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DNA Fingerprinting and Electrophoretic Karyotype of Environmental and Clinical Isolates of *Candida parapsilosis*

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The endonuclease restriction pattern (DNA fingerprinting) and the electrophoretic karyotype of 16 *Candida parapsilosis* isolates from environmental and clinical sources were investigated. DNA from both whole cells and separated mitochondria was digested with enzymes, including *Eco*RI, *Bam*HI, *Kpn*I, *Bgl*II, *Hpa*II, *Pvu*II, and *Hin*dIII. Regardless of their source and pathogenic properties, all isolates showed a uniform, reproducible, and overlapping whole-cell DNA fingerprinting with each endonuclease digest. Mitochondrial DNA fragments were, in all cases, major contributors to the total cellular DNA restriction pattern. In contrast, the electrophoretic karyotype generated by rotating field gel electrophoresis (RFGE) or contour clamped homogeneous field electrophoresis (CHEF) showed a remarkable polymorphism among the isolates. This polymorphism concerned the smaller molecular size section of the karyotype (range, 1.8 to 0.7 Mb), where at least two to five chromosomal bands could be consistently detected by both RFGE and CHEF. Larger (≥3.0 to 1.9 Mb) chromosome-sized DNA bands (four in CHEF and three in RFGE) were quite distinct and common to all isolates. Thus, seven karyotype classes could be defined, on the basis of both the number and size of putative chromosomes. The three categories of isolates (soil, vaginal, and hematological) were not randomly distributed among the seven classes. In particular, the four hematological isolates had a karyotype pattern which was clearly distinct from that shown by the three environmental isolates, and of the nine vaginal isolates only one shared a class with isolates from another source (soil). Although tentative, the classification was totally consistent with the independent and reproducible results obtained by the two pulse-field electrophoretic techniques employed. It is suggested that the electrophoretic analysis of the karyotype might be particularly useful for epidemiological and pathogenicity studies on biotypes of *C. parapsilosis*.

Candida parapsilosis is a rather frequent cause of fungemia in neutropenic or otherwise severely debilitated subjects undergoing parenteral nutrition (6, 14, 18). Tissue invasion, in particular endocarditis, by *C. parapsilosis* may occur in neutropenic, bone marrow-transplanted patients with a central venous catheter (12). A role for this yeast as a frank agent of vulvovaginal candidiasis has recently been advocated (4, 5). It has been shown that vaginal isolates of this fungus, in contrast to laboratory-maintained, environmental strains, produce in vitro (4) and probably also in vivo (5) marked amounts of secretory aspartylproteinase, a virulence enzyme (3, 16, 22, 23). In addition, all fresh clinical isolates of *C. parapsilosis* (either from vaginitis patients or from subjects with hematologic malignancies) but not the environmental strains were appreciably pathogenic for mice rendered neutropenic by cyclophosphamide (5). All of these properties suggest the existence of separate strains or biotypes of *C. parapsilosis*, the identification of which would be important for both pathogenetic and epidemiological studies. These concepts have been increasingly and usefully verified with *Candida albicans* but have found so far little application with *C. parapsilosis*. In particular, DNA-typing methods have revealed a great potential for simple, reproducible, and powerful discrimination of strains or biotypes of *C. albicans*, eventually demonstrating the extraordinary genetic diversity and heterogeneity of this human commensal pathogen (8, 13, 20). The aim of this study was to apply DNA-typing methods, including electrophoretic analysis of the karyotype, to *C. parapsilosis* and to examine whether different environmental and clinical isolates of this fungus could be subdivided into a number of biotypes by these techniques.

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MATERIALS AND METHODS

Yeasts. Sixteen isolates of *C. parapsilosis* were used throughout this study. Table 1 shows the sources and some differential features of these isolates. Overall, they could be grouped into three categories (environmental, vaginal, and hematologic isolates) which also differed in pathogenicity aspects (5) (Table 1). The environmental isolates were from stock cultures of laboratory collections, while both vaginal and hematologic yeasts were recently isolated in our clinical laboratories. All isolates were identified according to established laboratory identification procedures, inclusive of morphology on corn meal agar, germ tube test in serum, assimilation of carbon sources in the API 20C gallery system (Biomerieux), and agglutination with adsorbed anticandida sera (candida check kit; Iatron), as previously described (4, 5). The vaginal isolates were either from subjects affected by candidal vaginitis or from carriers (Table 1). The ability to secrete an aspartylproteinase in bovine serum albumin (BSA) medium and the experimental pathogenicity for cyclophosphamide-immunodepressed mice were assessed as described elsewhere (1, 3). Briefly, the yeast was pregrown in YPD medium (glucose, 2%; yeast extract, 1%; and Bacto-Peptone, 2%; Difco) and induced to proteinase secretion in BSA medium (yeast carbon base, 1.17%; yeast extract, 0.01%; and BSA [BDH], 0.2% [wt/vol]; pH 5.0). Enzyme activity was measured as the diameter of a lytic area on BSA

TABLE 1. Clinical and microbiological data for isolates of *C. parapsilosis*

Isolate (laboratory code)	Source of isolation	API 20C assimilation profile	Proteinase secretion[a]	Exptl pathogenicity results[b]	
				D/T	MST (days)
SA-17	Candida vaginitis	6652171	++	2/6	>30
SA-19	Candida vaginitis	6756131	++	4/15	>30
SA-23	Candida vaginitis	6756131	++	3/6	>30
SA-25	Candida vaginitis	6656171	++	7/10	3
SA-36	Candida vaginitis	6656171	++	1/5	>30
SA-42	Candida vaginitis	2756171	+	ND	
SA-28	Vaginal carriage	2756171	+	1/10	>30
SA-38	Vaginal carriage	6756171	+	0/10	>30
SA-197	Vaginal carriage	6756131	+	0/10	>30
HEM-1	Candidemia and endocarditis	6752131	+	5/5	4
HEM-2	Candidemia	6756131	++	ND	
HEM-3	Candidemia	6756131	+	ND	
HEM-4	Candidemia	6756131	+	2/5	>30
ISS-4879	Soil sample	6756131	−	0/10	>30
ISS-4884	Soil sample	6776171	−	0/10	>30
ISS-4888	Soil sample	6756131	−	0/10	>30

[a] Measured as the diameter of the clear zone of BSA hydrolysis (−, no hydrolysis; +, 1 to 2 mm; ++, 3 to 5 mm). For further details, see references 3 and 4.

[b] Mice were treated with cyclophosphamide (Cytoxan; Sigma) (150 mg/kg intraperitoneally) and after 2 days challenged intravenously with 10^6 cells of *C. parapsilosis* in saline. For further details, see references 1 and 5; D/T, Number of dead animals over total (ND, not determined); MST, median survival time.

agar and expressed as a − to ++ score, as described previously (3, 4). For experimental infections, the yeast inoculum was prepared from cells grown in Winge broth (glucose, 0.2%; yeast extract, 0.3%) for 48 h at 28°C on a reciprocal shaker, washed, counted in a hemacytometer, and diluted to an appropriate concentration in physiological saline. A total of 10^6 cells of each strain of *C. parapsilosis* were therefore used to produce systemic (by the intravenous route) infections in inbred, male CD2 F1 mice (Charles River, Calco, Italy), 18 to 21 g, pretreated with cyclophosphamide (CY; Cytoxan; Sigma) (150 mg/kg by the intraperitoneal route), 2 days before the infectious challenge.

Growth conditions and spheroplast preparation. Each isolate of *C. parapsilosis* was grown in YPD medium. The culture (1 liter) was incubated at 30°C with good aeration and was grown to stationary phase (optical density at 600 nm = 1.5 at 1 cm). For spheroplast formation, aliquots of the culture (50 ml) were washed and the cells were suspended in 6 ml of SE medium (1.2 M sorbitol and 0.1 M EDTA, pH 7.5) with 2 mg of Zymolyase 20T (Miles) added. After incubation for 1 h at 37°C, >90% of the cells were osmotically fragile spheroplasts as observed under the light microscope.

DNA preparation from spheroplasts. For the extraction of the whole cellular DNA (wDNA), the spheroplasts were centrifuged (4,000 rpm, 5 min) and incubated in a lysis buffer (50 mM Tris HCl, 20 mM EDTA, 1% sodium dodecyl sulfate, pH 7.5) in a final volume of 4 ml for 30 min at 65°C. Potassium acetate (1.5 ml, 3 M) was added, and the mixture was kept for 1 h on ice and then centrifuged (15 min, 10,000 rpm) in a Sorvall centrifuge equipped with an SS34 rotor. The pellet was discarded, and the supernatant was treated for DNA purification as described by Sherman et al. (21).

Preparation of mDNA. To obtain a purified preparation of mitochondrial DNA (mDNA) from cells of *C. parapsilosis*, essentially the method of Wills et al. (26) was used. Briefly, washed spheroplasts (see above) were lysed by homogenizing for 1 min in a Dounce homogenizer after suspension in a mixture of 0.7 M sorbitol, 60 mM Tris HCl, 1 mM EDTA, and 0.1% BSA. The homogenate was centrifuged twice for 10 min at 2,500 rpm, and the resultant two supernatants containing the mitochondrial fraction were mixed and centrifuged (10 min at 15,000 rpm) in a Sorvall centrifuge equipped with an SS34 rotor. Further purification, including a treatment with DNase I, was carried out as described by Wills et al. (26). The DNA was extracted by the phenol method (see above), and after dialysis of the aqueous phase, the nucleic acid was purified by a gradient of CsCl (BDH), as described elsewhere (19, 26).

Restriction endonuclease analysis (REA). The following restriction endonucleases, purchased from Boehringer, Mannheim, Germany, were used throughout this study: *Eco*RI, *Hin*dIII, *Bam*HI, *Bgl*II, *Hpa*II, *Pvu*II, and *Kpn*I. wDNA or mDNA (10 or 3 μg respectively) was incubated with 80 or 20 U of each enzyme. The conditions for DNA digestion, electrophoresis on agarose (1%, Bio-Rad), and ethidium bromide staining were those indicated in common laboratory texts (11).

Preparation of samples for karyotype analysis. Cells of *C. parapsilosis* were grown to the stationary phase in YPD medium and then washed in 1.2 M sorbitol solution containing 20 mM EDTA, pH 8.0. The pellet was resuspended to a cell concentration of 10^9/ml in a solution of EDTA-sorbitol as described above, but containing 20 mM β-mercapthoethanol, and incubated for 15 min at 37°C. The samples were then embedded in low-melting-point agar (Bio-Rad), and the spheroplast lysis method described by Vollrath and Davis (25) was used to prepare the chromosomal DNA. A similar DNA preparation from a laboratory strain of *Saccharomyces cerevisiae* was used as a standard.

Determination of the electrophoretic karyotype. The electrophoretic karyotype of *C. parapsilosis* was determined by pulsed electrophoresis in two different technical approaches. One used rotating field gel electrophoresis (RFGE), and the other used contour clamped homogeneous field gel electrophoresis (CHEF). The RFGE analysis was performed with a Rotaphor R22 gel apparatus from Biometra (Biomedinische Analytic GmbH, Gottingen, Germany). In this apparatus, chromosome separation is achieved through periodic changes in the direction of the electrical field due to the mechanical rotation of the electrodes. Free choice of separation angle and handling of large gels (20 by 20 cm) are advantageous features of this instrument. The operating

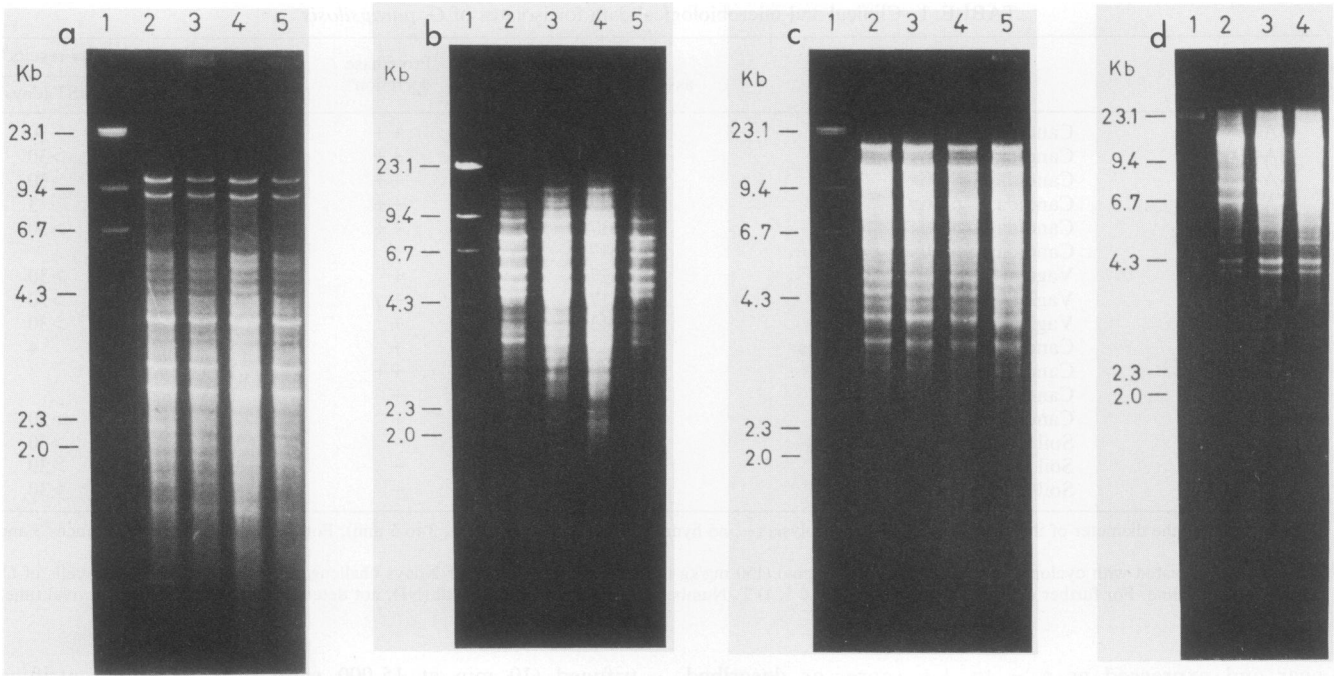

FIG. 1. REA of wDNA of *C. parapsilosis* after digestion with *Hpa*II (a), *Hin*dIII (b), *Eco*RI (c), or *Bgl*II (d) and electrophoresis on an agarose gel as described in the text. Lanes: 1, *Hin*dIII digest of lambda DNA; 2, environmental isolate ISS-4884; 3, vaginitis isolate SA-17; 4, vaginal carriage isolate SA-28; 5, hematological isolate HEM-1.

conditions were slightly modified from the standard ones given by the constructors. Briefly, three consecutive runs were done on each gel (20 by 20 cm; 0.5-cm-thick 1% GTG agarose; Biometra) containing the DNA samples in agarose inserts, immersed in running buffer composed of 25 mM Tris HCl, 25 mM boric acid, 0.75 mM EDTA, pH 8.2. Common as well as differential parameters of each run (duration, temperature, electrode rotation angle and period of rotation, and voltage of electrophoresis) are specified in single experiments. After the electrophoresis the gel was stained with ethidium bromide (0.5 μg/ml; 30 min), destained, and photographed with Polaroid film no. 667. The CHEF analysis was performed with the CHEF-DR II apparatus from Bio-Rad. The operating conditions included two consecutive runs on each gel (14.5 by 12.7 cm; 0.5-cm-thick 1% agarose, Bio-Rad) containing the agarose inserts of DNA immersed in running buffer (50 mM Tris HCl, 50 mM boric acid, and 1.5 mM EDTA, pH 8.2). The parameters of each run are specified in the legend of the relevant figure. After electrophoresis, the gel was stained with ethidium bromide and photographed, as described above.

RESULTS

(i) REA of wDNA. The patterns of endonuclease restriction of wDNA from the distinct isolates of *C. parapsilosis* were generated by using a set of enzymes inclusive of those which proved most useful for DNA fingerprinting of *C. albicans* (8, 20). Whatever the enzyme used, the REA pattern was identical for wDNA of all isolates of *C. parapsilosis* tested, and only slight differences in the intensities of major bands could occasionally be observed. The largest number of distinct bands was generated by *Eco*RI, *Hin*dIII, *Bgl*II, and *Hpa*II enzymes, while with the other enzymes used (in particular, with *Kpn*I) a less discriminative pattern was

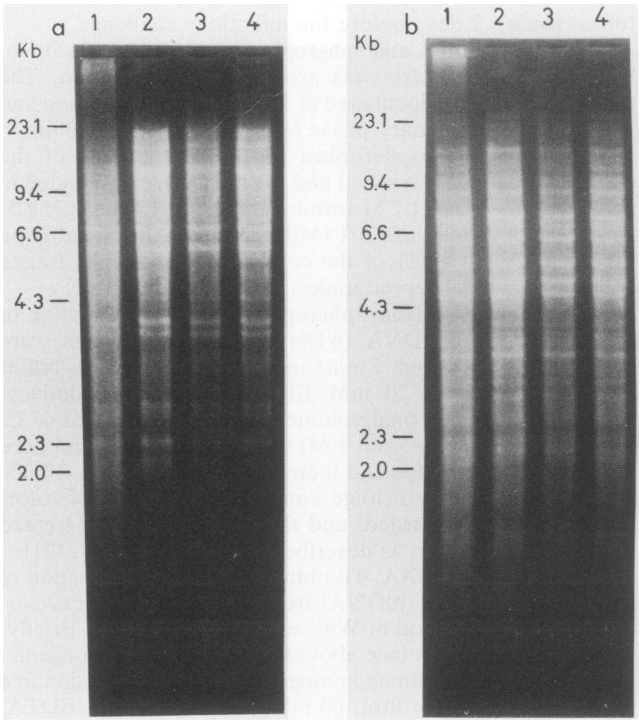

FIG. 2. REA of *C. parapsilosis* wDNA after digestion with *Bgl*II (a) or *Hin*dIII (b) and electrophoresis on agarose as described in the text. Lanes: 1, ISS-4888; 2, SA-23; 3, SA-38; 4, HEM-3.

TABLE 2. Sizes of major endonuclease restriction fragments of wDNA and mDNA of *C. parapsilosis*

Restriction fragment	Size (kbp) of:	
	wDNA	mDNA[a]
*Eco*RI	19.0, 16.0, 13.0, 8.4, 7.3, 5.7, 4.9, 4.4, 3.8, 3.4, 0.8	16.0, 13.0, 0.8
*Hin*dIII	14.0, 9.1, 7.5, 6.8, 6.0, 5.3, 5.0, 4.5, 3.6, 2.7, 2.3, 0.8	6.8, 6.0, 5.3, 5.0, 3.6, 2.3
*Bam*HI	20.0, 9.2, 8.5, 7.2	20.0, 9.2
*Bgl*II	13.7, 7.3, 6.6, 6.3, 6.2, 4.7, 4.4, 4.2, 3.5, 2.9, 2.5 (×2), 2.3	6.6, 4.4, 4.2, 3.5, 2.9, 2.5 (×2), 2.3
*Hpa*II	10.8, 8.8, 4.9, 4.6, 3.7, 3.1, 2.7	10.8, 8.8, 3.7, 3.1, 2.7

[a] The sizes of mDNA fragments were calculated on wDNA electrophoretic runs in which all fragments were better separated because of longer runs.

observed. Figures 1 and 2 show the REAs of strains representative of the different categories of isolates investigated. Table 2 reports the molecular sizes of the major fragments generated by the five more common enzymes as determined by comparison with standard-M_r fragments of *Hin*dIII digests of phage DNA. The pattern was independently studied after a number of passages in cultures and growth in different media (Sabouraud dextrose, or Winge yeast extract-glucose); it was consistent and stable for each isolate tested.

(ii) mDNA analysis. Camougrand et al. (2) suggested that different groups of *C. parapsilosis* could be discriminated on the basis of the restriction pattern of mDNA. Thus, we investigated particularly this aspect with purified preparations of mDNA from selected isolates of each category of *C. parapsilosis*, using the panel of endonucleases indicated in Table 2, which also indicates the number and sizes of the fragments obtained in each digestion. The most discriminative pattern was that obtained with *Hin*dIII, which gave at least 6 fragments ranging from 2.2 to 6.8 kb. Nonetheless, no appreciable differences were detected among the various isolates examined with either *Hin*dIII or any other enzyme digestion pattern. Figure 3 shows the *Eco*RI, *Hin*dIII, and *Hpa*II REA patterns of mDNA from representative isolates of *C. parapsilosis*. From the above patterns, the size of this DNA was calculated to fall around 30 kb in each isolate, confirming estimations by others (2). A comparison of the mDNA fragment pattern (Fig. 3) with the digestion pattern of wDNA (Fig. 1 and 2) clearly shows that the bands generated by digestion of the mDNA are major components of the digestion pattern of wDNA, as previously observed in *C. albicans* (26).

(iii) Electrophoretic karyotypes of *C. parapsilosis*. Variations in the electrophoretic karyotype of *C. albicans* are being intensely investigated, and reports suggest that they can be of great value in defining biotypes of this fungus (7, 10, 13, 15). Also in view of the established uniformity of DNA restriction patterns of *C. parapsilosis* (see above) as opposed to those of *C. albicans* (8, 20), we considered it to be of particular interest to examine the electrophoretic karyotypes of our isolates of *C. parapsilosis*. For comparison and approximate mass determination, the karyotype of a strain of *S. cerevisiae* was also examined. Both RFGE and CHEF patterns were without gross distortions and consistently revealed both common and differential features among the isolates. Common to all isolates were, besides a large amount of material banding closely to the origin of the gel, three (in RFGE) or four (in CHEF) well-defined chromosome-sized DNA molecules in the relatively large molecular size range (≥3 to 1.9 Mb). In a lower segment of the gel (1.8 to 0.7 Mb), there were appreciable variations in the karyotypes of the different isolates, resulting from changes in both

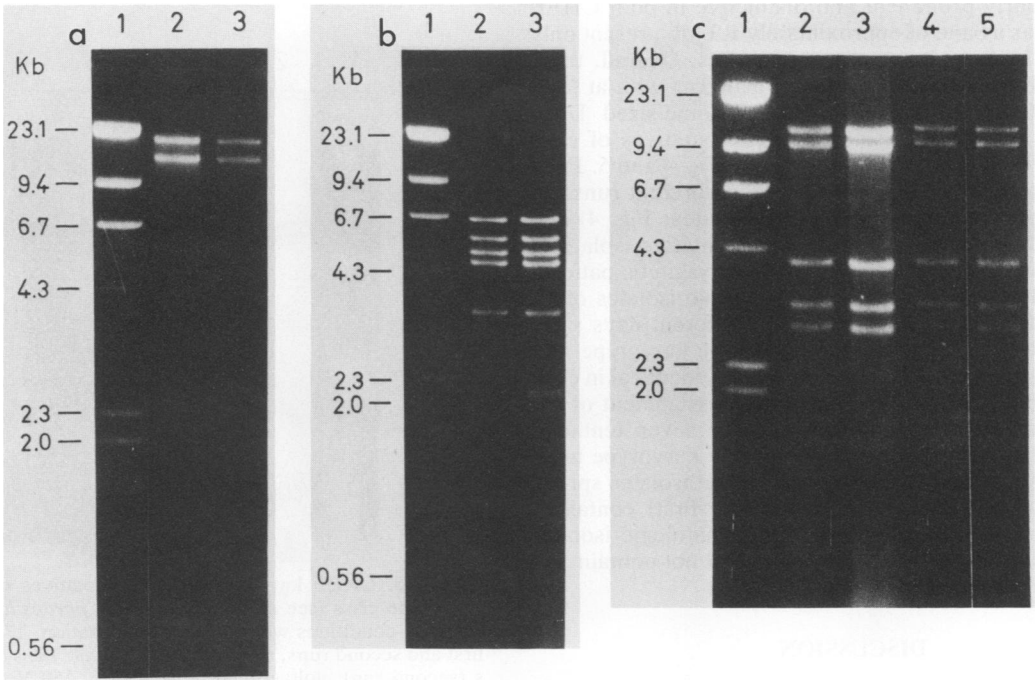

FIG. 3. REA of mDNA from *C. parapsilosis* after digestion with *Eco*RI (a), *Hin*dIII (b), or *Hpa*II (c) and electrophoresis on an agarose gel as described in the text. Lanes: 1, *Hin*dIII digest of lambda DNA; 2, environmental isolate ISS-4888; 3, vaginitis isolate SA-19; 4, vaginal carriage isolate, SA-28; 5, hematological isolate HEM-1.

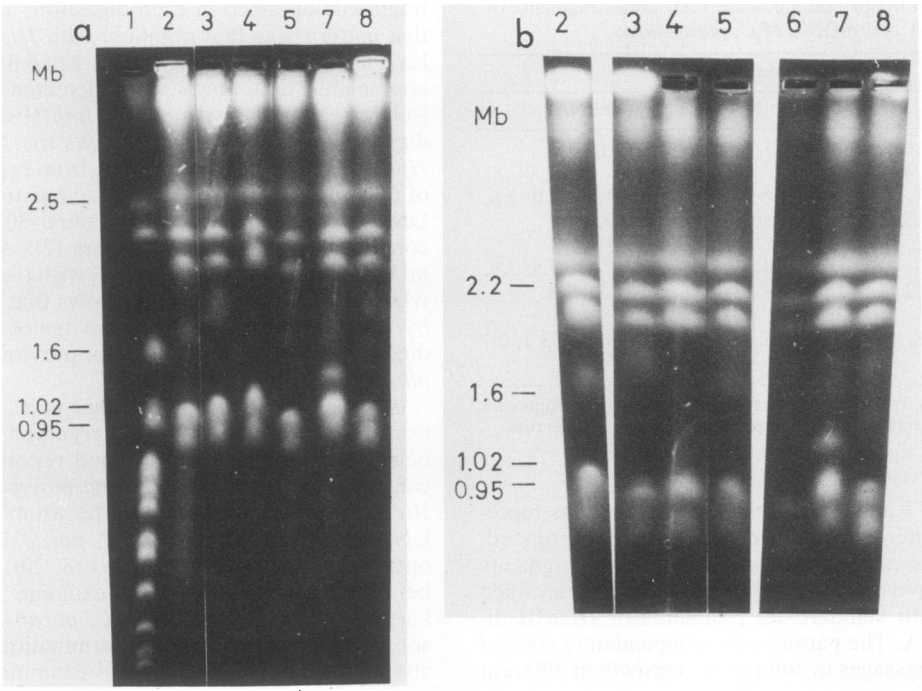

FIG. 4. RFGE karyotype of representatives of each proposed karyotype class (see also Table 3) of *C. parapsilosis* obtained under two distinct sets of RFGE conditions. The electrophoresis conditions for panel a were as follows: duration, 22 h (first run) and 24 h (second and third runs); rotation angle, 120°; interval of rotation, 75 s (first run), 150 s (second run), and 600 s (third run); voltage, 240 V (first run) and 120 V (second and third runs). The conditions for panel b were the same, except that the first run lasted for 24 h and the rotation intervals were 70 and 1,000 s for the first and third runs, respectively. Lanes: 1, *S. cerevisiae*; 2, ISS-4884; 3, SA-17; 4, SA-19; 5, SA-28; 6, SA-197; 7, HEM-1; 8, HEM-2. For other experimental details, see the text.

the number and sizes of the bands. Although some constituents either were not well resolved or were faintly visible, at least two to five DNA-sized bands could be detected in each case. Particularly prominent and distinctive in both CHEF and RFGE was a band of approximately 1.3 Mb present only in three (of the four) hematological isolates. Overall, there appeared to be seven electrophoretic patterns with at least five to eight easily discernible chromosome-sized DNA molecules in the distinct isolates. Representatives of each pattern in RFGE and CHEF are shown in Fig. 4 and 5. Each single pattern was totally reproducible in different runs, by both RFGE (four repetitions with all isolates; Fig. 4) and CHEF (two repetitions with each representative isolate of the different classes). In addition, for two vaginitis patients and all four hematological subjects, multiple isolates of *C. parapsilosis* from the same subject on different days were available and examined: the electrophoretic karyotype pattern obtained by either RFGE or CHEF was identical in each isolation. Table 3 proposes a preliminary assignment of the different isolates of *C. parapsilosis* to the seven tentative electrophoretic classes individuated by the karyotype analysis. As shown in the table, the nine vaginal isolates spread into five classes, only one of which (the first) contained isolates from other source (soil). The hematologic isolates fell into two classes (VI and VII) which did not contain any isolates from another source.

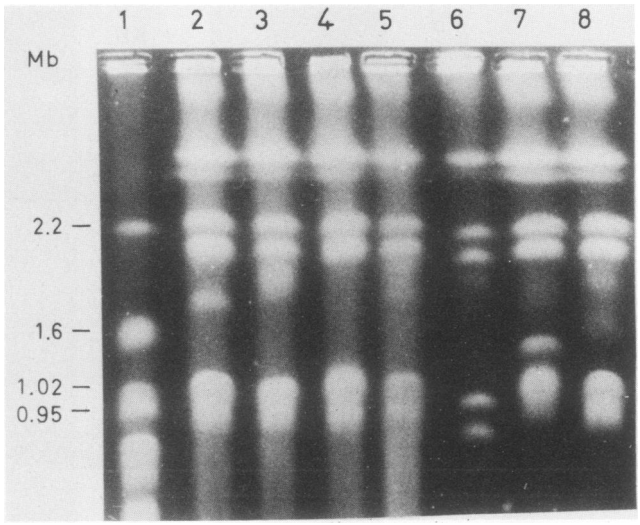

FIG. 5. CHEF karyotype of representatives of each proposed karyotype class (see also Table 3) of *C. parapsilosis*. The electrophoresis conditions were as follows: duration, 17 and 24 h for the first and second runs, respectively; interval, 180 s (first run) and 100 s (second run); voltage and temperature, 150 V and 10°C in both runs. The lanes are as those described in the legend to Fig. 4 (lane 1 contains only the higher portion of *S. cerevisiae* karyotype [Fig. 4]).

DISCUSSION

This investigation represents, to our knowledge, the first application of both DNA fingerprinting and electrophoretic karyotype analysis exclusively devoted to a panel of envi-

TABLE 3. Tentative class subdivision of isolates of *C. parapsilosis* according to the karyotype in RFGE and CHEF

Class	No. of isolates	Laboratory identification	Source of isolation	No. of putative chromosome-sized DNA bands in[a]:	
				CHEF	RFGE
I	4	ISS-4879	Soil	7	6
		ISS-4884[b]	Soil		
		ISS-4888[b]	Soil		
		SA-25	Vaginitis		
II	1	SA-17[b]	Vaginitis	7	6–7
III	5	SA-19[b]	Vaginitis	6	5–6
		SA-23[b]	Vaginitis		
		SA-36	Vaginitis		
		SA-38[b]	Vaginial carriage		
		SA-42	Vaginitis		
IV	1	SA-28[b]	Vaginal carriage	7–8	6–7
V	1	SA-197[b]	Vaginal carriage	6–7	5
VI	3	HEM-1[b]	Candidemia and endocarditis	7–8	6
		HEM-3[b]	Candidemia		
		HEM-4	Candidemia		
VII	1	HEM-2[b]	Candidemia	7–8	6–7

[a] The highest-M_r, large-DNA material close to the origin of the gel, present in all isolates and seen with both RFGE and CHEF, was not considered to be a distinct chromosome-sized band. Uncertainties in the attribution of the number of DNA-sized bands depend on the presence of constituents in the region from 2.0 to 1.6 Mb which were faintly discernible, but nonetheless consistently observed in separate runs, by either RFGE or CHEF.

[b] Isolates shown in Fig. 4 and 5.

ronmental and clinical isolates of *C. parapsilosis*. Previous results were obtained mostly with *C. albicans* and demonstrated the potency of these molecular approaches in discriminating among isolates of the fungus (7, 8, 10, 13, 20). In their studies on the electrophoretic karyotype of *Candida* spp., Magee and Magee (10) and, very recently, Monod et al. (15) also observed the karyotype of one or more isolates of *C. parapsilosis* but did not investigate biotyping isolates of this fungus from different sources and endowed with different pathogenicity potentials (4, 5). *C. parapsilosis* has been classically considered a low-pathogenic or nonpathogenic fungus, but it is increasingly implicated as a cause of fungemia and endocarditis in severely neutropenic, bone marrow-transplanted patients with underlying malignancies (6, 12). Moreover, we obtained suggestive evidence for this fungus being a cause of candidal vaginitis (4, 5). Thus, genetic discrimination among isolates of *C. parapsilosis* may offer important clues to the understanding of the pathogenetic mechanisms operating in invasive or superficial candidiasis due to *C. parapsilosis*, as well as the modalities of fungal infection and transmission.

In this study, we employed 16 isolates of *C. parapsilosis* from different sources and endowed with different pathogenicity properties. Nonetheless, a rather uniform DNA restriction pattern (fingerprinting) lacking significant polymorphism was noticed. In particular, this uniformity was verified with the examination of mDNA restriction fragments that had previously been reported to discriminate among closely related species (and varieties) such as *C. parapsilosis* and *Candida rhagii* (2). The mass of restriction fragments of mDNA was absolutely identical in our isolates and in those of Camougrand et al. (2) when typical *C. parapsilosis* strains were compared. mDNA was, as in *C. albicans* (17), a major contributor of the most abundant (repetitive) restriction fragments of cellular DNA, but it contained fewer *Eco*RI restriction sites and was smaller (30 kb) than the mDNA of *C. albicans* (41 kb) (26). *Eco*RI digests of total DNA of *C. parapsilosis* were also produced by Scherer and Stevens (20). These authors divided their seven isolates into three classes and suggested that *C. parapsilosis* is genotypically more heterogeneous than *C. albicans*. From a comparison of our *Eco*RI pattern (Fig. 1a) and that in Fig. 3 and Table 1 of the aforementioned paper (20), it seems that all of our 16 isolates belong to the group VII of Scherer and Stevens (20). Studies with the use of more-discriminative restriction enzymes and suitable DNA probes for a further assessment of DNA fingerprinting in *C. parapsilosis* are in progress in our laboratories.

Other results of our investigations which suggest remarkable genetic heterogeneity in *C. parapsilosis* are those derived from the karyotype analysis generated by RFGE and by CHEF. In particular, the former technique, which has not been used in previous studies of *Candida* chromosomes, offered an efficient, undistorted separation of DNA-sized chromosomes of *C. parapsilosis* over a wide range of sizes (3.0 to 0.5 Mb) which required, in previous investigations dealing with *C. albicans* (9, 10), the combined use of three different techniques. In general, the resolving power of RFGE was comparable to that of a most widely used CHEF, but the latter gave an additional band in the high-M_r range. In this range, however, the electrophoretic pattern was common to all isolates studied. Karyotype variations among the isolates occurred only in the lower portion of the karyogram, where chromosomes of 700 to 1,300 kb were located. In some isolates, the differences concerned faintly visible bands which nonetheless were reproducible in different runs and were consistently detected by both RFGE and CHEF.

Overall, the karyotype of *C. parapsilosis* could consist of five to eight chromosomes as a minimum estimate, and the 16 isolates examined could be separated into a number of distinct electrophoretic groups potentially useful for biotyping. The classification proposed in this paper must, however, be taken as a truly preliminary one, and more-refined experiments inclusive of chromosome hybridization with cloned genes are needed to gain more-precise insight into the electrophoretic karyotyping of *C. parapsilosis*.

Several authors have recently emphasized the value of the electrophoretic karyotype in discriminating among isolates of various species of *Candida* (7, 10). Vazquez et al. (24) suggest that this approach may be more specific than REA in biotyping *C. albicans*. The present data appear to support this idea also for *C. parapsilosis*, in view of the remarkable homogeneity of DNA fingerprinting for different clinical isolates of the fungus and, conversely, the documented polymorphism of the electrophoretic karyotype.

From this preliminary investigation, dealing with a relatively low number of isolates, no definitive conclusions can be drawn concerning any relationship between the karyotype pattern and the source or the virulence of *C. parapsilosis* isolates. Nonetheless, the isolates from the three sources do not appear to be randomly distributed in the seven classes. In particular, none of the hematologic isolates belonged to a class containing isolates from other sources, the three environmental isolates did not split into different classes, and of the nine vaginal isolates, only one showed an

electrophoretic karyotype identical to that of soil isolates. The existence of a particular niche for particularly aggressive biotypes of *C. parapsilosis* is not demonstrated, but this clearly warrants further investigation. For this investigation, the genetic analysis of the karyotype appears particularly promising.

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