon1 and PCon2) added to blood (1.0 ml). The sensitivity of

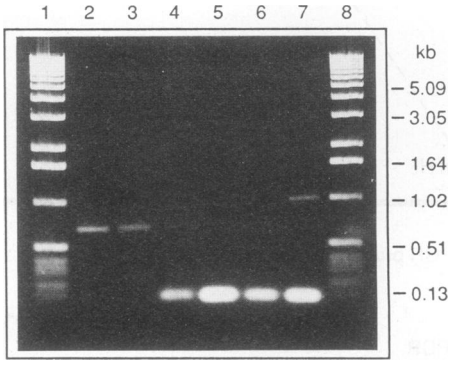

FIG. 3. Ethidium bromide-stained agarose gel of products amplified by PCR from blood spiked with cells of *C. albicans* ATCC 10261 or *C. krusei* 90.147. Lanes contained the following amplifications: 2 and 3, *C. albicans* DNA from 10^3 and 15 ± 5 (mean ± standard error) CFU per ml of blood, respectively, primers PSpA1 and PSpA2; 4, *C. albicans* DNA from 15 ± 5 CFU per ml of blood, primers PCon1 and PCon2; 5, *C. krusei* DNA from 64 ± 8 CFU per ml of blood, primers PCon1 and PCon2; 6, *C. krusei* DNA from 64 ± 8 CFU per ml of blood, primers PCon1, PCon2, and PSpA2; and 7, *C. albicans* DNA from 10^3 CFU per ml of blood, primers PCon1, PCon2, and PSpA2. Lanes 1 and 8 contained DNA markers (1-kb ladder).

detection (15 ± 5 CFU of *C. albicans* per ml of blood) was 10-fold higher than that achieved with hybridization detection of nonamplified yeast rDNA (7) and was also higher than the previously reported sensitivities of PCR amplifications for clinical specimens which detected 10^2 CFU of *C. albicans* per ml of blood (1), 10^5 CFU of *C. albicans* per ml of blood (19), or 10^2 CFU of *C. albicans* per ml of urine (12). Fifteen CFU/ml may not be the lower limit of sensitivity for *C. albicans*, but at this cell concentration the presence of PCR products correlated reproducibly with viable cell counts.

When primers PCon1, PCon2, and PSpA1 were used in a single reaction with *C. albicans* DNA extracted from spiked blood, two PCR products were obtained (Fig. 3, lane 7). The major fragment amplified (105 bp) was from the conserved 5S coding region. A smaller amount of a 1,015-bp fragment which included both the 5S and the NTS sequences (Fig. 1) was only amplified when *C. albicans* DNA was present. When *C. krusei* DNA from blood was used as a template with these three primers, only the 105-bp fragment was amplified (Fig. 3, lane 6). The same effects were observed when *C. parapsilosis*, *C. kefyr*, *C. tropicalis*, or *C. glabrata* was used in place of *C. krusei* (data not shown). Therefore, a species-specific sequence and a sequence common to at least six pathogenic yeasts may be coamplified in a single reaction. This possibility allows the detection firstly of the presence of a yeast and secondly of the presence of *C. albicans* by a single PCR amplification of a blood sample.

Future work will determine whether specificity and sensitivity similar to those obtained with spiked blood samples can be obtained with clinical specimens. In addition, it may be possible to extend this application to the detection of other clinically significant fungi. The development of strategies that enable yeast identification and species identification in one step will provide considerable time-saving benefits in the diagnosis of fungemias.

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