Discrimination among the Clinical Isolates of Candida albicans by Amplification of the Repetitive Sequences, alts

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Received August 4, 1997; in revised form, November 10, 1997. Accepted December 3, 1997

Abstract: A primer pair, PB and BSH, which amplified alts, a portion of Candida albicans-specific repetitive sequence, RPS, gave stable and reproducible fingerprint patterns of the strains by polymerase chain reaction (PCR). We applied this method to clinical isolates of C. albicans for strain discrimination. Using PCR fingerprint patterns, we could analyze the relatedness of C. albicans strains including those isolated from children with leukemia and their bedside parents. The results indicated that PCR analysis targeting an alt region gives rise to the same conclusion as the previous study obtained by SmaI RFLP analysis.

Key words: PCR, Candida albicans, Repetitive sequence

Genetic typing methods such as pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphism (RFLP) of total DNA, and Southern hybridization with DNA probe have been prevalent in the epidemiological studies of Candida albicans (11, 12). In addition, recently, typing methods by PCR have been used to determine genetic variations among C. albicans strains (10, 14). For Southern blot analysis, mid-repeat sequences Ca3 or 27A have been most frequently used as a probe to assess the genetic relatedness of commensal and infecting strains in C. albicans, and these sequences contain homologous regions to the C. albicans-specific repetitive sequence, RPS element (1, 8). RPS was found during research on the karyotype variation among the strains (6), and it was assumed to be involved in chromosome rearrangement (4, 6). It was composed of 2,114 bp in length, and contained several inner repeats of a short stretch (172 bp), alt, whose repeating number gave rise to variation in the molecular sizes of RPSs (3) (Fig. 1). The distribution of the sizes of RPSs that were intrinsic to each respective strain allowed us to use a RPS as a probe for delineation of the C. albicans strains (5). In a previous study (5), we demonstrated the relationship between strains taken from patients and those from their bedside parents, suggesting the occurrence of horizontal transmission of the yeast.

Here, we developed a PCR assay directing short repetitive sequences, alts, for fingerprinting and applied it to clinical isolates as well as the same isolates used in our previous study to compare its usefulness with other methods such as RFLP for strain delineation (5). By reconfirming our previous results, we could demonstrate that PCR using alts sequences as a target is a faster, simpler and more reliable method for the delineation of C. albicans strains.

The 55 clinical strains of C. albicans, NUM and TCH strains, including one laboratory stock strain (FC18) and three C. stellatoidea strains (IFO1397, IFO0692 and IFO1398) were used. Thirty of the 55 strains of C. albicans were isolated from different individuals. The other yeast strains, C. glabrata (TIMM1063), C. guilliermondii (NUM4), C. parapsilosis (NUM303), C. tropicalis (NUM37), C. kefyr (IFO0586), C. krusei (IFO0013), Saccharomyces cerevisiae (X2180) and Schizosaccharomyces pombe (HM422) were also used as reference species. All NUM strains were isolated at Nagoya University Hospital. TCH strains were isolated in the Gynecology Department of Chita City Hospital. Cells grown aerobically in YPD (2% glucose, 2% peptone, and 1% yeast extract) in the middle of the loga-

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Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.
rhythmic phase were harvested and subjected to the isolation of genomic DNA. The growth temperature was 30°C throughout the experiment. The template DNA was prepared as described by Schonian et al (13).

A primer pair for the PCR, PB and BSH, was designed to amplify alts in the RPS in C. albicans chromosomes. PB was a sequence from the position 733 to 749 within an alt region in the RPS and BSH was from 1436 to 1421, outside of the alt (Fig. 1). PCR was performed with a PJ-2000 thermal cycler (Takara Shuzo, Kyoto, Japan). The 20 µl of reaction solution contained 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 50 mM KCl, 200 µM of each dNTP, 50 pmol each primer, 2.5 units of Taq DNA polymerase (Takara Shuzo) and 25 ng template DNA. The reaction mixture was subjected to an initial denaturation at 94°C for 7 min. The conditions of amplification were as follows; 30 cycles of 20 sec at 95°C, 60 sec at 50°C and 45 sec at 72°C. Control tubes with no template DNA were included in each run. Amplified products were resolved by agarose gel electrophoresis, photographed and subjected to image analysis by an IS-1000 digital imaging system (Alpha Innotech Corp., Calif., U.S.A.).

Amplification with the primer pair, PB and BSH, worked only in C. albicans. The PCR products resolved into 3 to 7 bands ranging in size from 200 to 1,500 bp in each strain by electrophoresis, respectively. A band around 560 bp in size was in common with all 30 strains from different individuals. The region above 1,500 bp in the gel was not resolved into bands but appeared as smears. As it was confirmed that these smears were not hybridized with genes coding for the RPS1 probe (6), only the bands ranging in size from 200 to 1,500 bp were taken into account for PCR fingerprinting.

Next, we applied this method to 30 strains of C. albicans isolated from different individuals to see whether these strains could be differentiated. To estimate the banding patterns of these strains, we took into account two parameters, the intensity and position of each band by image analysis. Thus, we could differentiate the 30 strains into 28 banding patterns (data not shown). This result indicated that the PCR amplification method using PB and BSH as a primer set could be used for strain lineage analysis as well as the RPS labeling method that we had adopted previously.

We analyzed the PCR fingerprint patterns of 28 strains from 3 patients with leukemia and their parents, and compared them with the results of our previous report, obtained by other molecular typing methods (5). Figure 2 shows the PCR fingerprint patterns of multiple strains from patient A and his mother. The PCR fingerprint patterns, positions and intensities of the bands were identi-
cal among the strains from patient A and his mother, whereas the karyotypes of several strains were different (5). The results obtained by PCR were consistent with the results of RFLP by Smal digestion, and those of Southern blot analysis using a RPS probe (5). As a result, PCR analysis using the PB and BSH primer pair also indicated a close relatedness between strains from patient A and his mother. The results also showed that the variation in karyotypes did not affect the PCR fingerprint pattern. Similar observations have been reported in comparative studies of the molecular typing methods for C. albicans (2, 14).

Figure 3 shows the PCR fingerprint patterns of strains from patient B and her mother, and patient C and her mother (CM). Numbers above the lane denote the origins of the strains as in the following examples. Number 1 indicates the strain of the first recovery, number 2 indicates the second recovery. PCR pattern from a reference strain, FC18, is shown in the right panel. Numbers on the left indicate the size of PCR products estimated from the molecular size markers Hinf1-digested fx174 and Styl-digested DNA.

Furthermore our method is more advantageous in that it uses a single primer pair, whereas other established PCR methods require the use of a combination of multiple primers for the differentiation of a number of C. albicans isolates (9, 10, 14).

References


