JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1989, p. 2165–2168
0095-1137/89/102165-04$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 27, No. 10

Electrophoretic Karyotypes of *Torulopsis glabrata*

C. S. KAUFMANN AND W. G. MERZ*

Department of Laboratory Medicine (Pathology), The Johns Hopkins Medical Institutions, Baltimore, Maryland 21205

Received 17 March 1989/Accepted 15 June 1989

Chromosome-sized DNA molecules of clinical isolates of *Torulopsis glabrata* were resolved by a pulsed-field electrophoretic method, contour-clamped homogeneous electric fields. With the conditions established in this study, 8 to 12 bands (ranging from 445 to 3,000 kilobases) were observed. There were differences in the intensities and migrations of bands, consistent with *T. glabrata* being either haploid or diploid. A total of 22 distinctive electrophoretic patterns were noted among single isolates of *T. glabrata* recovered from 33 patients. When strains were delineated by an electrophoretic pattern, individuals usually harbored only one strain.

Torulopsis glabrata is a yeast that may be found as a member of normal flora in humans; colonization of the gastrointestinal tract and female genitourinary tract is most frequent. This species, although less virulent than *Candida albicans*, is capable of causing opportunistic infections, including vaginitis, pyelonephritis, endophthalmitis, peritonitis, persistent fungemia, and disseminated infection (17).

As with many other fungi, the genetic make-up of this species is virtually unknown. DNA homology studies have demonstrated the significant relatedness of this species to members of the genus *Candida* (24), although taxonomically it is often still retained in the genus *Torulopsis* (15). Pulsed-field electrophoretic methods were first developed by Schwartz and Cantor (19) and were initially employed to study *Saccharomyces cerevisiae* (1, 2, 19). These procedures permit separation of large DNA molecules, including the chromosomes of lower eucaryotic organisms (9). They are also invaluable tools for establishing an electrophoretic karyotype of yeast species incapable of completing a sexual cycle. Although the electrophoretic karyotypes of a limited number of medically important yeast species have been reported (4, 6, 13, 25), only *C. albicans* (8, 10, 12, 16, 20, 21) and *Cryptococcus neoformans* (18) have been studied in depth. Therefore, this study was designed (i) to define a standard electrophoretic karyotype of *T. glabrata* by studying a large sample of strains with contour-clamp homogeneous field gel electrophoresis (CHEF) (3), (ii) to determine the extent of variations of the electrophoretic karyotypes and (iii) to determine whether an electrophoretic karyotype can be used as a stable genetic marker to delineate strains for use in epidemiologic studies.

MATERIALS AND METHODS

Organisms. Isolates of *S. cerevisiae* (YPH-149) and *Schizosaccharomyces pombe* (Leopold strain 972) were used as controls for preparation of intact DNA and as relative DNA size markers in CHEF.

A total of 86 recent clinical isolates of *T. glabrata* recovered from 33 inpatients in 15 hospital units were analyzed. Criteria for isolate inclusion were (i) *T. glabrata* isolates recovered from the same patient and (ii) *T. glabrata* isolates recovered from multiple patients on the same hospital unit within a 3-week period. Determination of species was based on fermentation of glucose and trehalose, lack of urease activity, and the production of small yeast cells without filamentation on corn meal agar with Tween 80 and caffeic acid (7). All control and test isolates were maintained on Sabouraud dextrose agar at 4°C.

Preparation of yeast DNA for CHEF. Preparation of intact yeast DNA was modified from the methods of Schwartz and Cantor (19) and Merz et al. (16). Yeast cells were grown for 18 h at 30°C on a rotary wheel in 4 ml of glucose (2%)–yeast extract (1%)–peptone (2%) broth medium. A 0.35-ml sample of cell growth was washed twice by centrifugation with 50 mM sodium EDTA–10 mM Tris (pH 7.5) and was then suspended in 0.15 ml of the 50 mM sodium EDTA–10 mM Tris (pH 7.5) buffer in 2.2-ml microcentrifuge tubes (PGC Scientific, Gaithersburg, Md.). Each tube was placed in a 39°C temperature block. After the addition of 2 μl of a 20-mg/ml stock of zymolyase (ICN Pharmaceuticals, Lisle, Ill.) in 10 mM Na_2HPO_4, 0.25 ml of 1% low-melting-point agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in 0.125 M sodium EDTA (pH 8.0) was added, and tubes were mixed gently and placed immediately into ice. The agarose plugs were overlaid with 0.4 ml of 0.5 M sodium EDTA–10 mM Tris (pH 7.5) and incubated at 37°C for 18 to 24 h. The plugs were transferred to polypropylene tubes (12 by 75 mm; Becton Dickinson Labware, Lincoln Park, N.J.), and 0.4 ml of a 2-mg/ml solution of proteinase K (Boehringer-Mannheim, Indianapolis, Ind.) in 0.5 M sodium EDTA–10 mM Tris (pH 7.5)–1% *N*-lauroyl sarcosine was added. The plugs were incubated at 50°C for 18 to 24 h and then washed four times with 50 mM sodium EDTA–10 mM Tris (pH 7.5) before they were stored at 4°C in 0.25 M sodium EDTA–10 mM Tris (pH 7.5). Agarose DNA plugs were stable for approximately 1 month.

CHEF. Small amounts 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]). CHEF was performed as described by Chu et al. (3). The circulating buffer was 0.5× TBE maintained at 9°C. Electrophoresis was conducted with 3-min alternating pulses of 120 V for 20 h followed by 6-min alternating pulses of 120 V for an additional 20 h. Gels were stained with ethidium bromide (1 μg/ml in distilled water) for 15 min and then destained with distilled water overnight. DNA bands were visualized with shortwave UV light (254 nm).

* Corresponding author.

Downloaded from http://jcm.asm.org/ on November 13, 2019 by guest

TABLE 1. Distribution of recovery of clinical isolates of *T. glabrata*

Source	No. of isolates (n = 86)	No. of patients (n = 33[a])
Blood	5	3
Vascular catheter tip	2	2
Urinary tract	45	20
Gastrointestinal tract	25	13
Respiratory tract	9	8

[a] Thirteen patients had *T. glabrata* recovered from multiple anatomic sites.

RESULTS

The 86 clinical isolates analyzed in this study are presented in Table 1; 52% were recovered from the urinary tract, 29% were recovered from the gastrointestinal tract, 11% were recovered from the respiratory tract, and 8% were recovered from the vascular system. The 33 patients were hospitalized in 15 separate inpatient units.

The electrophoretic karyotypes of single isolates from each of the 33 patients are summarized in Table 2, and five representatives are illustrated in Fig. 1. Eight to 12 chromosome-sized DNA bands were observed within this population, with the majority of isolates having either 10 or 11 bands. The smallest DNA bands of most isolates were estimated to be 445 kilobases (kb) by comigration with chromosome 9 of *S. cerevisiae*, whereas a few isolates had smaller DNA bands, 100 to 200 kb, as estimated by comigration with *S. cerevisiae* chromosome 1. Under these conditions, the largest *T. glabrata* DNA molecule was estimated to be 1 to 3 Mb by comparison with the chromosomal DNA bands of *S. pombe*. Differences in the widths and intensities of the DNA bands were also observed. For example, the bottom band of lane 5 is an example of a wide, intense band which may represent comigration of two homologs or of two different chromosomes that resolved into two bands (the second and third bands from the bottom) with the isolate in lane 3. Confirmation of these assumptions would require supportive data such as DNA-DNA hybridization data. Interestingly, the smaller DNA band of ~100 to 200 kb (lane 3) was noted among all four isolates recovered from only one patient.

Variation was also observed in the patterns of the DNA bands among the *T. glabrata* isolates recovered from the 33 study patients. Of the two strains with eight DNA bands, both had different patterns. Within the three strains with nine bands, all had different electrophoretic patterns. Among the 10 strains with 10 bands, 8 had different patterns. Of the 17 strains with 11 bands, eight different patterns were discernible plus the 1 strain with 12 bands. Therefore, a total of 22 distinct electrophoretic karyotype patterns were distinguishable among the 33 single isolates tested from the 33 patients.

TABLE 2. Variation of electrophoretic karyotypes among isolates of *T. glabrata*

Number of chromosome-sized DNA bands	No. of patients	No. of distinct electrophoretic patterns
8	2	2
9	3	3
10	10	8
11	17	8
12	1	1

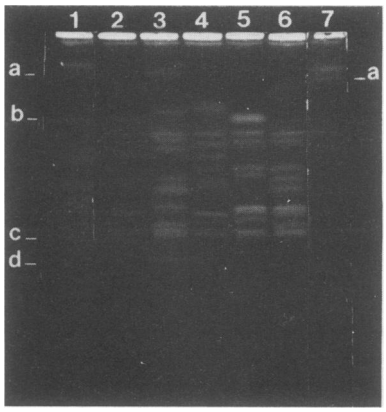

FIG. 1. Electrophoretic patterns of single isolates of *T. glabrata* recovered from five representative patients. Lanes: 1, *S. pombe* and *S. cerevisiae*; 2, pleural fluid isolate; 3, throat isolate; 4, stool isolate; 5, urine isolate; 6, urine isolate; 7, *S. pombe*. Bands: a, *S. pombe* chromosome III, 3 Mb; b, *S. cerevisiae* chromosome 12, 1,600 to 2,500 kb; c, *S. cerevisiae* chromosome 9, 445 kb; d, *S. cerevisiae* chromosome 1, 220 kb.

This population of *T. glabrata* also included multiple isolates recovered from different anatomic sites or isolates recovered from the same site on a different day from individual patients. Twelve patients with two isolates, 3 patients with three and four isolates, 2 patients with five and seven isolates, and 1 patient with six isolates, for a total of 23 patients, were tested. No variation in the electrophoretic patterns was noted within isolates of 19 of the 23 patients. Figure 2 demonstrates this stability with three isolates of *T. glabrata* recovered from a single patient over a 1-month interval. One of these patients had positive blood cultures and prior positive cultures from other anatomic sites; the electrophoretic pattern of isolates recovered from stool, urine, and blood were all identical. Two different electrophoretic patterns were noted within multiple isolates recovered from four patients. With three of these patients, the different strains were recovered from different anatomic sites, whereas with the fourth patient, the two strains were recovered at different times from the same site.

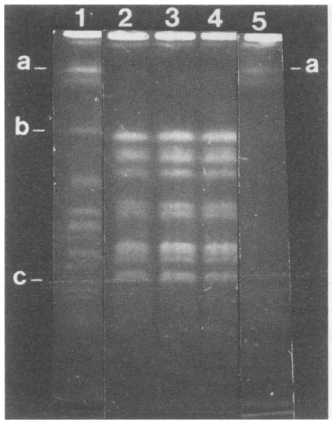

FIG. 2. Electrophoretic patterns of three isolates recovered from an individual patient over a 1-month interval. Lanes: 1, *S. pombe* and *S. cerevisiae*; 2, throat isolate; 3, stool isolate; 4, urine isolate; 5, *S. pombe*. Bands: a, *S. pombe* chromosome III, 3 Mb; b, *S. cerevisiae* chromosome 12, 1,600 to 2,500 kb; c, *S. cerevisiae* chromosome 9, 445 kb.

Downloaded from http://jcm.asm.org/ on November 13, 2019 by guest

Strains of *T. glabrata* recovered from multiple individuals hospitalized in the same hospital units within a 3-week period were tested to assess possible transmission of the organism. The units studied included three units with two patients positive for *T. glabrata*, one unit with three positive patients, one unit with four positive patients, one unit with five positive patients, and one unit with eight positive patients. The seven units included one surgical unit, a medical intensive care unit, one neurology intensive care unit, and four separate oncology units. Transmission of strains among patients hospitalized in the same unit was not noted; no isolates from different patients within any single hospital unit had identical electrophoretic karyotypes.

DISCUSSION

Chromosome-sized DNA bands of *T. glabrata* were resolved by the use of CHEF, a type of pulsed-field electrophoresis. The electrophoretic conditions established in this study permitted the clear separation of both the smaller chromosomes (<1,000 kb) and the separation of the larger chromosomes (1 to 3 Mb) within a single gel. This was accomplished by a two-step ramping procedure (a 3-min alternating pulse time for 20 h followed by a 6-min alternating pulse time for 20 h) to optimize the resolution of the chromosomes. The smallest chromosome of *T. glabrata* resolved uniformly among all isolates at a size of 445 kb, as estimated by comigration with *S. cerevisiae* chromosome 9. In addition, the 100- to 200-kb band noted with all four isolates from one patient could be a stable chromosome fragment. There was extensive chromosome length polymorphism in the largest chromosome among the isolates. The largest chromosome of some *T. glabrata* isolates migrated similarly to the three-Mb chromosome of *S. pombe*, whereas the largest chromosomes of other strains were between 1,100 and 2,500 kb, as estimated by migration similar to that of chromosome 12 of *S. cerevisiae* or chromosome III of *S. pombe*.

The chromosome number and ploidy of *T. glabrata* can only be estimated from analysis of the numbers, patterns, and intensities of the DNA bands. The results could be consistent with *T. glabrata* having a haploid number of 14 if each band represented one chromosome. The conclusion that *T. glabrata* was haploid would agree with conclusions drawn from protoplast fusion hybrid analysis and irradiation studies of this species (5, 22, 23). However, if one assumes that intense bands represent comigration of homologs and narrower bands represent a single homolog of an individual chromosome, the data would be consistent with *T. glabrata* being diploid with a minimum haploid number of 7. Conformation of the ploidy and the haploid chromosome number of *T. glabrata* will require the development of chromosome-specific probes. Hybridization studies will then permit location of specific genes and recognition of homologs as has recently been established with *C. albicans* by Magee et al. (12). They were able to resolve 11 chromosomal DNA bands among isolates of *C. albicans* by pulsed-field electrophoresis, and they confirmed 7 chromosomes through the use of 14 cloned genes and three nonspecific DNA probes.

There was significant variation of the electrophoretic karyotype patterns among the population of these clinical isolates, although all isolates studied had identical phenotypic characteristics. Within single isolates recovered from 33 patients, 22 distinct electrophoretic karyotype patterns were discernible. These patterns were distinguished by both differences in the number of chromosomal bands observed and differences in the migration of the chromosomal bands assumed to be chromosome length polymorphisms due to genetic translocations and deletions. This same phenomenon has also been observed among isolates of *C. albicans*, another asexual yeast species (16).

The significant variation of the electrophoretic karyotype pattern did not permit the establishment of a species-specific karyotype; rather the pattern was like a fingerprint that permitted strain delineation. When multiple isolates of *T. glabrata* recovered from 19 individual patients were studied for up to 1.5 months, the electrophoretic karyotype patterns did not change. This suggests that most individuals harbor only one strain of *T. glabrata*. Therefore, strain delineation by electrophoretic karyotyping, a reproducible, stable genetic marker, will be an important tool for epidemiologic studies, since there are no biotyping procedures currently available for this species. A method that might permit strain delineation with the same advantages as an electrophoretic karyotype may be the use of restriction enzyme fragment length polymorphisms. Two recent studies have shown that *T. glabrata* can be distinguished from other species of yeasts by restriction enzyme fragment length polymorphism analysis (11, 14). However, variation of restriction enzyme fragment length polymorphisms within this species was not addressed.

In conclusion, chromosomal DNA bands of clinical isolates of *T. glabrata* were resolved with a pulsed-field electrophoretic technique, CHEF. Use of this technique will permit molecular genetic analyses of this asexual species of yeast, which analyses have not been previously possible. In addition, there was significant variation in the electrophoretic karyotype, which permitted strain delineation based on a reproducible, stable genetic marker. Epidemiologic analysis demonstrated that an individual usually harbored one strain and that transmission of the organism within a hospital unit did not occur. Therefore, electrophoretic karyotype strain delineation will permit further genetic, pathogenetic, and epidemiologic studies of this medically important yeast species.

ACKNOWLEDGMENTS

We appreciate the critical advice of Philip Hieter and Carla Connelly, the personnel of The Johns Hopkins Mycology Laboratory for collecting isolates, and Alan O'Neill for patience in preparation of the manuscript.

This study was supported by a Johns Hopkins University Institutional Grant, Biomedical Research Support Grant RR5378, to W.G.M.

LITERATURE CITED

1. **Carle, G. F., and M. V. Olson.** 1984. Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. Nucleic Acids Res. **12**:5647–5663.
2. **Carle, G. F., and M. V. Olson.** 1985. An electrophoretic karyotype for yeast. Proc. Natl. Acad. Sci. USA **82**:3756–3760.
3. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogenous electric fields. Science **234**:1582–1585.
4. **DeJonge, P., F. C. M. DeJonge, R. Meijers, H. Yde Steensma, and W. A. Scheffers.** 1986. Orthogonal-field-alternation gel electrophoresis banding patterns of DNA for yeasts. Yeast **2**: 193–204.
5. **Galeotti, C. L., K. S. Sriprakash, C. M. Batum, and G. D. Clark-Walker.** 1981. An unexpected response of *Torulopsis glabrata* fusion products to X-irradiation. Mutat. Res. **81**: 155–164.
6. **Johnston, J. R., C. R. Contopoulou, and R. K. Mortimer.** 1988. Karyotyping of yeast strains of several genera by field inversion

Downloaded from http://jcm.asm.org/ on November 13, 2019 by guest

gel electrophoresis. Yeast **4**:191–198.
7. **Kaufmann, C. S., and W. G. Merz.** 1982. Two rapid pigmentation tests for identification of *Cryptococcus neoformans*. J. Clin. Microbiol. **15**:339–341.
8. **Kwon-Chung, K. J., B. L. Wickes, and W. G. Merz.** 1988. Association of electrophoretic karyotype of *Candida stellatoidea* with virulence for mice. Infect. Immun. **56**:1814–1819.
9. **Lai, E., B. W. Birren, S. M. Clark, M. I. Simon, and L. Hood.** 1989. Pulsed-field gel electrophoresis. BioTechniques **7**:34–42.
10. **Lott, T. J., P. Boiron, and E. Reiss.** 1987. An electrophoretic karyotype for *Candida albicans* reveals large chromosomes in multiples. Mol. Gen. Genet. **209**:170–174.
11. **Magee, B. B., T. M. D'Souza, and P. T. Magee.** 1987. Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. J. Bacteriol. **169**:1639–1643.
12. **Magee, B. B., Y. Koltin, J. A. Gorman, and P. T. Magee.** 1989. Assignment of cloned genes to the seven electrophoretically separated *Candida albicans* chromosomes. Mol. Cell. Biol. **8**:4721–4726.
13. **Magee, B. B., and P. T. Magee.** 1987. Electrophoretic karyotypes and chromosome numbers in *Candida* species. J. Gen. Microbiol. **133**:425–430.
14. **Mason, M. M., B. A. Lasker, and W. S. Riggsby.** 1987. Molecular probe for identification of medically important *Candida* species and *Torulopsis glabrata*. J. Clin. Microbiol. **25**:563–566.
15. **McGinnis, M. R., L. Ajello, E. S. Beneke, E. Drouhet, N. L. Goodman, C. J. Halde, L. D. Haley, J. Kane, G. A. Land, A. A. Padhye, D. H. Pincus, M. G. Rinaldi, A. L. Rogers, I. F. Salkin, W. A. Shell, and I. Weitzman.** 1984. Taxonomic and nomenclature evaluation of the genera *Candida* and *Torulopsis*. J. Clin. Microbiol. **20**:813–814.
16. **Merz, W. G., C. Connelly, and P. Hieter.** 1988. Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. J. Clin. Microbiol. **26**:842–845.
17. **Odds, F. C.** 1988. Candida and candidosis: a review and bibliography, 2nd ed. Bailliere Tindall, Philadelphia.
18. **Polacheck, I., and G. A. Lebens.** 1989. Electrophoretic karyotype of the pathogenic yeast *Cryptococcus neoformans*. J. Gen. Microbiol. **135**:65–71.
19. **Schwartz, D. C., and C. R. Cantor.** 1984. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell **37**:67–75.
20. **Snell, R. G., I. F. Hermans, R. J. Wilkins, and B. E. Corner.** 1987. Chromosomal variation in *Candida albicans*. Nucleic Acids Res. **15**:3625.
21. **Snell, R. G., and R. J. Wilkins.** 1986. Separation of chromosomal DNA molecules with *C. albicans* by pulsed field gel electrophoresis. Nucleic Acids Res. **14**:4401–4406.
22. **Whelan, W. L., and K. J. Kwon-Chung.** 1987. Parasexual genetics of *Torulopsis glabrata*. J. Bacteriol. **169**:4991–4994.
23. **Whelan, W. L., S. Simon, E. S. Beneke, and A. L. Rogers.** 1984. Auxotrophic variants of *Torulopsis glabrata*. FEMS Microbiol. Lett. **24**:1–4.
24. **Yarrow, D., and S. A. Meyer.** 1978. Proposal for amendment of the diagnosis of the genus *Candida* Berkhout nom. cons. Int. J. Syst. Bacteriol. **28**:611–615.
25. **Zimmerman, M., M. Hoffmann-Hintz, M. Kolvenback, and C. C. Emeis.** 1988. OFAGE banding patterns of different yeast genera and intergeneric hybrids. J. Basic Microbiol. **28**:241–247.

Downloaded from http://jcm.asm.org/ on November 13, 2019 by guest