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Clamped Homogeneous Electric Field Gel Electrophoresis Typing of *Torulopsis glabrata* Isolates Causing Nosocomial Infections

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Thirty isolates of *Torulopsis glabrata* were examined by pulsed-field gel electrophoresis, which resolved 13 DNA pieces, allowing the identification of 12 types. Bands at 1,400, 1,200, 1,070, 1,025, 681, and 500 kbp were conserved. When applied to 18 isolates from an outbreak, 10 distinct types were identified by this technique. Seven patients had isolates which were identical.

The opportunistic pathogen *Torulopsis glabrata* has recently been described as a cause of cross infection (8). The outbreak was defined by DNA fingerprinting of the chromosomal DNA by the restriction enzyme *Xba*I. Similar protocols have been developed to delineate cross infection due to *Candida albicans* (1, 10), *Candida parapsilosis* (3), and *Candida tropicalis* (4). They have been superseded in the case of *C. albicans* by techniques such as Southern hybridization analysis with DNA probes (5) and pulsed-field gel electrophoresis (14), which show a higher degree of discrimination.

The success of DNA fingerprinting is dependent on variation in the repetitive sequences, which are detected as bright bands by agarose gel electrophoresis. For *T. glabrata*, the degree of discrimination with the enzyme *Eco*RI was poor even when the technique was supplemented by Southern hybridization with *Saccharomyces*-derived probes (11). Recently, the technique of clamped homogenous electric field gel electrophoresis (CHEF) has been applied to 33 isolates (6). This method demonstrated 22 distinctive electrophoretic patterns, with the number of chromosomes varying from 8 to 12. This technique has been used here to type isolates by both the original protocol (6) and a new protocol.

Eighteen isolates from the original outbreak were available from patients either with leukemia or who had had a bone marrow transplant (Table 1). Also examined were three control isolates from the same hospital (Table 1, no. 24 to 26), three additional control isolates (Table 1, no. 28 and 29), and *T. glabrata* NCPF 3240. *Saccharomyces cerevisiae* YP148 (2) and *C. albicans* SGY126 (9) were the controls for the preparation of intact DNA and as relative DNA size markers.

Cell samples were prepared by a modification of the method of Schwartz and Cantor (13). The different strains were grown to a stationary phase in 25 ml of YPD media (1% yeast extract, 2% dextrose, 2% peptone). The cells were pelleted, washed twice with 50 mM EDTA (pH 7.5), and resuspended in the same buffer at a ratio of 3 volumes of EDTA solution to 2 volumes of cells.

To make spheroplasts, 200 μl of 2 mg/ml-Zymolyase 100T (ICN Biomedicals Ltd., High Wycombe, United Kingdom) (in 0.01 M sodium phosphate containing 50% glycerol) was added to 0.3 ml of cell suspension. This mixture was incubated at 37°C for 20 min. The cell enzyme suspension was mixed with 0.9 ml of 2% low-temperature-gelling agarose, distributed into molds, and allowed to solidify at 20°C for 5 min. The agarose plugs were transferred to buffer (0.5 M EDTA [pH 8.0], 0.01 M Tris-HCl [pH 7.5], 7.5% [vol/vol] 2-mercaptoethanol) and incubated overnight at 37°C. The buffer was removed, and the plugs were washed three times in 50 mM EDTA (pH 8.0). Cell lysis was completed by adding 0.01 M Tris-HCl (pH 7.5)–0.5 M EDTA (pH 8.0)–1% lauryl sarcosine–1 mg of Proteinase K per ml to the plugs. This mixture was incubated for 2 days with gentle shaking. The plugs were washed three times with 50 mM EDTA (pH 8.0) and loaded directly onto the gels.

Electrophoresis was conducted in a Bio-Rad CHEF-DRII system. The plugs were loaded into 1% (wt/vol) agarose gels in 0.5% TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM sodium EDTA [pH 8.0]). The DNA was resolved by CHEF for 40 h at 12°C. In protocol 1, the electric field was 4.5 V/cm with a two-step pulse time of 120 s for 20 h and 180 s for a further 20 h. The electric field in protocol 2 was 3.6 V/cm, with a two-step pulse time of 180 s for 20 h and 360 s for a further 20 h (6).

Gels were stained with ethidium bromide (0.5 μg/ml in distilled water) for 15 min and destained in distilled water for 3 h. DNA bands were visualized with shortwave UV light (254 nm) and photographed.

CHEF typing of the 30 isolates by protocol 1 (Fig. 1 and 2) produced a better degree of resolution than protocol 2 (Fig. 3). Thus, the detailed interpretation of the results is based on the results obtained with protocol 1. This identified bands in 13 separate areas (Table 2). The total number of bands for each isolate varied from 9 to 11, and bands 4, 5, 7, 8, 11, and 13 were conserved, with the exception of CHEF type 4, for which bands 4 and 5 consistently ran slightly high. The molecular weight of each band (Table 3) was determined by comparison with the known standards. The running conditions meant that the *C. albicans* chromosomes at 3,000, 2,600, 2,200, and 1,700 kbp resolved into only two bands (Fig. 1, track 10; Fig. 2, track 10). This finding underlines the problem of assuming that a single band on electrophoresis is due to one chromosome. Both protocols confirmed that the

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TABLE 1. Details of the outbreak isolates (no. 1 to 23), unrelated isolates (no. 24 to 26) from the same hospital, control *T. glabrata* isolate (no. 27), and isolates (no. 28 and 29) from a different hospital

Outbreak isolate no.	Date isolated (mo and yr) or isolate	Identification site of isolate	Systemic antifungal therapy	Outcome[a] of patient	CHEF type	Figure
1	June 1988	Feces	+	d	2	1, lane 2 3, lane 2
2	Sept. 1988	Feces	+	d	8	2, lane 6 3, lane 3
3	Sept. 1988	Blood	+	s	9	2, lane 7 3, lane 4
4	Oct. 1988	Throat	+	s	6	1, lane 8 3, lane 9
5	Oct. 1988	Throat	−	s	1	1, lane 7 3, lane 5
6	Dec. 1988	Feces	−	s	3	1, lane 3 3, lane 6
7	Dec. 1988	Feces	−	s	5	1, lane 6 3, lane 7
8	Dec. 1988	Feces	−	s	1	2, lane 9
9	Dec. 1988	Feces	−	s	12	2, lane 8
10	Jan. 1989	Feces	−	s	1	2, lane 4 3, lane 8
11	Feb. 1989	Urethra	+	d	1	
12	Feb. 1989	Feces	−	s	4	1, lane 5
13	Feb. 1989	Feces	−	s	7	2, lane 5
14	Feb. 1989	Feces	−	s	UN[b]	
15	Mar. 1989	Feces	−	s	1	1, lane 9
16	Apr. 1989	Feces	−	s	UN	
17	Apr. 1989	Feces	−	s	UN	
18	May 1989	Feces	−	s	UN	
19	May 1989	Feces	−	s	1	
20	May 1989	Blood	+	s	1	1, lane 4
21	June 1989	Blood	+	d	UN	
22	July 1989	Feces	−	s	3	
23	Sept. 1989	Feces	−	s	5	
24	1989	Feces	−	s	2	
25	1989	Feces	−	s	3	2, lane 3
26	1989	Feces	−	s	1	2, lane 2
27	NCPF 3240				2	
28	Jan. 1990	Blood	+	s	10	
29	Jan. 1992	Blood/urine	+	s	11/11	

[a] s, survived; d, died.
[b] UN, unavailable.

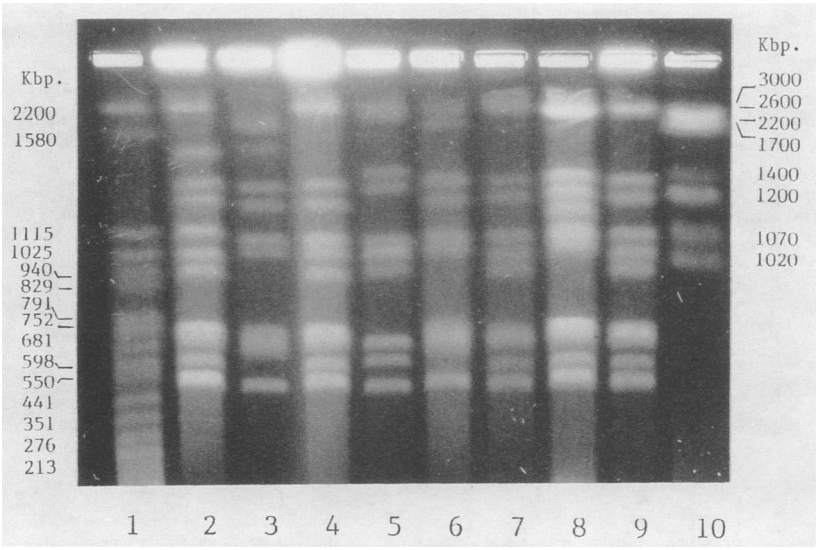

FIG. 1. Lanes: 1, *S. cerevisiae* YP148; 10, *C. albicans* SGY126; 2 to 9, *T. glabrata* isolates as described in Table 1. Molecular sizes (in kilobase pairs) are indicated.

smallest chromosome of *T. glabrata* resolved with *S. cerevisiae* chromosome IX. Kaufmann and Merz (6) gave it a molecular size of 445 kbp, but in the present study, the band ran consistently nearer the origin than *S. cerevisiae* chromosome IX and below *S. cerevisiae* chromosome VIII so that it had an apparent molecular mass of approximately 500 kbp. *T. glabrata* bands migrated as follows: band 1 with *S. cerevisiae* chromosome XII, band 2 with *S. cerevisiae* chromosome IV, band 3 between *S. cerevisiae* chromosome IV and *C. albicans* chromosome IV, band 4 with *C. albicans* chromosome V, band 6 with *S. cerevisiae* chromosomes XV and VII, band 7 with *C. albicans* chromosome II, band 8 with *S. cerevisiae* chromosome XVI, band 9 with *S. cerevisiae* chromosome XIII, band 10 with *S. cerevisiae* chromosome X, band 11 with *S. cerevisiae* chromosome XI, and band 12 between *S. cerevisiae* chromosomes XI and V (Table 3).

In contrast to the findings of Kaufmann and Merz 1989 (6), it was possible to define a characteristic karyotype for *T. glabrata*. Bands 4, 5, 7, 8, 11, and 13 were constant and defined a yeast isolate as *T. glabrata*. The karyotypes of isolates from cases 1 to 10, 12, 13, 15, 20, 25, and 26 are illustrated in Fig. 1, 2, and 3. Typing systems can be judged by three criteria: typeability, reproducibility, and discrimination. All isolates of *T. glabrata* were typeable by this method. Provided that the running condition of the gel was standardized, the reproducibility was excellent. All isolates were tested at least three times. Unlike DNA or immunoblot fingerprinting, it was unnecessary to define prominent bands and restrict the typing system to variation in these bands (7,

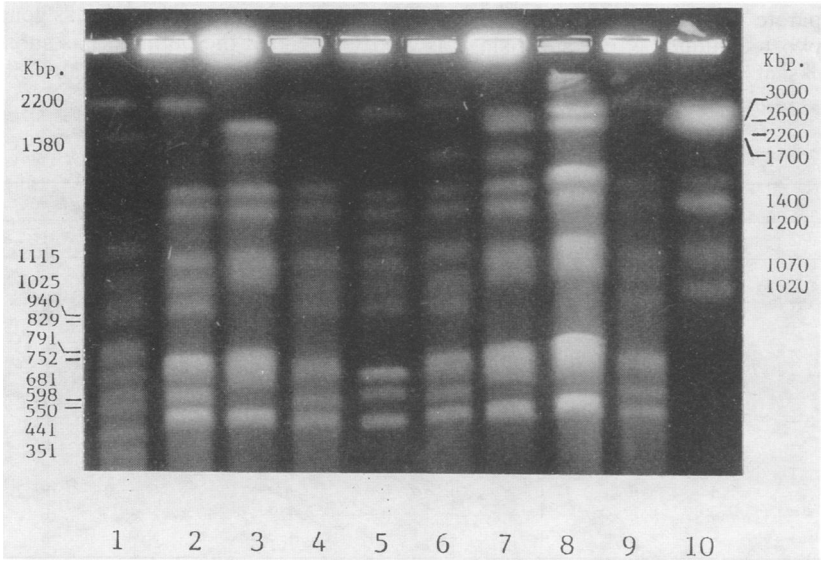

FIG. 2. Lanes: 1, *S. cerevisiae* YP148; 10, *C. albicans* SGY126; 2 to 9, *T. glabrata* isolates as described in Table 1. Molecular sizes (in kilobase pairs) are indicated.

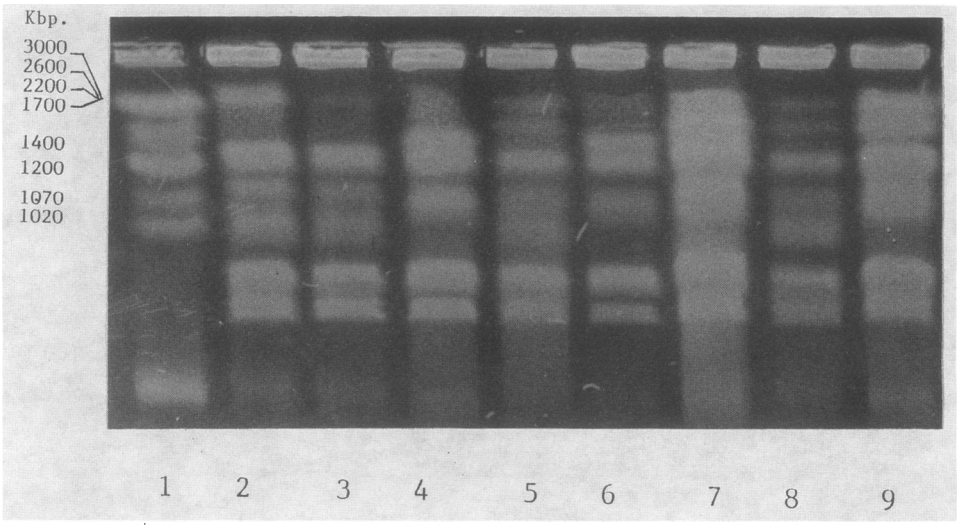

FIG. 3. Lanes: 1, *C. albicans* SGY126; 2 to 9, *T. glabrata* isolates as described in Table 1. Molecular sizes (in kilobase pairs) are indicated.

8). Discrimination was based on differences in bands 1, 2, 3, 6, 9, 10, and 12. A minority of CHEF types had bands 2, 3, and 6, while the majority had bands 9, 10, and 12. The largest-molecular-weight band was the most variable, as previously described (6), being absent (type 3), present (types 1, 2, 4, 6, 8, 11, and 12), of a smaller size (types 7, 9, and 10), or double (type 5). The original outbreak was defined by DNA fingerprinting with *Eco*RI and *Xba*I (8). All isolates, including nine control isolates, were identical when cut by *Eco*RI. *Xba*I produced five types, of which all the outbreak strains and three of the controls were type 1. These three controls are the isolates from cases 24, 25, and 26 (Table 1). This low degree of discrimination agreed with the results of Reagan et al. (11) in which five of six *T. glabrata* isolates were indistinguishable by either DNA fingerprinting or Southern hybridization with a *Saccharomyces*-derived probe. CHEF had a much higher degree of discrimination in that the three control isolates identical by DNA fingerprinting produced three separate CHEF types (Table 1). The disparity between the two techniques is not surprising, as DNA fingerprinting looks at only the variation in the sequences of DNA which are highly repetitive. By their nature, these sequences are likely to be ribosomal, conserved, and confined, as with *C. albicans* (12), to a single chromosome.

CHEF typing showed that the original outbreak was not as extensive as was first reported (8), as the 18 isolates identical by DNA fingerprinting produced 10 distinct types. It still showed a cluster of isolates of CHEF type 1 from patients 5, 8, 10, 11, 15, 19, and 20 between October 1988 and May 1989. In May 1989, the suggestion that the problem was nosocomial was made, and infection control improved (8). There was a thorough survey of the unit involving screening of the food, the throats and hands of staff, settle plates, and environmental swabs. These swabs failed to reveal either the cycle of cross infection or the source of the outbreak. In the original report, it was stated that the outbreak ended spontaneously in September 1989 (8). The CHEF results show that the heightened awareness generated in May 1989 effectively ended the outbreak, as subsequent isolates (cases 22 and 23, Table 1) were unrelated.

TABLE 2. Details of the bands found in each CHEF type

CHEF type	Band													Total no. of bands
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1	+	−	−	+	+	−	+	+	+	+	+	+	+	10
2	+	+	−	+	+	−	+	+	+	+	+	+	+	11
3	−	+	+	+	+	−	+	+	−	+	+	−	+	9
4	+	−	−	+[a]	+[a]	−	+	+	+	−	+	+	+	9
5	DB[b]	−	−	+	+	−	+	+	−	+	+	−	+	9
6	+	−	−	+	+	+	+	+	−	+	+	+	+	10
7	+[c]	−	−	+	+	+	+	+	+	−	+	+	+	10
8	+	−	+	+	+	−	+	+	+	+	+	+	+	11
9	+[c]	+	+	+	+	−	+	+	−	+	+	−	+	10
10	+[c]	−	+	+	+	+	+	+	−	−	+	+	+	10
11	+	−	+	+	+	+	+	+	+	−	+	+	+	11
12	+	+	−	+	+	−	+	+	−	+	+	−	+	9

[a] Band higher.
[b] DB, double band.
[c] Band lower.

TABLE 3. Approximate molecular sizes of chromosomes

C. albicans SGY126		*T. glabrata*		*S. cerevisiae* YP148	
Chromosome no.	Approx molecular size (kbp)	Band no.	Approx molecular size (kbp)	Chromosome no.	Approx molecular size (kbp)
V, VI, VII, VIII	3,000–1,700	1	2,200	XII	2,200
		2	1,580	IV	1,580
		3	1,480		
IV	1,400	4	1,400		
III	1,200	5	1,200		
		6	1,115	XV, VII	1,115
II	1,070	7	1,070		
I	1,020	8	1,025	XVI	1,025
		9	940	XIII	940
				II	829
				XIV	791
		10	752	X	752
		11	681	XI	681
		12	620	V	598
				VIII	550
		13	500	IX	441
				III	351
				VI	276
				I	213

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