Journal of Clinical Microbiology, Dec. 2000, p. 4554–4559 Vol. 38, No. 12
0095-1137/00/$04.00+0
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Comparative Genotyping of *Candida albicans* Bloodstream and Nonbloodstream Isolates at a Polymorphic Microsatellite Locus

FRÉDÉRIC DALLE,[1,2] NORELIE FRANCO,[3] JOSÉ LOPEZ,[1,2] ODILE VAGNER,[1,2] DENIS CAILLOT,[4] PASCAL CHAVANET,[2,5] BERNADETTE CUISENIER,[1,2] SERGE AHO,[6] SARAB LIZARD,[3] AND ALAIN BONNIN[1,2]*

Laboratoire de Parasitologie et Mycologie,[1] Laboratoire de Microbiologie Médicale et Moléculaire,[2] Service d'Hématologie Clinique,[4] Service des Maladies Infectieuses et Tropicales,[5] and Service d'Epidémiologie et d'Hygiène Hospitalière,[6] CHU et Faculté de Médecine, and Laboratoire de Génétique Moléculaire, Centre George François Leclerc,[3] Dijon, France

Received 26 June 2000/Returned for modification 9 August 2000/Accepted 21 September 2000

Molecular typing studies have shown that the predominant form of reproduction of *Candida albicans* is clonal and that, in a majority of situations, persistent or recurrent infections are due to a unique strain. Characterization of distinct subpopulations and correlation with clinical features may thus be important to understanding the pathogenesis of candidiasis. In a clonal model, a unique polymorphic marker may identify populations with different biological properties. We therefore compared 48 bloodstream isolates and 48 nonbloodstream matched strains of *C. albicans* at the elongation factor 3-encoding gene (CEF3) polymorphic microsatellite locus of *C. albicans*. Sizing of the alleles was performed by automated capillary electrophoresis. A new, 137-bp allele was characterized, and seven nondescribed combinations were observed, resulting in 15 and 11 distinct CEF3 profiles in bloodstream and control strains, respectively. Genotypes 126-135, 130-136, and 131-131 accounted for 60.4% of both bloodstream and control strains. Four bloodstream isolates but no control strains displayed the 135-135 combination. None of the other genotypes was present at an increased frequency in bloodstream isolates. Bloodstream and nonbloodstream strains of *C. albicans* thus have a heterogeneous structure at the CEF3 locus, with three major and multiple minor allelic combinations.

Yeasts of the genus *Candida* are ubiquitous eucaryotic organisms that develop as saprophytes of the mucosa and skin in humans and other vertebrates. In immunocompromised or intensive-care patients, however, these normally harmless organisms may overcome host defenses, resulting in increased mucosal colonization and eventually invasion of the fungus into the bloodstream through epithelial and endothelial layers. A striking rise in candidemia has occurred in the past 15 years in intensive-care, surgical, neutropenic, and cancer patients. Candidemia accounts for 8 to 15% of bloodstream infections and is associated with a substantial, 38%, attribuable mortality (1, 7, 34, 35). Within the genus *Candida*, the species *Candida albicans* is the most common fungus isolated in humans. It is detected in one or more body locations in 70% of healthy women and accounts for 50 to 70% of all disseminated *Candida* infections (8, 10).

This clinical and epidemiological context led to the development of a variety of molecular typing techniques to distinguish *Candida* isolates, including multilocus enzyme electrophoresis, total genomic DNA digestion profiles, electrophoretic karyotyping, randomly amplified polymorphic DNA analysis, and Southern blot hybridization with repetitive DNA probes (5, 11, 15, 16, 19, 20, 24, 26, 28–30). These methods confirmed endogenous acquisition through mucosal colonization as a major source of disseminated infection, but exogenous infection from the hospital staff or environment was also shown to occur (11). In addition to molecular epidemiology, the availability of molecular markers allowed for population structure studies. Several investigators reported a linkage disequilibrium between independent markers, indicating that *C. albicans* is primarily clonal (2, 12, 17, 24, 25, 30). According to a clonal model of reproduction, *C. albicans* would comprise different clonal lineages that propagate independently. If such clones differ in properties such as virulence or host adaptation, major clones should in fact be the object of biomedical studies (31). Correlation of *C. albicans* subpopulations with clinical features is therefore critical to understanding the mechanisms of virulence and host adaptation of this microorganism. Establishing such correlations, however, implies identification of appropriate markers. In a clonal organism, multilocus allele combinations are typically linked across the genome (30). Therefore, distinct alleles of a unique polymorphic marker might be associated with gene combinations involved in biological properties and could provide a means for identifying important isolate subpopulations. To be useful in the clinical microbiology setting, however, this marker should be stable, easy to assay, adaptable to large series, and discriminatory.

Among the molecular methods aimed at characterizing infectious agents, microsatellite regions have recently been proposed as markers of eucaryotic genomes (4). Indeed, these simple, tandemly repeated oligonucleotides (mono- to hexanucleotides) are highly polymorphic, codominantly inherited (allowing distinction of heterozygotes in diploid organisms), and amenable to precise, robust, and highly reproducible allele characterization through sizing or sequencing of PCR products. They thus compare favorably with other DNA-based methods that may be time-consuming and poorly reproducible and that may rarely assay codominantly inherited polymorphisms. Several groups have recently characterized microsatellite markers of the *C. albicans* genome (3, 9, 18, 21). One such microsatellite, present in the promoter region of the elongation factor 3-encoding gene (CEF3) of *C. albicans*, was

* Corresponding author. Mailing address: Laboratoire de Parasitologie et Mycologie, Hôpital du Bocage, 21034 Dijon Cedex, France. Phone: 33 (0)3 80 29 36 03 or 33 (0)3 80 29 35 23. Fax: 33 (0)3 80 29 32 80. E-mail: alain.bonnin@chu-dijon.fr.

TABLE 1. CEF3 allele associations and origins of the *C. albicans* isolates tested

Bloodstream isolates		Control isolates		
Designation	Allele association	Designation	Site of isolation	Allele association
1561	133-136	1551	Stool	126-135
1543	130-135	1547	Urine	126-135
1525	126-135	1508	Bronchoalveolar lavage	126-135
1521	126-135	1495	Ascites	131-131
1492	130-130	1476	Vaginal mucosa	126-135
1471	135-135	1449	Peritoneal fluid	133-144
1467	135-135	1442	Stool	130-136
1445	131-131	1427	Skin	136-145
1439	131-131	1407	Bronchoalveolar lavage	131-131
1412	131-131	1393	Bronchoalveolar lavage	130-136
1383	126-135	1381	Upper respiratory tract	137-139
1367	126-135	1368	Urine	130-136
1344	130-144	1346	Upper respiratory tract	136-144
1315	126-135	1333	Esophageal mucosa	126-135
1304	135-139	1317	Colonic mucosa	136-144
1248	126-135	1305	Oral mucosa	131-131
1153	130-136	1300	Peritoneal fluid	131-131
1141	136-137	1288	Sperm	126-135
1134	131-131	1256	Skin	131-131
1100	130-136	1211	Sinus mucosa	130-144
1098	130-136	1199	Bronchoalveolar lavage	130-136
1058	131-131	1155	Bronchoalveolar lavage	126-135
1043	130-136	1138	Bronchoalveolar lavage	136-145
1042	130-136	1103	Mediastinal fluid	136-136
953	126-126	1060	Oral mucosa	131-131
931	130-136	1045	Skin	131-131
930	135-135	960	Oral mucosa	136-144
926	131-131	936	Oral mucosa	126-135
868	130-136	854	Throat mucosa	126-135
843	130-136	800	Penis mucosa	126-126
829	135-135	794	Sputum	130-136
796	126-135	742	Vaginal mucosa	126-135
795	131-131	712	Nasal secretions	133-144
778	131-131	665	Anal mucosa	133-144
677	136-144	658	Urethra	136-145
650	126-135	643	Oral mucosa	136-145
649	126-135	626	Skin	126-135
610	126-135	622	Urine	126-135
598	130-144	603	Penis mucosa	130-136
537	126-126	551	Vulva	126-135
517	136-145	539	Vaginal mucosa	130-136
516	126-135	520	Upper respiratory tract	130-136
483	136-144	505	Skin	126-126
482	126-135	491	Stool	136-145
478	130-131	484	Sputum	130-130
461	126-135	477	Oral mucosa	130-130
443	136-144	460	Ascites	126-135
429	133-144	445	Oral mucosa	126-126

shown to have 11 alleles organized in 16 different combinations and was characterized in terms of discriminatory power (3).

We report here the genotyping of this CEF3 microsatellite in 48 bloodstream isolates and 48 nonbloodstream control strains of *C. albicans* obtained from patients treated at the University Hospital of Dijon. Three allele combinations were shown to be present at almost identical frequencies in the two series of isolates and altogether accounted for 60.4% of the strains tested, suggesting that the three corresponding *C. albicans* populations may develop more efficiently in hospitalized humans than isolates with underrepresented alleles. Except for one allelic combination that was identified in four bloodstream isolates but in none of the control strains, no CEF3 genotype was present at an increased frequency in the bloodstream group.

MATERIALS AND METHODS

***C. albicans* strains.** Bloodstream strains of *C. albicans* were obtained from patients treated at the University Hospital of Dijon between January 1994 and July 1999. Blood culturing was performed with Sabouraud broth from 1994 to December 1997 and with BACTEC 9240 blood culture bottles (Becton Dickinson, Le Pont de Claix, France) since January 1998. Control strains originated from a variety of clinical samples that had been cultured on Sabouraud dextrose agar (Table 1). They were selected on the basis of the following criteria. One control strain was included for each bloodstream isolate included in this study. The control strain was taken from a patient who did not have blood cultures positive for *Candida* spp. and who was treated during the same period (within a 4-week period) as the bloodstream isolate counterpart to overcome a possible fluctuation of *C. albicans* populations over the 4.5-year duration of bloodstream isolate collection. A control strain was also selected from a patient treated in a distinct clinical unit to avoid any artificial homogenization of the two groups due to transmission of nosocomial strains. Only one isolate per patient was used to compare allelic frequencies in bloodstream and control isolates (Table 2). For two patients for whom two or three blood culture isolates were available, geno-

TABLE 2. Frequencies of CEF3 allele associations in bloodstream and control isolates

CEF3 allele association	No. (%) of isolates with the indicated allele association		
	Bloodstream	Control (noninvasive)	Total
126-135	13 (27)	14 (29.2)	27 (28.1)
130-136	8 (16.7)	8 (16.7)	16 (16.7)
131-131	8 (16.7)	7 (14.6)	15 (15.6)
135-135	4 (8.3)	0 (0)	4 (4.2)
136-144	3 (6.25)	3 (6.25)	6 (6.25)
136-145	1 (2)	5 (10.4)	6 (6.25)
130-135	1 (2)	0 (0)	1 (1)
130-130	1 (2)	2 (4.2)	3 (3)
130-144	2 (4.2)	1 (2)	3 (3)
130-131	1 (2)	0 (0)	1 (1)
126-126	2 (4.2)	3 (6.25)	5 (5.2)
133-136	1 (2)	0 (0)	1 (1)
133-144	1 (2)	3 (6.25)	4 (4.2)
135-139	1 (2)	0 (0)	1 (1)
136-137	1 (2)	0 (0)	1 (1)
137-139	0 (0)	1 (2)	1 (1)
136-136	0 (0)	1 (2)	1 (1)

typing of sequential isolates was performed (Table 3). For each of these patients, all isolates displayed the same profile, and the first strain of each sequence (strains 829 and 1467 for patients ID1 and GE1, respectively) was therefore included in the comparison of bloodstream and control isolates. Therefore, altogether, 48 bloodstream strains originating from 48 patients and 48 matched control strains originating from 48 distinct patients were included in the comparison of allelic combinations.

Each strain was determined to be *C. albicans* by filamentation at 37°C for 4 h in human serum, followed by determination of the sugar assimilation profile with an API 32C kit (Biomerieux, Marcy l'Étoile, France). In addition to clinical isolates, three reference strains of *C. albicans* (ATCC 26278, ATCC 38245, and ATCC 38248) previously characterized at the CEF3 locus were included as controls. Yeasts were stored at −20°C on Sabouraud dextrose agar until typed.

DNA isolation. DNA extraction was adapted from a previously described protocol (13). Briefly, for each *C. albicans* isolate, two colonies grown on Sabouraud-chloramphenicol-gentamicin plates (Sanofi Diagnostic Pasteur, Marnes la Coquette, France) were suspended in 5-ml Sabouraud broth aliquots and incubated at 30°C for 18 h. After centrifugation for 2 min, the pellet was suspended in 0.5 ml of distilled water and washed by centrifugation for 20 s. The resulting supernatant was discarded, and the pellet was suspended in 200 μl of lysis buffer containing 10 mM Tris HCl (pH 8), 1 mM disodium EDTA, 100 mM NaCl, 2% (wt/vol) Triton X-100, and 1% (wt/vol) sodium dodecyl sulfate. After the addition of 200 μl of phenol-chloroform-isoamyl alcohol (25:24:1) and 300 mg of 0.5-mm-diameter glass beads (Sigma, l'Isle d'Abeau Chesnes, France), the sample was vortexed for 3 min, 200 μl of TE buffer (10 mM Tris HCl [pH 8], 1 mM disodium EDTA) was added to the tube, and the sample was centrifuged for 5 min. The upper, aqueous layer was recovered, and the nucleic acids were precipitated in ethanol at −20°C. After treatment with RNase (Sigma) and ethanol precipitation, the DNA was suspended in 50 μl of distilled water and stored at −30°C prior to PCR amplification.

PCR amplification. For each sample analyzed, 1 μl of DNA lysate was used as a template in 25-μl reaction mixtures containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.25 mM $MgCl_2$, 0.2 mM (each) deoxynucleoside triphosphate, 12.5 pmol (each) primers CAND1 (sense) (5′-TTT CCT CTT CCT TTC ATA TAG AA-3′) (positions 193 to 215; GenBank accession no. Z11484) and CAND2 (antisense) (5′-GGA TTC ACT AGC AGC AGA CA-3′) (positions 308 to 327; Genbank accession no. Z11484), and 1.25 U of *Taq* DNA polymerase (Ampli Taq Gold; Perkin-Elmer). Primer CAND2 was 5′ end labeled with fluorescein (Oligoexpress, Grenoble, France) for determination of the sizes of PCR products with an automatic sequencer (Perkin-Elmer 9700 DNA thermal cycler). Initial denaturation and activation of the Ampli Taq Gold polymerase were achieved by treating samples at 94°C for 10 min; the reactions were then subjected to 40 cycles of 30 s at 94°C, 30 s at 50°C, and 60 s at 72°C, followed by a 7-min extension at 72°C. Following completion of the PCR, 10-μl aliquots of the PCR products were analyzed on horizontal 2% agarose gels in TAE buffer (40 mM Tris acetate, 2 mM disodium EDTA · $2H_2O$) to control DNA amplification. Each amplification run contained a negative control (TE buffer only).

Fragment analysis by automated fluorescent capillary electrophoresis. Fragment size analysis was performed on an ABI PRISM 310 genetic analyzer (Perkin-Elmer Applied Biosystems, Courtaboeuf, France). Data were collected with ABI PRISM 310 collection software and analyzed with Genescan 2.1 software (Perkin-Elmer Applied Biosystems). For capillary electrophoresis, PCR products were diluted to 5 to 20 ng/μl in distilled water, and 1-μl aliquots were added to the capillary electrophoresis mixture, containing 13.5 μl of the template suppression reagent and 0.5 μl of the GeneScan-500 size standard (Perkin-Elmer Applied Biosystem). The capillary sample mixture was denatured for 5 min at 95°C and rapidly cooled on ice prior to analysis. The ABI PRISM 310 genetic analyzer was set up according to the manufacturer's instructions to use performance-optimized polymer 4 for microsatellite analysis (Perkin-Elmer Applied Biosystems). A 47-cm-long, 50-μm-inside-diameter capillary was installed, and performance-optimized polymer 4 was used as the liquid polymer matrix for electrophoresis. Electrophoresis parameters were 5-s injection time, 15-kV injection voltage, 15-kV electrophoresis voltage, 150-s syringe pump time, 120-s preinjection electrophoresis, 60°C migration temperature, and 28-min collection time to ensure detection of PCR fragments smaller than 450 bp.

Sequencing of the 137-bp CEF3 alleles. Capillary electrophoresis PCR fragment analysis identified a fragment at 137 bp that had not been described previously. In order to characterize the sequence of the repetitive elements TTC and TTTC in this new allele, PCR products were cloned with a TOPO TA cloning kit (Invitrogen, Groningen, The Netherlands) according to the manufacturer's recommendations. The cloned PCR products were analyzed by capillary electrophoresis to check for the presence of the allele of interest, and nucleotide sequencing of the new allele was carried out with an ABI PRISM BigDye terminator cycle sequencing ready reaction kit on an automated sequencer (model ABI PRISM 373A; Perkin-Elmer).

TABLE 3. Genotyping of sequential bloodstream isolates for two patients

Clinical isolate	Patient	Department	Date of positive blood culture[a]	Genotype
1475	GE1	Gastroenterology	15/01/1999	135-135
1467	GE1	Gastroenterology	29/12/1998	135-135
956	ID1	Infectious diseases	23/05/1996	135-135
853	ID1	Infectious diseases	27/01/1996	135-135
829	ID1	Infectious diseases	04/01/1996	135-135

[a] Day/month/year.

RESULTS

Genotyping of reference strains. In a first series of experiments, three reference strains of *C. albicans* previously genotyped at the CEF3 locus (3) were analyzed by fluorescent capillary electrophoresis. This preliminary step was necessary to ensure fragment size comparisons between the two methods of electrophoretic separation of the PCR fragments: gel electrophoresis, which was performed in the initial description of the CEF3 microsatellite, and capillary electrophoresis, which was used in the present work. Indeed, variations in fragment size determination may occur depending on the electrophoresis method (27, 33). Since *C. albicans* is a diploid organism (36) and the CEF3 gene is a single-copy locus in the *C. albicans* genome (22), one or two distinct PCR fragments (i.e., alleles) may result from amplification of the CEF3 microsatellite in homozygote or heterozygote isolates, respectively. The three reference strains were selected on the basis of their CEF3 allele combinations to ensure comparative analysis of small fragments (heterozygote ATCC strain 26278; alleles, 126 and 135 bp), large fragments (heterozygote ATCC strain 38248; alleles, 130 and 144 bp), and intermediate fragments (homozygote ATCC strain 38245; association, 130-130). These experiments showed a constant discrepancy between gel electrophoresis and capillary electrophoresis analyses, the latter giving values shorter by 7 bp. Analysis of the newly described 137-bp allele confirmed this discrepancy, since this allele, which displayed an apparent size of 130 bp upon capillary electrophoresis analysis, in fact had a 137-bp sequence upon sequencing of the cloned PCR products. As suggested by Wenz et al. (33), a normalizing factor was applied, consisting of the addition of 7 bp to the apparent size of the fragments analyzed

by fluorescent capillary electrophoresis. This 7-bp correction was applied to all data presented in this article.

Reproducibility of the assay. For reference strain ATCC 26278, three separate DNA extractions were performed. The resulting DNA samples were used as templates in distinct PCRs. All three PCR products displayed the 126-135 profile. Subsequently, PCR products from strain ATCC 26278 were run in every capillary electrophoresis experiment as a control. Two separate DNA extractions and typing experiments were also performed with isolate 1381 in order to clone and sequence the 137-bp allele. Finally, three separate amplifications and fragment size analyses were performed to confirm any allele combination that had not been described in the initial characterization of the CEF3 microsatellite (3). This procedure concerned isolates 1561 (combination 133-136), 1543 (combination 130-135), 1304 (combination 135-139), 1141 (combination 136-137), 1381 (combination 137-139), 478 (combination 130-131), and 960 and 1317 (representing allele combination 136-144). In all these experiments, repeated genotyping of an isolate always gave identical profiles, indicating that the assay is reproducible and that the CEF3 microsatellite locus is stable upon freezing and thawing of strains stored at −20°C.

Genotyping of bloodstream and colonizing isolates. Ten different alleles organized in 17 distinct profiles were found among 96 clinical isolates genotyped at the CEF3 locus. Both groups of isolates demonstrated an important polymorphism at the CEF3 locus, with 15 and 11 distinct allele combinations being found in bloodstream and control isolates, respectively (Table 2). However, most of these profiles were represented by one to three isolates only, whereas genotypes 126-135, 130-136, and 131-131 altogether accounted for 60.4% of both bloodstream and control isolates. In contrast, genotype 135-135 was overrepresented in bloodstream isolates compared with control isolates (8.3% versus 0), whereas genotype 136-145 accounted for 10.4% of control isolates and 2% of bloodstream isolates.

New alleles and allele-combinations: the discriminatory power of the assay. Among the 10 alleles described in the present work, 1 had not been observed in the initial characterization of the CEF3 microsatellite. Sequencing of the corresponding PCR fragments was performed to characterize the molecular structure of this allele. The sequence of the variable region was $(TTC)_7$ $(TTTC)_4$. Along with this new allele, two new associations, 136-137 and 137-139, that had not been previously described were observed. Moreover, new associations of previously described alleles were also observed in the current series: 133-136, 130-135, 135-139, 136-144, and 130-131. Altogether, when the results for the bloodstream and control strains analyzed here are pooled with previous data from Bretagne et al. (3), 12 distinct alleles are now characterized at the CEF3 locus of *C. albicans*, leading to 23 distinct allele combinations. Based on the 156 isolates currently genotyped at this locus, the numerical index of discriminatory power of this assay (3) is 0.87.

Stability of the CEF3 locus in vivo. When sequential isolates from patients ID1 and GE1 were genotyped, both series of isolates displayed the 135-135 genotype (Table 3). These experiments suggest that the CEF3 locus is stable during infection in vivo.

DISCUSSION

Multilocus analysis of *C. albicans* isolates from human immunodeficiency virus-positive and -negative individuals has provided evidence for linkage disequilibrium between independent markers (2, 12, 17, 24, 25, 30, 38), and although some recombination may occur (12, 30), available data support the hypothesis that the population structure of *C. albicans* is predominantly clonal. If distinct clonal lineages of *C. albicans* have variations in biological traits such as pathogenicity, identification of the corresponding genotypes should contribute to a better understanding of the natural history of candidiasis. The goal of the present study was to address this question by comparing *C. albicans* isolates from bloodstream cultures (i.e., invasive isolates) to clinical isolates obtained from other clinical samples from patients who did not have candidemia (i.e., control isolates).

The typing approach used here was to be developed in the context of a clinical mycology laboratory. It thus needed to be discriminatory for assessing nosocomial transmission, robust, reproducible, and simple to use. Analysis of PCR-amplified microsatellite regions appeared to fulfill these requirements. We chose to target a microsatellite in the promoter region of the CEF3 gene of *C. albicans*. Indeed, the CEF3 gene is a single-copy locus in the *C. albicans* genome (22), and variability at this locus cannot result from polymorphic copies interspersed in the genome. Moreover, this marker was shown to be stable upon subculturing in vitro and was characterized in terms of discriminatory power (3). Each genotype identified at the CEF3 locus was considered a marker, on the assumption that in a clonal model of development, a unique variable DNA region provides sufficient information to identify distinct genotypes (30). The technical approach used, automated capillary electrophoresis, has several advantages in the context of a clinical laboratory, since each run is completed within 30 min and fragment size can be determined as soon as the sample has been processed, providing immediate information. In addition, this technique is extremely flexible; between 1 and 96 samples can be analyzed in a single run.

Among all possible profiles, genotypes 126-135, 130-136, and 131-131 were overrepresented in both invasive and noninvasive strains at our institution. In addition, the frequencies of these three combinations were almost identical in our two series of strains described in the present report and in the series of Bretagne et al. (3). The stability of these allelic combinations among strains obtained in distinct clinical contexts and in distinct geographical areas is a striking result of the current investigation. Interestingly, Xu et al. (38) mentioned three main genotypes in a multilocus analysis of strains from healthy individuals and patients with or without AIDS. Altogether, these three major genotypes accounted for 54% of the strains tested. Further studies will be necessary to determine if the three major genotypes described in the present investigation segregate with those identified by Xu et al. (38). Taken together, these data suggest that some *C. albicans* populations may have a preferential tropism for development in humans. We must emphasize, however, that all the strains genotyped in the current study, including the so-called control strains, were cultured from patients treated in a university hospital, i.e., originated from nonhealthy individuals. This fact applies also to at least 31 of the 60 isolates previously genotyped at this locus (3). Therefore, these allelic frequencies may differ from those encountered in the general population and may reflect the natural selection of parasite populations more prone than others to undergo increased proliferation in a variety of clinical disorders. In this respect, Xu et al. mentioned reduced genetic diversity among *C. albicans* strains from nonhealthy patients compared with strains from healthy persons, suggesting the possibility of the selection of strains involved in clinical disorders (38).

In contrast to the three major genotypic populations ac-

counting for about 60% of the strains, multiple minor genotypes were identified in both bloodstream and control isolates tested here. Similarly, in the study by Xu et al., 63 minor genotypes accounted for 46% of the isolates (38). A comparison of frequencies of the minor genotypes among bloodstream and control strains showed that two combinations were present in control strains only (137-139 and 136-136), while five others were detected in bloodstream strains only (130-135, 130-131, 133-136, 135-139, and 136-137). The seven genotypic combinations reported in this study and not present in the series of Bretagne et al. (3) (133-136, 130-135, 135-139, 136-137, 137-139, 136-144, and 130-131) belong to this subgroup of minor genotypic combinations. The significance of these so-called minor genotypes in pathogenic processes deserves attention in future studies. Recent population structure studies showed that most *C. albicans* isolates were grouped in one of three major clusters (17, 24). Whether and how the genotypes identified here and by Xu et al. (38) segregate in these three clusters will need to be investigated.

The current study was initiated to determine whether some CEF3 allelic combinations were associated with invasive *C. albicans* strains. Genotype 135-135 was the only one that apparently met this expectation. However, four patients only had a genotype 135-135 candidemia, and more data will be necessary to determine the significance of this finding. None of the other genotypes, including the three overrepresented genotypes 126-135, 130-136, and 131-131, was present at an increased frequency in bloodstream isolates. Similarly, in a previous study based on isoenzyme typing and including 14 bloodstream isolates, no specific *C. albicans* population was associated with invasion (2). Other investigators reported that bloodstream isolates are often identical or highly similar to colonizing isolates obtained from the same patients (18, 20, 32). Interestingly, mucosal populations may be heterogeneous (16, 23, 26). Therefore, one cannot rule out the possibility that within a complex *C. albicans* colonizing population, only particular subpopulations are capable of bloodstream invasion. Cloning of colonizing strains and genotyping of the resulting clones in comparison with that of the corresponding bloodstream strains will be necessary to address this question, and the genotyping assay developed here will provide a useful tool for this purpose.

In a clonal model of development, different profiles of a unique polymorphic marker may be associated with variations in pathogenicity, whether or not this polymorphic marker is physically close to the genes involved in pathogenic mechanisms. Therefore, our approach was to use a well-characterized microsatellite marker of *C. albicans* and to compare its organization in bloodstream and nonbloodstream strains. Indeed, the CEF3 microsatellite locus analyzed here was shown to be stable upon repeated subculturing in vitro (3). Considering this stability, other features of this marker, such as its characterized discriminatory power, and the fact that the procedure is robust and accessible to a clinical mycology laboratory, the CEF3 marker provided a suitable means for identifying *C. albicans* isolates. Despite the size of the blood culture and control populations analyzed, no firm correlation could be established between bloodstream invasion and a CEF3 genotype. This result may reflect the fact that the pathogenicity of *C. albicans* is a complex and multiparametric process. The *C. albicans* genome may be subject to more recombination than previously assumed. Indeed, and despite the fact that *C. albicans* is essentially clonal, some recombination has been shown to occur in this organism (12, 30, 37). Lockhart et al. (14) also demonstrated that mucosal *C. albicans* may undergo a microevolution process. Finally, the pH of the infection site has been shown to regulate the gene expression and virulence of *C. albicans* (6). Therefore, regulation of the expression of genes encoding virulence factors upon exposure to different environmental signals could be a key mechanism of pathogenicity. If such is the case, profiles of gene expression and in vitro modulation of cytopathogenicity under different microenvironmental conditions should be investigated in parallel with genotypes in future studies aimed at understanding the pathobiology of *C. albicans*.

ACKNOWLEDGMENTS

We thank Françoise Dromer for providing control isolates, Fabienne Bon for sequencing the 137-bp allele, Mafoud Assem and David Masson for technical support in cloning the 137-bp PCR products, and Stéphane Bretagne for help in the development of the method.

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