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Rapid PCR Method for Detecting *Candida albicans* Using Primers Derived from the Integrin-like Protein Gene *αINT1* of *Candida albicans*

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Oligonucleotide primers amplifying a 344 bp fragment on the integrin-like protein alpha-INT1p gene (*αINT1*) of *Candida albicans* were synthesized for screening of *C. albicans* from clinicalsamples by the polymerase chain reaction (PCR). The PCR specifically amplified DNA from *C. albicans* and none from any other *Candida*, fungal, or human DNA in standard strains used here. The PCR assay showed that the primers (LH1 and LH2) were specific for 26 isolates of *C. albicans* from clinical samples, whereas the positive fragment, 344 bp, was not amplified from 15 clinical isolates including 14 other medically important *Candida* species and an isolate of *Saccharomyces cerevisiae*. PCR was conducted on the urine samples of 20 patients and 4 samples were *C. albicans* positive. The detection limit of the PCR assay for *C. albicans* was shown to be approximately 10 cells/ml saline. The PCR system using 344 bp *αINT1* as a target is more specific and rapid than the conventional culture method, and the sensitive detection method is applicable to clinical diagnosis of *C. albicans* infections.

Key words: *Candida albicans,* PCR, αINT1p, integrin

The opportunistic pathogen, *Candida albicans*, is the most common cause of candidiasis in neonates, diabetics, and immunosuppressed patients (4) and the leading cause of infectious esophagitis in AIDS patients (15). In most cases *Candida* esophagitis recurs after 3 months, while in patients with AIDS, candidiasis is not cured easily. At the most serious level, the mortality rate from systemic candidiasis is as high as 50% (14). Thus, defining the most effective diagnostic and therapeutic approach to curing candidiasis in such patients is especially important. Using the classical culture method, identification of *Candida* takes several days, thus a more rapid, sensitive, and specific method is needed to diagnose the yeast infection. A rapid identification of yeasts provides timely information for patient management, which allows effective and early antifungal therapy. Classical methods that rely on cultivation of presumptive pathogens are time consuming and are often labor intensive. The polymerase chain reaction offers an alternative approach for the specific and rapid detection of pathogenic yeast. Procedures suitable for identifying *Candida* directly from clinical specimens are now emerging. They are based on genomic amplification methods and seem to detect and identify a large number of related species of pathogenic yeast using distinct target DNA sequences (9, 11). Immunological methods have been also reported as well (12). Several *C. albicans*-specific DNA sequences have been reported. These genes encode proteins such as the cytochrome P_{450} (1), actin (8), heat shock protein 90 (2), and mitochondrial DNA (13).

The existence of integrin-like protein in *Candida albicans* has been reported recently and the gene *αINT1* was cloned and its DNA sequence was reported (5, 6). It was clarified that *αINT1* was unique to *C. albicans*. The species-specific DNA sequences within the gene *αINT1* are attractive targets for PCR-based detection methods. Primers complementary to species-specific DNA sequences would be suitable for the detection of *Candida albicans*.

In this study, a PCR assay with the DNA fragment encoding an integrin-like protein as a target sequence is shown to be useful for specific identification and sensitive detection of *C. albicans* in clinical samples without DNA purification.

Materials and Methods

Strains

The strains of *C. albicans*, other *Candida* spp., *Saccha-*

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Table 1. Strains used in this study

	Species	No. of strains	Source
Standard strains	*Candida albicans*	1	ATCC 10231[b]
	Candida krusei	1	ATCC 32196
	Candida famata	1	ATCC 12790
	Candida kefyr	1	ATCC 9767
	Cryptococcus neoformans	1	ATCC 2344
Clinical isolates[a]	*Candida albicans*	26	
	Candida glabrata	7	
	Candida parapsilosis	5	
	Candida intermedia	1	
	Candida tropicalis	1	
	Saccharomyces cerevisiae	1	
Animal cell	Human cell, *Caco-2*		ATCC HTB-37

[a] Clinically isolated strains from Korea University Kuro Hospital
[b] American Type Culture Collection, Rockville, MD, USA

romyces cerevisiae, *Cryptococcus neoformans*, and human cell used are summarized in Table 1. Yeast strains were cultured on Sabouraud Dextrose Agar (SDA) from Difco (Detroit, USA).

Preparation of genomic DNA and processing of urine samples for PCR

The test strains were cultured on SDA and the DNA from the test strains was prepared as described by Holm *et al.* (7). Crude DNA of the human cell line was prepared according to the method described by Maniatis *et al.* (10). The crude DNA was used in the PCR assay. The PCR samples from urine specimens were prepared according to the method described by Miyakawa *et al.* (13). One milliliter of human urine was centrifuged at 10,000 $\times$ g for 10 min in an Eppendorf microcentrifuge, and the pellet was washed twice with distilled water. The pellets were resuspended in 100 μl of 1 M sorbitol-0.1 M EDTA (pH 8.0) containing 25 μg of Lyticase (Sigma, St. Louis, MO. USA) and 2% 2-mercapto-ethanol, and the mixture was incubated at 37°C for 15 min. After centrifugation at 10,000 $\times$ g for 2 min, the spheroplasts were washed twice with 1 M sorbitol-0.1 M EDTA (pH 7.5). The spheroplasts were suspended in PCR buffer containing 0.1% Nonidet P-40 and 0.1 mg of proteinase K (Sigma, St. Louis, MO. USA) per ml, and incubated at 55°C for 30 min. The samples were heated at 95°C for 10 min to remove enzyme activity and then used for the PCR assay.

PCR

PCR was performed as follows: the reaction solution consisted of 2.5 μl of PCR reaction buffer (50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3)), 100 ng of target genomic DNA, 200 μM dNTP, 1 μM of each primer, 0.5 U *Taq* polymerase, and sterilized distilled water to a final volume to 25 μl. The synthetic oligonucleotides used were primer LH1 (5'-AGC CAC AAC AAC AAC AAC AAC TCT) and LH2 (5'-TTG AGA AGG ATC TTT CCA TTG ATG), which were derived from the integrin-like protein alpha-INT1p (*αINT1*) gene of *C. albicans* (GenBank Accession number U35070). The PCR was performed in a Peltier Thermal Cycler (PTC-200, MJ Research Inc. Watertown, MA. USA). The first cycle was 3 min of denaturation at 92°C and this was followed by 30 cycles, each consisting of 1 min of denaturation at 92°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C. At the final cycle, an additional 10 min of incubation at 72°C was performed for complete extension. For each PCR run, a negative control was also included. The PCR product was detected by electrophoresis on a 2% agarose gel using TAE buffer (40 mM Tris acetate, 1 mM EDTA (pH 8.4)), the gel was stained with ethidium bromide and photographed under UV light.

Results and Discussion

Species specificity

The PCR primers, LH1 and LH2, are based on sequence of the gene encoding the integrin-like protein alpha-INT1p from *C. albicans* (11). The *C. albicans* gene *INT1* is similar to vertebrate leukocyte integrins (12). Primer LH1 is located between nucleotides 401 and 424 on the sequence, while primer LH2 is located between 721 and 744 nucleotide sequence. These primers have been evaluated for specificity in detecting *C. albicans*. The primers amplified

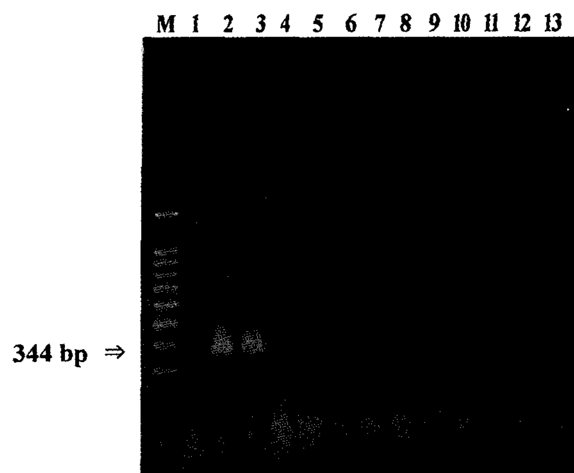

Fig. 1. PCR-amplified products. Lanes contained the following samples: 1, negative control; 2, positive control with *C. albicans* ATCC10231; 3, *C. albicans* from urine sample; 4, *C. famata* ATCC 12790; 5, *C. krusei* ATCC 32196; 6, *C. kefyr* ATCC 9767; 7, *C. tropicalis*; 8, *C. intermedia*; 9, *C. parapsilosis*; 10, *C. glabrata*; 11, *Cryptococcus neoformans* ATCC 2344; 12, *Saccharomyces cerevisiae*; 13, human cell (*Caco-2*) ATCC HTB-37; M, DNA marker. Lanes 7, 8, 9, 10, and 12 are from clinical isolates as described in Table 1.

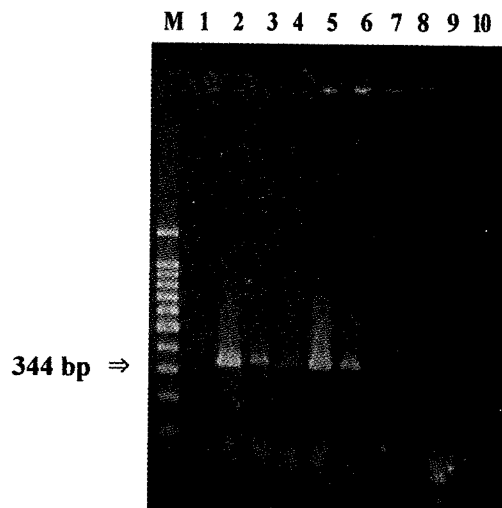

Fig. 2. PCR-amplified products from clinical urine samples. Lanes contained the following samples: 1, negative control; 2, positive control with *C. albicans* ATCC10231; 3 to 6, *C. albicans* positives from urine samples; 7 to 10, *C. albicans* negatives from urine samples; M, DNA marker.

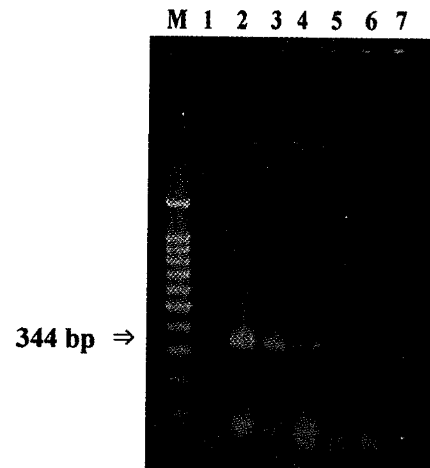

Fig. 3. Sensitivity of DNA detection by PCR of *C. albicans* cells in saline, determined by ethidium bromide staining. Template DNAs were prepared from 1ml of saline containing cells as follows: 1, no cell; 2, 10^4 cells/ml; 3, 10^3 cells/ml; 4, 10^2 cells/ml; 5, 10 cells/ml; 6, 1 cell/ml; 7, 1 cell/ml; M, DNA marker.

a fragment 344 bp in length (Fig. 1) from the *C. albicans* strain. On the other hand, the primers did not amplify a product from the other *Candida* species, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and the human colorectal adenocarcinoma *Caco-2* cell line. Using the primers, PCR was performed with 41 clinical samples. The 344 bp fragments were detected in 26 clinical isolates of *C. albicans* and the positive fragment was amplified in neither 14 other medically important *Candida* species nor an isolate of *Saccharomyces cerevisiae* (data not shown). These results suggest a possibility for the clinical application of the primers.

Identification of C. albicans from clinical specimens

We investigated whether the species-specific DNA product from PCR could be used to identify *C. albicans* from clinical specimens which contain yeast strains. Among 20 urine samples, 4 samples were *C. albicans*-positive by the PCR assay and *C. albicans* was isolated from 3 samples by the culture method (data not shown). Fig. 2 shows the result from 4 positive samples with 4 negative urine specimens, and the data of the other 12 negative urine samples is not shown here. The result of the PCR assay and culture method showed a significant correlation in this study. The PCR assay proved to be more sensitive than the classical culture method and to be more useful for the clinical diagnosis of *C. albicans*. More trials are needed concerning the detection of *C. albicans* in clinical samples other than urine. However, it is expected that the PCR assay with the LH1 and LH2 primers may be a useful and valuable tool for the detection of *C. albicans* in the clinical sample, especially when the growth of *C. albicans* inhibited by the treatment with antifungal agents.

Sensitivity of detection for C. albicans DNA from cells in saline by PCR assay

To examine the detection sensitivity, 10-fold dilution of *C. albicans* cells in saline was made. The PCR samples were prepared from *C. albicans* cells in saline with the method described in Materials and Methods. The detection limit of *C. albicans* cells was approximately 10 cells/ml in saline by the PCR assay described here (Fig. 3). Dupont reported that approximately 10^3 yeast cells per milliliter of urine were suspected to colonize in asymptomatic patients with renal candidiasis (3). It has been reported that the detection limit of the saline sample in the PCR assay was 10-fold lower than that of the urine sample by ethidium bromide staining (13).

Many candidiasis patients have been lost due to the insufficient amount of time used to treat the disease. This is due to the lack of simple, rapid, and sensitive identification procedures. Since there is no need for culture and DNA purification using this method, it can be conducted in several hours using intact yeast cells. The other advantage of this method is that only 10 cells can be detected from saline, showing high sensitivity. Thus, the PCR procedure with the primers, LH1 and LH2, would be useful for detecting *C. albicans* cells in clinical samples.

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