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Variation of Electrophoretic Karyotypes among Clinical Isolates of *Candida albicans*

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Orthogonal-field-alternation gel electrophoresis was used to compare clinical isolates of *Candida albicans* by resolving chromosome-sized DNA molecules into an electrophoretic karyotype. Seven to nine bands were observed among isolates recovered from 17 patients. In addition, 14 distinct electrophoretic patterns were noted among the isolates from these patients. In a given individual, isolates were likely to have identical electrophoretic patterns. Therefore, the electrophoretic karyotype patterns demonstrated by orthogonal-field-alternation gel electrophoresis can be used to designate a strain for epidemiologic studies.

Candida albicans is an important cause of a wide spectrum of diseases in compromised human hosts. Our knowledge of variation among strains of this yeast has been limited to patterns of phenotype characteristics. Strain definition and characterization depend on phenotypic biotyping schemes (10, 11, 18, 19, 29), resistograms (8, 9, 26), killer types (14), and mitochondrial and genomic DNA restriction endonuclease polymorphisms (13, 20). In *Saccharomyces cerevisiae*, on the other hand, genetic approaches have proven to be important to define strains with specific genotypes and to determine linkage groups and chromosome number. Use of these same genetic techniques with strains of the medically important yeast *C. albicans*, however, has not been possible due to the polyploid nature of this organism and its inability to induce meiosis to complete an alternatively haploid-diploid life cycle. Recently, convincing data have been reported to support the fact that *C. albicans* is a stable diploid organism. Evidence for this conclusion has come from different approaches including genetic analysis of mitotic recombinants (4, 15, 16, 19, 23, 28), DNA content studies (12, 17, 27), and molecular biological studies (5).

Very little is known about the organization of genes on chromosomes in *C. albicans*, although limited genetic linkage groups have been established between a small number of genes by studying induced mutations, heterokaryon production, and mitotic crossing over events. The definitive chromosome number of *C. albicans* is unknown due to the lack of genetic data from a sexual cycle, the inability to visualize the condensed chromosomes during the metaphase, and the inability, until recently, to separate intact DNA molecules larger than 50 kilobases (kb).

Separation of large-molecular-size DNA (1,000 kb) was accomplished by a pulsed-field electrophoresis method developed by Schwartz and Cantor (21). With conventional gel electrophoresis (unidirectional electrophoretic field), DNA molecules greater than 50 kb comigrate. With pulsed-field electrophoresis, larger DNA molecules are separated based, theoretically, on their ability to relax and realign through the agarose pores in a changing electric field. Orthogonal-field-alternation gel electrophoresis (OFAGE) has been optimized to resolve 15 of the 16 chromosomes as chromosome-sized DNA molecules of *S. cerevisiae* (1, 2, 21). It has been used to directly map specific genes to chromosomes, to determine ploidy, and to demonstrate chromosome length polymorphisms. OFAGE has also been used to determine an electrophoretic karyotype of other eucaryotic organisms, including the protozoans *Plasmodium falciparum*, *Leishmania braziliensis*, and *Trypanosoma brucei*, as reviewed by van der Ploeg (24).

Pulsed-gel electrophoresis has recently been applied to investigate the chromosome-sized DNA bands of certain medically important yeasts (3, 6, 7, 22, 25). Studies of multiple strains of *C. albicans* by OFAGE (7) have estimated that the chromosome number is 9 or 10 and have shown variation among the five strains tested. Field-inversion gel electrophoresis also has been used to resolve chromosome-sized DNA bands of *C. albicans*, and five distinct chromosomal mobility groups have been described with eight strains (6).

The goals of the present study were to (i) define a standard electrophoretic karyotype of *C. albicans* by optimizing OFAGE conditions which resolve chromosome-sized DNA molecules, (ii) determine the extent of variations in electrophoretic karyotypes among clinical isolates of *C. albicans*, and (iii) determine whether an electrophoretic karyotype could be used as a reliable genetic marker to define strains of *C. albicans* for epidemiologic purposes.

MATERIALS AND METHODS

Organisms. Stock reference strains of *C. albicans* included B311-10 and V6, a germ tube-negative variant of strain B311-10, kindly provided by Helen Buckley, Temple University (Philadelphia, Pa.), and a diploid strain of *S. cerevisiae* (JH-SC-7). Test strains of *C. albicans* (34 isolates) recently recovered from clinical specimens submitted to the Mycology Laboratory of The Johns Hopkins Hospital from 17 different patients were also studied. The 34 isolates included multiple isolates recovered from 12 patients and single isolates recovered from 5 patients. Identification of *C. albicans* was based on the production of germ tubes by all isolates and compatible sugar assimilation patterns for many of the isolates. All isolates were grown at 30°C on Sabouraud dextrose agar and were maintained at 4°C.

Preparation of yeast DNA for OFAGE. The method for preparation of intact yeast DNA was modified from that of Schwartz and Cantor (21). Yeast were grown for 18 h at 30°C on a rotatory wheel in 5 ml of yeast nitrogen broth (Difco Laboratories, Detroit, Mich.) supplemented with 2% glu-

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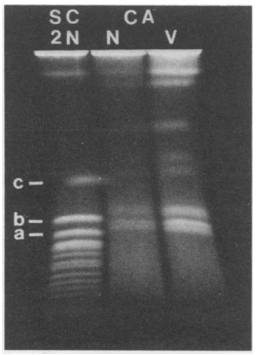

FIG. 1. Chromosome-sized DNA bands of a reference diploid strain of *S. cerevisiae* (SC) and the reference strain *C. albicans* (CA) B311-10 (N) and the germ tube-negative variant V-6 (V). Band a, *S. cerevisiae* chromosome 10; size, ~950 kb; band b, *S. cerevisiae* chromosomes 11; size, ~1,110 kb; band c, *S. cerevisiae* chromosome 4; size, ~1,580 kb. Ethidium bromide stain was used.

cose. Cells were centrifuged, washed 2 times with 50 mM sodium EDTA–10 mM Tris (Tris-EDTA; pH 7.5), and suspended in 0.15 ml of the Tris-EDTA in 2.2-ml microcentrifuge tubes (PGC Scientific, Gaithersburg, Md.). Each tube was placed in a 39°C temperature block after the addition of 1 μl of a 20-mg/ml stock of zymolyase (20,000 units/g; Seikagaku Kogyo Co., Tokyo, Japan) in 10 mM Na_2HPO_4 (pH 7.5). To each tube, 0.25 ml of 1% low-melt agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in 0.125 M sodium EDTA (pH 8.0) was added, mixed gently, and then placed immediately into ice. The agarose plugs were incubated with 0.4 ml of 0.5 M sodium EDTA–0.01 M Tris (pH 7.5) for 18 to 24 h at 37°C. The plugs were then transferred to polystyrene tubes (12 by 75 mm; Becton Dickinson Labware, Oxnard, Calif.), and 0.4 ml of 2 mg of proteinase K (Boehringer GmbH, Mannheim, Federal Republic of Germany) per ml in 0.5 M sodium EDTA–0.01M Tris (pH 7.5)–1% *N*-lauroyl sarcosine was added. The plugs were kept at 50°C for 18 to 24 h and then washed 4 times with Tris-EDTA before they were stored at 4°C.

OFAGE. Small portions of agarose plugs with yeast DNA were melted at 65°C, and approximately 25 μl was loaded into dry wells in 1% agarose in 0.5× TBE (44 mM Tris, 44 mM boric acid, 2 mM sodium EDTA [pH 8.3]). OFAGE was performed as described by Carle and Olson (1). The circulating buffer was 0.5× TBE maintained at 10°C, and electrophoresis was conducted with 5-min alternating pulses of 120 V for 48 h. Gels were stained with ethidium bromide (1 μg/ml in distilled water) for 10 min and then washed in 200 ml of distilled water for 30 min. DNA bands were visualized with short-wave UV light (254 nm).

RESULTS

The chromosome-sized DNA bands of a diploid strain of *S. cerevisiae* and two related strains of *C. albicans* are presented for comparison in Fig. 1. At least 13 ethidium bromide-stained DNA bands were observed with the *S. cerevisiae* strain under these conditions of electrophoresis, whereas 8 bands were observed with both *C. albicans* isolates. The majority of the DNA bands of *C. albicans* migrated more slowly than 10 of the *S. cerevisiae* bands, and DNA was retained in the wells, as evidenced by ethidium bromide staining. The electrophoretic DNA patterns of the two variants of the same *C. albicans* strain were identical; all eight DNA bands migrated identically.

OFAGE results for single isolates recovered from 8 of the 17 patients are presented in Fig. 2. There were differences observed in both the number of bands visualized and the electrophoretic patterns among the isolates. The number of discrete DNA bands among the isolates from all 17 patients varied from seven to nine. Seven, eight, and nine bands were noted among isolates from 10, 5, and 2 patients, respectively. Therefore, three distinct groups of organisms, based solely on the number of discrete chromosome-sized DNA bands, could be separated.

There was also significant variation in the electrophoretic patterns observed among the *C. albicans* recovered from the 17 patients. Within the 10 strains with seven bands, seven different patterns were discernible; within the 5 strains with eight bands all five had distinct patterns; and the 2 strains with nine bands were also distinct. Therefore, a total of 14 electrophoretic patterns were distinguishable among single isolates from the 17 patients. Of the 14 patterns, 3 were represented twice (i.e., strains from three pairs of patients were indistinguishable). One identical pair was from two patients hospitalized in the same hospital unit at the same time.

Multiple isolates (seven patients with two isolates and five patients with three isolates) of *C. albicans* recovered from different body sites or from the same body site on different days within a single patient were tested. Results of the karyotypes within an individual are presented in Table 1, and Fig. 3 illustrates the identity of electrophoretic karyotypes among isolates from individual patients. No variation in electrophoretic pattern was noted within isolates tested from 10 of the 12 individuals. Differences were noted in the electrophoretic patterns of isolates recovered from two patients. Each patient had isolates with two different electrophoretic patterns. Strains from four patients from whom multiple isolates were recovered from blood and other body sites were evaluable. In all four cases, the electrophoretic patterns of the *C. albicans* isolates from blood were identical with patterns for isolates from throat, urine, or stool specimens from the same patient.

The chromosome-sized DNA bands of *C. albicans* recovered from 17 patients revealed three groups of DNA bands based on their migration. Starting from the bottom of the gels, the first distinct group consisted of two to four smaller DNA bands. There was another group of between one and four bands which were larger than the first group, and finally,

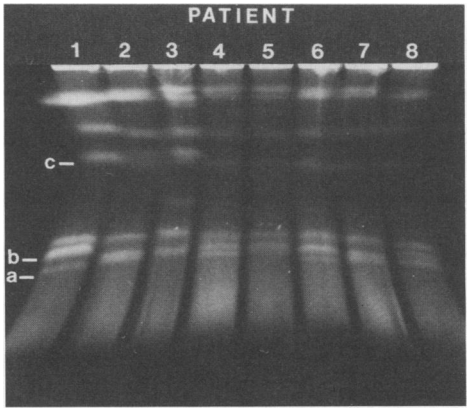

FIG. 2. Chromosome-sized DNA bands of single isolates recovered from eight representative, individual patients. Band a, ~950 kb; band b, ~1,110 kb; band c, ~1,580 kb. Ethidium bromide stain was used.

there was a group of between one and three bands which migrated at the top of the gels closest to the wells. In addition, there were differences in the widths or intensities of individual bands.

DISCUSSION

Chromosome-sized DNA bands of *C. albicans* were resolved by OFAGE. By using the conditions standardized in this study, preliminary conclusions on two major areas of importance were drawn. First, the electrophoretic patterns provided an initial insight into the genetic makeup of *C. albicans*; and second, electrophoretic karyotypes exhibited dramatic variations among clinical isolates from different patients, yet a relative stability of the karyotypes of isolates obtained from the same patient.

The sizes of the *C. albicans* DNA bands were probably greater than 1,200 kb, as determined by comparison with migration of *S. cerevisiae* chromosomes of known size. The larger DNA bands were probably in the 1- to 2-megabase range in size, in agreement with the results of Vollrath and Davis (25). This is an assumption, since the mobility of large molecules of DNA may not completely be related to molecular size, and DNA standards larger than 1,000 kb were not available.

The chromosome number of *C. albicans* can only be estimated by OFAGE. Seven to nine bands were visualized among the 34 clinical isolates of *C. albicans* tested. Analysis of these patterns suggests that *C. albicans* has a minimum of six to seven chromosomes. The group of smaller DNA bands could be consistent with the presence of a minimum of two chromosomes; some strain homologs comigrated, while in other strains homologs could be resolved. Similarly, the central group of larger DNA bands could also be consistent with the presence of two chromosomes, and the extremely large group could represent two or three chromosomes. These numbers depend on the assumptions that two different chromosomes did not comigrate and that the two less intense bands that were resolved could be homologs of a single chromosome that migrated differently due to chromosome length polymorphisms. Recognition of these as homologs that migrated differently due to polymorphisms will require chromosome-specific probes. In addition, larger, more complex chromosomes may not have entered the gel under the conditions tested. The present results are consistent with those of Snell and Wilkins (22), Magee and Magee (7), and Lott et al. (6), in that chromosome-sized DNA bands of *C. albicans* were resolved. Differences in the number of bands may be due to different electrophoretic conditions, and the determination of a definitive chromosome number will require specific probes or strains capable of completing a sexual cycle.

TABLE 1. Electrophoretic karyotypes of multiple isolates recovered from individual patients[a]

Patient no.	Karyotype of isolate recovered from the following body sites[b]:				Maximum no. of days between recovery of isolates	No. of distinct karyotypes
	Blood	Urine	Throat	Stool		
1	S		S, D		10	2
2			S	S	2	1
3			S	S	2	1
4			S	S	2	1
5			S	S	3	1
6	S	S	S		5	1
7	S		S	S	9	1
8	S, S				2	1
9	S, S, S				2	1
10	S	S	D		8	2
11	S, S				2	1
12	S, S, S				10	1

[a] Isolates were recovered from different body sites or from the same body site on different days.
[b] Abbreviations: S, same karyotype; D, different karyotype.

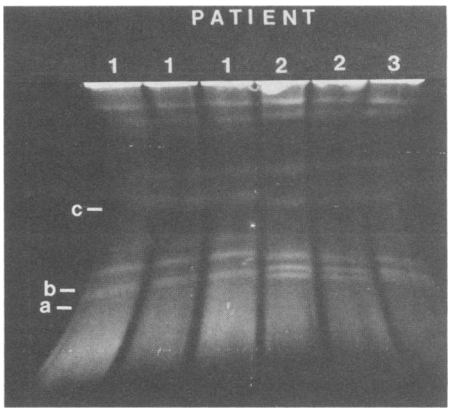

FIG. 3. Chromosome-sized DNA bands from three representative patients; for patient 1, three isolates were recovered from blood over 10 days; for patient 2, two isolates were recovered from blood 1 day apart; and for patient 3, one isolate was recovered from blood. Band a, ~950 kb; band b, ~1,110 kb; band c, ~1,580 kb. Ethidium bromide stain was used.

The electrophoretic separation and identification of chromosomes of *C. albicans* will permit genetic studies that were not previously possible. The location of specific genes by use of Southern blot analysis with DNA-DNA hybridization procedures will open new avenues for the genetic analysis of *C. albicans*. Specific genes on specific chromosomes can then be mapped, and linkage groups can be established. Investigation of the chromosomes may also aid in determining the ploidy (or aneuploidy) and variation among strains of *C. albicans*.

The chromosome-sized DNA bands permitted the establishment of an electrophoretic karyotype for *C. albicans* based on the number of bands visualized and the patterns of bands under standard conditions. A major finding was the significant variation in the electrophoretic karyotype noted when *C. albicans* isolates recovered from different patients were compared. Fourteen discrete patterns were noted among single isolates tested from the 17 patients in this study. The differences in electrophoretic karyotypes reflected both the total number and differences in the electrophoretic mobilities of the DNA bands. This dramatic variation in the genetic makeup among these strains of *C. albicans* (presumably due to translocations, deletions, etc.) reflects variation within this asexual species. This variation permits definition of a strain based on a reproducible genetic marker, the electrophoretic karyotype. Interestingly, in the present study, of the three pairs of patients with *C. albicans* strains exhibiting similar electrophoretic karyotypes, one pair of patients was hospitalized in the same hospital unit at the same time. It is possible that these two strains are genetically identical and of common origin. The diversity of electrophoretic karyotype patterns may permit investigations of hospital outbreaks.

In general, all isolates of *C. albicans* recovered from an individual patient had identical electrophoretic karyotypic

patterns. This was true in a total of 10 of 12 patients when multiple isolates recovered from two or more body sites or the same site at two different times were tested, implying that most patients were colonized by only one genetic strain. Only two patients had isolates of *C. albicans* with different electrophoretic patterns. When *C. albicans* caused deep infection or fungemia, the isolates recovered from the blood had the same electrophoretic pattern as isolates from colonization sites recovered before infection developed.

Use of an electrophoretic karyotype to delineate a strain of *C. albicans* has advantages over methods based on phenotypic characteristics. The most important advantage is the extremely high degree of variability observed among strains isolated from different patients. In the present study, significant variation in *C. albicans* (14 distinct strains recovered from 17 patients) was demonstrated by use of electrophoretic karyotyping. The total number of distinct electrophoretic karyotypes that exists among all clinical isolates of *C. albicans* is probably even much greater. This variation will permit very specific strain delineation so that epidemiologic studies that were previously not possible can be performed. In contrast, with existing biotyping techniques, only 8 biotypes among 200 isolates (29) and a total of only 13 strains from 27 patients based on resistograms (9) have been noted. The small number of distinct biotypes may limit the use of these methods for definitive epidemiologic investigations, whereas killer systems (14) may also provide significant variation for strain delineation. Another advantage of OFAGE is that the procedure requires only a single test, not a battery of conventional biochemical or inhibitory reactions. Disadvantages of the use of electrophoretic karyotyping are the need for equipment not frequently found in clinical laboratories and the limitation that it is only able to include a limited number (~10) of isolates per gel with appropriate controls.

In conclusion, OFAGE was shown to be a powerful tool for investigating the genomic DNA of *C. albicans* and can be applied to other medically important yeasts. OFAGE or other pulsed-gel electrophoresis techniques will permit analysis of chromosome organization, determination of linkage groups, and definition of genetically defined strains that may be used as an epidemiologic tool.

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