Electrophoretic Karyotyping of Typical and Atypical Candida albicans

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Electrophoretic karyotypes of atypical isolates of Candida albicans, e.g., strains that were germ tube negative, failed to express proteinase activity, demonstrated low virulence for mice, formed hyperchlamydoconidia, produced hyperhyphae, or were sucrose negative (including the type strain of Candida stellatoidea), were compared with those of typical C. albicans. Karyotypes of whole-cell DNA of classical C. albicans examined with transverse alternating-field electrophoresis under specific conditions were composed of seven DNA bands with a specific migration pattern. Certain atypical strains and representatives of the three serotypes of C. stellatoidea produced discrete karyotypes with 5 to 10 bands. All isolates demonstrated a significant degree of DNA relatedness, suggesting their conspecificity. Densitometric tracings of DNA bands provided an objective and standardized method for comparing bands within the gels.

The increased incidence of deep candidiasis, particularly as an often fatal iatrogenic disease, has stimulated interest in developing more finite procedures for epidemiological studies of the most common etiological agent, Candida albicans. Various methods, including differences in streak morphology (2), resistance to chemicals (31), a combination of physiological characteristics and resistances to chemicals (20, 21), differences in carbohydrate assimilation (33), serotyping (5), and sensitivity to yeast toxins (25), have been proposed for subdividing C. albicans into subgroups or biotypes. Some success has been achieved, but in general the methods recognize either too many or an insufficient number of subgroups for practical use. In certain instances the procedures have lacked reproducibility in different laboratories (10, 22).

The development of karyotyping data and endonuclease digestion patterns of genomic DNA has recently offered more fundamental and possibly more reproducible biotyping capabilities (27). Lott et al. (11) observed, by using field inversion gel electrophoresis, five distinct chromosomal mobility groups among isolates of C. albicans. Seven to nine bands with 14 distinct electrophoretic patterns were observed by Merz et al. (15), who used orthogonal-field alternating-gel electrophoresis in an epidemiologic study of isolates of C. albicans from different patients; and Magee et al. (12) resolved up to 11 DNA bands by applying contour-clamped homogeneous-field gel electrophoresis, orthogonal-field alternating-gel electrophoresis, and field inversion gel electrophoresis. These procedures in conjunction with gene probes led Magee et al. to postulate the presence of seven chromosomes. Lasker et al. (9), with a similar protocol in a study of two isolates, indicated the presence of at least eight chromosomes. Kwon-Chung et al. (7, 8) with an orthogonal-field alternating-gel electrophoresis procedure found distinct karyotype patterns for two types of Candida stellatoidea (considered herein a variety of C. albicans). One form, type II, had seven DNA bands in a pattern similar to that of C. albicans serotype A; the other, type I, serotype B, had eight or nine bands.

In most investigations, conditions for obtaining karyotypes have not been fully described, and only strains readily identified as C. albicans or C. stellatoidea have been investigated. Atypical forms of C. albicans that lack classical identification characteristics have been of increasing incidence among yeast cultures submitted to the Division of Mycotic Diseases, Centers for Disease Control, for identification. Karyotyping of atypical C. albicans and the use of a transverse alternating-field electrophoresis (TAFE) system has not been reported. In this study we compare TAFE karyotypes of whole-cell DNA of classical and atypical C. albicans.

MATERIALS AND METHODS

Cultures. Isolates were selected from cultures submitted for identification to the Division of Mycotic Diseases, Centers for Disease Control (C isolates), from the American Type Culture Collection (A isolates), and from the culture collection at Georgia State University (G isolates). The yeast isolates were screened with the API 20C (Analytab Products, Plainview, N.Y.) yeast system and identified by standard assimilation and fermentation tests and morphological studies of pseudomycelium, chlamydospores, and germ tubes (1, 30). The sources and identifications of the cultures are presented in Table 1. Cultures were lyophilized within 1 month of their submission to Georgia State University, and working stocks were maintained on Sabouraud dextrose agar.

Biotyping. Selected characteristics, namely, resistance to fluconazole and saframycin, citrate utilization, pH tolerance, and proteinase production from the procedures of Odds and Abbott (20), were employed for biotyping. Proteinase production was detected by the bovine serum albumin-agar plate method of Rüchel et al. (26). Serotypes were determined with the Candida Check RM 302 kit (Iatron Lab, Inc., Tokyo, Japan). Strains previously serotyped by standard procedures were employed as controls (29).

Virulence to mice. C. albicans strains were grown in 50 ml of Sabouraud broth on a reciprocal shaker (200 rpm) at 37°C for 24 to 48 h. Cells were harvested by centrifugation at 6,000 x g for 10 min and washed three times with normal saline (0.9% [wt/vol] NaCl). The inoculum preparation consisted of cells suspended in saline to an optical density of 0.12 at 600...
nm (about 2 × 10^6 cells per ml). CFW White Swiss mice from Charles River Laboratories (Wilmington, Mass.) were inoculated with 0.1 ml of cell suspensions via the tail vein. Autopsy was performed on mice that died and mice that were sacrificed 14 days after inoculation.

**DNA characteristics.** DNA was isolated by the method of Marmur (13) as modified by Meyer and Pfaff (17) and the base compositions (moles percent guanine plus cytosine) were determined at least twice for each strain by the thermal denaturation method (14). The DNA relatedness of selected strains was obtained by the procedures of Seidler and Mandel (28) as modified by Kurztman et al. (6).

**Karyotyping by TAFE.** Gel electrophoresis of whole-cell DNA was determined by a modification of the method of Lott et al. (11). Cells grown for 14 to 18 h in 20 ml of 1% (wt/vol) yeast extract–2% (wt/vol) peptone–1% (wt/vol) glucose at 30°C were harvested by centrifugation, washed twice, and suspended at approximately 1:10 (vol/vol) in 0.05 M EDTA (pH 7.5, adjusted with NaOH). To 1 ml of this cell suspension, 100 μl of Zymolyase 60 T (Miles Laboratories, Inc., Naperville, Ill.; 2 mg/ml in Tris-EDTA buffer) and 1.0 ml of 1.0% (wt/vol) LE agarose (Beckman Instruments, Inc., Palo Alto, Calif.) in 0.125 M EDTA were added. This suspension was vortexed and poured into molds (4 by 9 by 2 mm). After the agarose in the molds solidified, the solidified agarose plugs were incubated overnight at 37°C in 0.5 M EDTA–0.01 M Tris (pH 7.5) with 7.5% (wt/vol) 2-mercaptoethanol. The plugs were rinsed with sterile distilled water and incubated for an additional 12 to 18 h at 50°C in 0.5 M EDTA–1% lauryl sarcosine–0.01 M Tris (pH 9.5) containing 2 mg of proteinase K (Sigma Chemical Co., St. Louis, Mo.) per ml. The excess effluent was removed, and the plugs were rinsed with sterile distilled water and were stored at 4°C until processed, which was always within 2 weeks. The agarose plugs were cut into smaller plugs to fit the size of the wells in gel plates (1% LE agarose in TAFE buffer). The smaller plugs were held in sterile distilled water for about 1 h at room temperature before they were loaded into the wells of the gel plates. The wells with plugs were sealed with melted agarose, and the gel plates were placed in the vertically positioned track in the chamber of a TAFE system (Beckman) filled with 1 × TAFE buffer. The temperature of the chamber was held at 10 to 13°C, and the voltage, switch time, and running time were varied for the optimal resolution of DNA bands. Gels were stained with ethidium bromide, destained in distilled water, and photographed under UV light.

The lanes on the negatives (a 1.6-mm width in the center of the lane, every 20 μm of migration distance) were scanned with an Ultrascan XL enhanced laser densitometer (LKB Produkter AB, Bromma, Sweden). The data obtained were analyzed with the computer software Gelscan XL (LKB) under the following conditions: peak width for peak search, 8; area rejected, 1.0; integration method, signal; and base line option, common.

**RESULTS**

**Phenotypes.** The characterization of the isolates is presented in Table 1. Sucrose-negative *C. albicans* strains were considered *C. albicans* var. *stellatoidea*. This variety is indicated by the API 20C profile that terminates in the digits 50. Other variations in profile numbers that indicated differences in assimilation of glucose, xylose, arabinose, xyitol, and methyl-d-glucoside fell within the profile codes for possible *C. albicans*. The assimilation and fermentation patterns (data not shown) of all strains by standard tests (30) were in general agreement with those established for *C. albicans*. Some reactions that were negative with the API 20C system (e.g., xylose assimilation) were positive in the liquid assimilation media. Isolates that readily produced germ tubes and chlamydospores and expressed virulence for mice, in conjunction with compatible physiological characteristics (e.g., G-30, G-M-44), were considered classical or typical isolates of the species. These typical isolates were proteinase positive, showed tolerance to pH 1.4, were citrate positive, and were resistant to safranin and fluocytosine. These properties grouped the typical isolates with two of the more common biotypes in the scheme of Odds and Abbott (20). These characteristics for distinguishing the common biotypes were not always reproducible; e.g., distinction of a positive or negative reaction was arbitrary for the atypical strains of *C. albicans*. All strains readily expressing proteolysis, except for strain C-655, a hyphal form, were virulent for mice. A representative of classical *C. albicans* (G-30) produced in mice intensive tissue invasion and necrosis. The yeast was recovered from the kidney, heart, and spleen and occurred in tissues in hyphal and yeast forms. Details of the virulence of
TABLE 2. G+C content and DNA relatedness of selected isolates of C. albicans

<table>
<thead>
<tr>
<th>Paired DNA (mol% G + C)</th>
<th>% DNA relatedness</th>
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<tbody>
<tr>
<td>G-364 (36) + G-30 (35)</td>
<td>100</td>
</tr>
<tr>
<td>G-655 (36) + G-364 (36)</td>
<td>100</td>
</tr>
<tr>
<td>C-448 (36) + G-30 (35)</td>
<td>95</td>
</tr>
<tr>
<td>A-11006 (34) + G-1114 (36)</td>
<td>98</td>
</tr>
<tr>
<td>G-1114 (36) + G-30 (35)</td>
<td>91</td>
</tr>
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this strain (G-30, also known as CA 30) for mice were presented by Cole et al. (3). Isolate G-364 (germ tube negative), even in conjunction with an adjuvant (gastric mucin), was recoverable from the kidney of only 1 sacrificed mouse out of the 10 examined. Microscopic examination of tissues from this mouse revealed negligible tissue invasion. The recovered yeast isolate showed the same characteristics (i.e., no germ tubes, proteinase negative, etc.) as the inoculum.

The moles percent G+C of the DNA and DNA reassociations of selected isolates are given in Table 2. Classical C. albicans G-30 showed significant (>90%) DNA relatedness with the representative morphologically and physiologically atypical strains, including C. albicans var. stellatoidea (G-1114).

Karyotypes. In preliminary studies with C. albicans G-30, the karyotype patterns obtained with pulsed-field electrophoresis showed differences in the number of DNA bands (and their resolution), varying mainly with alterations in the running time, voltage, and switching time. Maximal and usually comparable resolution of bands occurred at 72 to 96 h with test conditions of 50 mA and a 10-min switch time. Under the conditions of 50 mA and a 10-min switch time for 96 h, isolates G-30, G-9, G-M-44, and C-B-1071 demonstrated nearly identical karyotype patterns consisting of four obvious electrophoretic mobility groups or clusters, indicated as A 1, 2, B 3, C 4, 5, and D 6, 7. Two of these strains are shown in Fig. 1, lanes 1 and 3. With conditions recommended by the manufacturer (Beckman) of 140 mA and a 60-s switch time for 18 h for DNA from Saccharomyces cerevisiae (334 pulsed-field electrophoresis certified) and 38 mA and a 60-min switch time for 140 h for Schizosaccharomyces pombe (Y-12796), 13 and 3 bands, respectively, were produced. Only 11 and 2 bands, respectively, of DNA from these species were obtained with the standard conditions for C. albicans (data not shown).

The resolution of DNA bands and comparisons of karyotypes were facilitated by densitometric tracings. Computer analysis of scan data, under conditions that recognized seven bands for G-30, indicated that the broad and diffuse A cluster of C-402 and the D cluster of C-B-1071 were composed of two bands (Fig. 1, cluster D of lane 3 and cluster A of lane 4; Fig. 2, C-B-1071 and C-402). Karyotypes and densitometer scans of the same strain which were prepared separately and from different-age cultures were nearly identical.

Some atypical strains had distinct karyotypes from those of the classical group. This distinct karyotype was reproducible. Only six definite bands were resolved for isolate G-364 lacking one band in cluster A; probably this is a result of comigration of bands A 1, 2 (Fig. 1, lane 2). Isolate C-448 had additional bands in groups B and D (Fig. 3). A small shoulder corresponding in location to band 10 of C-448 was observed in G-30. This slight shoulder was observed for a few other strains at the same location, but our standard densitometric conditions did not recognize it as a peak except for C-448, which was the only strain where band 10 was detectable on photographs of gels. The karyotype of C. albicans C-655 (hypal form) was unique under a variety of test conditions because it gave a maximum of only five bands (Fig. 4).

Three serotypes of C. albicans var. stellatoidea (G-1114, serotype A; A-11006, serotype B; and C-B-1034, serotype C) produced three different electrophoretic karyotype patterns (Fig. 5). Photographs of the karyotyping gel of strain G-1114 appeared to have only a single band at cluster A. However, with densitometric analysis the karyotype of G-1114 was similar to that of classical C. albicans (G-30) (Fig. 5).

FIG. 1. Ethidium bromide-stained gel showing chromosome sizes of C. albicans DNA separated by TAFE at 50 mA with a 10-min switch time for 96 h. Lanes: 1, strain G-30; 2, G-364; 3, C-B-1071; 4, C-402.

FIG. 2. Densitometric tracings of chromosome-size DNA bands separated by TAFE at 50 mA with a 10-min switch time for 96 h.
KARYOTYPING OF CANDIDA ALBICANS

FIG. 3. Densitometric tracings of chromosome-size DNA bands separated by TAFE at 50 mA with 10-min switch time for 96 h.

DISCUSSION

The karyotypes of strains of *C. albicans* determined by TAFE varied with changes in electrophoretic conditions and, in some instances, between runs with the same conditions (gel-to-gel variations). A control DNA of a classical *C. albicans* (G-30) therefore was included in each gel. Densitometric tracings of gels facilitated the detection of bands not readily observed on photographs of gels, the discernment of broad or diffuse bands in the gels, and the comparison of karyotypes obtained in different runs. Classical isolates of *C. albicans* produced a distinctive karyotype of 7 DNA bands in four mobility groups, whereas some atypical strains produced different patterns of 5 bands (C-655, hyphal form), 9 or 10 bands (C-448), or 6 bands (G-364, avirulent form). Though the karyotypes of each of the three representatives of *C. albicans* var. *stellatoidea* were distinct, *C. albicans* and *C. albicans* var. *stellatoidea* demonstrated significant DNA relatedness, supporting their conspecificity as noted by Meyer (16). Kwon-Chung et al. (7) suggested that type II *C. stellatoidea* was a sucrose-negative mutant of *C. albicans* serotype A. Probably, type I *C. stellatoidea* strains of Kwon-Chung et al. (7) (represented herein by A-11006) are derived from *C. albicans* serotype B.

In comparison of DNA of *S. cerevisiae* with molecules that may be within a range of 260 to 2,200 kilobases (18, 24)

FIG. 4. Densitometric tracings of chromosome-size DNA bands separated by TAFE as follows: (A) 60 mA, 5-min switch time, 72 h; (B) 50 mA, 5-min switch time, 96 h; (C) 50 mA, 10-min switch time, 96 h; (D) 38 mA, 60-min switch time, 140 h.

FIG. 5. Densitometric tracings of chromosome-size DNA bands separated by TAFE at 50 mA with a 10-min switch time for 96 h.
and Schizosaccharomyces pombe with molecules of 3,000 to 9,000 kilobases (4), we estimated that chromosome-size DNA molecules of classical C. albicans range from about 1,000 to 5,000 kilobases. Perfect et al. (24) with a contour-clamped homogeneous-field gel electrophoresis procedure estimated that chromosome-size DNA molecules of C. albicans range from 900 to greater than 2,200 kilobases. However, for reasons cited in the paragraph below, particularly conformational influences, direct size comparisons of DNA bands from different species may be somewhat inaccurate. C. albicans, which has not demonstrated a sexual cycle, has been reported to be diploid with strains showing possible aneuploidy \(2n + n\) (32). Magee et al. (12) reported that homologous chromosomes may migrate separately, possibly because of deletions, translocations, or differences in the amount or distribution of repetitive DNA. Chromosomes of nearly the same molecular weight may comigrate as one band in a gel (15). Conversely, similarly sized chromosomes could confer in difference and the stereochemical state of their DNA and therefore migrate differently (19). Some of our data suggest that large DNA molecules or complexed DNA molecules in the wells may fail to enter the gel. This was possibly the case with C. albicans C-655. Also, the DNA bands in our system did not migrate at the same rate throughout the entire gel distance. For example, the first group of bands entering the gel (the D cluster) in classical C. albicans contained three closely packed bands at 150 mA with a 90-s switch time for 24 h; these bands converged in the gel to two bands by 96 h. Therefore the number of DNA bands in our TAFE system (as noted by others with different systems) does not necessarily represent the number of chromosomes.

The current status of gel electrophoretic karyotyping of C. albicans involves varied procedures that provide DNA band patterns of apparent value in biotyping, but the patterns are not comparable between laboratories. To our knowledge this is the first presentation of karyotypes for C. albicans with a TAFE procedure. It permitted distinction of strain types among phenotypically diverse isolates of C. albicans whose identification had been verified by molecular procedures. Further studies with establishment of karyotype data banks and refinement of procedures for additional species are required before karyotyping can be employed in practical epidemiological studies of unknown yeast species.

LITERATURE CITED