JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1994, p. 2253–2259
0095-1137/94/$04.00+0

Vol. 32, No. 9

Strain Relatedness of *Candida albicans* Strains Isolated from Children with Leukemia and Their Bedside Parents

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Received 4 April 1994/Returned for modification 10 May 1994/Accepted 18 June 1994

***Candida* yeasts are occasionally recovered from patients with leukemia in spite of antifungal therapy used during chemotherapy. It is not yet known whether yeasts in these patients are of endogenous or exogenous origin. We examined the strain relatedness of *Candida albicans* isolated from three patients with leukemia (A, B, and C) and their bedside parents using pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphism (RFLP) by *Sma*I digestion, and the Southern hybridization patterns of the RFLPs by the *C. albicans*-specific probe RPS1. *Sma*I digestion and Southern hybridization by RPS1 showed identical or similar patterns among *Candida* isolates in patient A and his mother, although their karyotypes were different. Isolates from patient B and both parents showed identical electrophoretic karyotypes, *Sma*I digestion patterns, and hybridization patterns. Since electrophoretic karyotypes are more variable than RFLPs and their hybridization patterns, the identity of the last two suggests a close relatedness between strains. Our results also suggest that transmission of yeast strains may have occurred between patient A and his mother and between patient B and her parents. Isolates from patient C and her mother are thought to have originated from different strains, since different patterns were obtained in electrophoretic karyotypes, *Sma*I digestion patterns, and Southern hybridization patterns.**

The imperfect yeast *Candida albicans*, an opportunistic pathogen in immunocompromised hosts, may cause a life-threatening infection. To prevent fungal infection, the compromised patient with cancer is administered antifungal drugs at the start of chemotherapy. However, such therapeutic regimens do not usually prevent the occasional recovery of *Candida* yeasts from specimens collected for clinical examination. Since *Candida* yeasts are present in humans as commensal organisms in the gastrointestinal tract, it is difficult to ascertain whether the infecting strain is derived from endogenous or exogenous sources and/or whether an initially susceptible strain is replaced by a genotypically distinct strain that manifests novel characteristics. In this regard, several reports have assumed that candidiasis arises from autoinoculation followed by proliferation in the gastrointestinal tract (5, 22). Others have suggested that an exogenous source, such as a nurse, could spread the organism, resulting in the outbreak of an infection (6, 17). However, these assumptions have not been fully examined because of a lack of discriminating and reproducible typing systems.

To define the infecting strain, effective typing systems capable of discriminating strains within the species are required. Recently, genetic typing systems such as electrophoretic karyotype by pulsed-field gel electrophoresis (PFGE) (1, 8, 18), restriction fragment length polymorphism (RFLP) of total DNA (17, 18, 24), and Southern hybridization pattern with the use of a DNA probe have been used to distinguish *C. albicans* strains (16, 18, 22, 27, 30). It has been suggested that the use of a combination of different systems may help distinguish certain isolates from each other (16). However, the relatedness between delineated strains should be elucidated first for epidemiological studies.

It has been established that isolates from the same individual probably originate from a single strain when their karyotypes are the same or very similar in each individual (1, 8, 19, 20). On the other hand, several other studies have demonstrated that clones of a single strain can exhibit significant changes in the size of certain chromosomes during short periods (15, 23, 31). Using PFGE to study *Candida* infection in pediatric wards, we found a diversity of karyotypes within isolates from the same individual, while we frequently detected identical karyotypes in isolates from different individuals. However, as sufficient evaluation of differences between clinically isolated strains cannot be accomplished by one method alone (16), we further analyzed our results, using RFLP combined with Southern hybridization by means of a DNA probe to study the relatedness between different strains.

In the present study, we obtained *Candida* yeasts repeatedly isolated from inpatients admitted to pediatric wards with leukemia who were treated with amphotericin B as well as anticancer chemotherapy. We examined the strain relatedness between those isolates by comparing karyotypes from PFGE, RFLPs from *Sma*I digestion, and hybridization patterns with a *C. albicans*-specific repetitive sequence RPS1 (13). At the same time, we also isolated *Candida* yeasts from bedside parents to examine strain relatedness and compared them with those of their children. The possibility of transmission between parents and their children is also discussed.

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MATERIALS AND METHODS

Reference strain. FC18 was used as a reference strain of *C. albicans*.

Isolation of yeasts. The experimental protocol was approved by the Ethics Committee in our institution. Yeasts were collected from five children with leukemia (A to E, aged 4, 3, 7, 10, and 4 years, respectively). Patients (see Table 1) were hospitalized in the pediatric ward of Nagoya University Hospital. All patients were administered an antifungal agent (amphotericin B, 30 to 50 mg/kg of body weight per day orally and 10 mg/day by inhalation) at the onset of chemotherapy (12) as a prevention against opportunistic fungal infection. Samples of various specimens (saliva, stool, urine, and blood) were collected twice a month for 6 months during the period from February 1990 to May 1992. After centrifugation of 1.0 ml of saliva or 5.0 ml of urine at 2,800 rpm, the pellet was quantitatively streaked on a YPD plate (1% yeast extract, 1% polypeptone, 2% glucose, 1.5% agar, all wt/vol) containing 0.1 mg of chloramphenicol per ml. One-milliliter samples of blood were cultured in 70-ml BC bottles (Roche Co., Basel, Switzerland) and observed for 2 weeks. Anal swabs were streaked onto YPD plates containing 0.1 mg of chloramphenicol per ml. Saliva samples were also taken from bedside parents during examinations of their children. With a pinhole air sampler (Sanki Kagaku Co., Tokyo, Japan), a volume of 54 liters of air, collected from the patient's room, was applied on a YPD plate and cultured to examine airborne yeasts. The number of recovered colonies was determined for each sample, and five of the colonies or all of them (if the total number was smaller than five) were subjected to further examination. Isolates from inpatients of other wards in the same hospital used in a previous study (8, 9) were also used as controls in this study.

Identification of yeast isolates. The morphology of yeast isolates was initially confirmed by microscopy. Each isolate was identified by serological tests with the Candida Check test (Iatron Laboratories, Tokyo, Japan) (28) and by hyphal and chlamydospore formation. Yeast species were determined from electrophoretic karyotypes analyzed by PFGE with reference to the patterns of standard strains (7, 14). When isolates could not be identified by the above methods, they were tested for assimilation patterns with API 20C aux (API Systems; BioMerieux SA, Marcy-l'Etoile, France) (3).

Preparation of yeast chromosomal DNA for PFGE. The sample plug containing yeast chromosome DNA for PFGE was prepared according to the method described by Iwaguchi et al. (14).

PFGE. PFGE was performed according to the contour-clamped homogeneous electric field method (4) with a Pulsaphor system with a hexagonal electrode array (Pharmacia-LKB, Uppsala, Sweden), as described previously (14). Yeast chromosomal DNAs were separated in 0.8% agarose gels under two different conditions (7). The first condition, resolving the entire ensemble of chromosomal DNAs of *C. albicans*, included a 300-s switch time at 140 V for 24 h followed by a period of 1,000 s at 90 V for 48 h. The second method, separating DNAs smaller than 1.4 Mb with a higher resolution, included a 100-s switch time at 180 V for 15 h followed by a period of 300 s at 140 V for 20 h. Yeast chromosomal DNAs digested by a restriction enzyme including *Sma*I, *Sfi*I, *Cla*I, or *Eco*RI were separated in 0.85% agarose gels with a ramp of 20 to 100 s at 180 V for 15 h. Gels were stained with 0.5 μg of ethidium bromide per ml for 1 h, destained with distilled water, and observed under UV light (302 nm). Chromosomes of *Saccharomyces cerevisiae* x2180-1A (21), *Schizosaccharomyces pombe* HM422-h$^-$ (10), and lambda DNA digested by *Eco*T14I (Takara Shuzo Co., Kyoto, Japan) were subjected to the same fractionation and served as reference markers for the molecular size of DNA bands in PFGE.

Southern hybridization. Southern hybridization was performed with genes coding for rRNA (rDNA) (14) and RPS1 probes (15) as described by Iwaguchi et al. (14).

RESULTS

Isolation and identification of *Candida* yeasts. The presence of yeast-like fungi was examined in various specimens of five leukemia patients and in saliva of their bedside parents. Yeast-like colonies were isolated from saliva of all patients except D and from stool samples from three patients (A, B, and D) only (Table 1). No yeast-like fungi were identified in urine and blood samples or in samples of air from patients' rooms. The number of colonies recovered from saliva varied widely among subjects, with more than 100 colonies recovered repeatedly in patient A. Patients B and E had a few colonies identified only once during the period of examination, while a moderate number of colonies were identified from patient C. Various numbers of yeast colonies were observed in samples of saliva from bedside parents ranging from a few to hundreds, with the highest number found in the mother of patient A. In contrast, relatively few colonies were recovered from stool samples except in one patient (patient B). One to five colonies in each sample (numbers in parentheses in Table 1) were selected for further analysis. Isolation and identification of 239 colonies from a total of 4,416 were performed. This analysis yielded a distribution of 229 colonies of *C. albicans*, 5 colonies of *Candida glabrata*, 4 colonies of *Candida guilliermondii*, and only 1 colony of *Candida parapsilosis* (1). *C. glabrata* and *C. parapsilosis* were recovered only from patients D and B, respectively, while *C. guilliermondii* was recovered from patients B and E. Isolated species from patients D and E were found only once and were different from those from their mothers, in whom *C. albicans* colonies were isolated. Thus, the probability of a transmission of yeasts from the mother to her child was excluded in these cases. *C. albicans* strains isolated from patients A, B, and C and their parents were the subject of this study from an epidemiological point of view. More than 100 colonies were recurrently recovered from saliva from patient A irrespective of the presence or absence of symptoms of infection such as fever or elevated C-reactive protein. In contrast, colonies were recovered in patients B and C only when they had high fevers and elevated levels of C-reactive protein. *C. albicans* colonies were always recovered from their bedside parents.

Karyotypes of *C. albicans*. Chromosomes of 229 isolates of *C. albicans* from patients and their parents were analyzed by PFGE. Separation of entire chromosome of *C. albicans* showed 7 to 11 bands ranging in size from 0.82 to 3.80 Mb (Fig. 1a). Chromosome bands of sizes greater than 2.2 Mb varied from one clone to another even in a single strain. The variability among chromosomes seemed to be derived from chromosome 2, containing genes coding for rRNA. The change in size in this chromosome may explain the variability in chromosome size (1, 15).

On the basis of findings of previous studies that karyotypes of isolates could be more effectively distinguished from each other when chromosomes smaller than 2.2 Mb are separated (1, 9), karyotypes were analyzed under the recommended conditions as demonstrated in Fig. 1b. Of two to five colonies from any sample at a given time, all showed the same or a few different karyotypes depending on the sample tested. Multiple isolates taken at different times from some individuals (B, B's

TABLE 1. Numbers of yeast colonies recovered from patients and members of their families[a]

Patient and sample no.	Date	No. of colonies recovered from: St	Sa	Sa (patient's mother)
A				
1	05/29/90	0	120 (5)	ND
2	06/06/90	2	58 (5)	102 (5)
3	06/20/90	0	62 (5)	121 (5)
4	07/04/90	0	68 (5)	72 (5)
5	07/18/90	0	5 (5)	152 (5)
6	08/01/90	0	15 (5)	ND
7	08/22/90	0	185 (5)	153 (5)
8	09/19/90	0	121 (5)	164 (5)
9	10/03/90	0	243 (5)	151 (5)
10	10/17/90	0	103 (5)	88 (5)
11	10/30/90	0	253 (5)	472 (5)
12	12/13/90	0	261 (5)	116 (5)
13	11/27/91	0	87 (5)	2 (2)
Totals		2	1,581 (65)	1,593 (52)
B				
1	10/23/90	0	0	103 (5)
2	10/30/90	0	0	175 (5)
3	11/14/90	4 (4)	1 (1)[b]	106 (5)
4	11/29/90	250 (5)	0	78 (5)
5	12/14/90	0	0	121 (5)
6	01/25/91	0	0	58 (5)[c]
7	02/27/91	0	0	ND
8	03/20/91	0	0	0
9	11/28/91	2 (2)[d]	0	18 (5)
Totals		256 (11)	1 (1)	659 (35)
C				
1	02/06/90	ND	ND	ND
2	02/21/90	ND	1 (1)	ND
3	03/22/90	0	1 (1)	2 (2)
4	04/06/90	0	0	5 (5)
5	04/18/90	0	1 (1)	8 (5)
6	04/28/90	0	32 (5)	ND
7	05/09/90	0	11 (5)	ND
8	05/23/90	0	61 (5)	ND
Totals		0	107 (18)	15 (12)
D				
1	09/28/90	29 (5)[e]	0	60 (5)
2	10/17/90	0	0	5 (5)
3	10/31/90	0	0	54 (5)
4	11/29/90	0	0	6 (5)
5	12/13/90	0	0	21
6	01/25/91	0	0	6 (5)
7	02/27/91	0	0	3
8	11/28/91	0	0	3 (3)
Totals		29 (5)	0	158 (28)
E				
1	05/30/90	0	0	0
2	05/09/90	0	0	2 (2)
3	06/06/90	0	0	0
4	07/18/90	0	0	0
5	08/22/90	0	0	8 (5)
6	09/19/90	0	0	0
7	10/17/90	0	0	0
8	11/21/91	0	2 (2)[d]	3 (3)
Totals		0	2 (2)	13 (10)

[a] Colonies are *C. albicans* unless otherwise noted. The numbers in parentheses are the numbers of isolates used in the present study. Dates are given as month/day/year. St, stool; Sa, saliva; ND, not determined.
[b] *C. parapsilopsis.*
[c] Isolates from B's father.
[d] *C. guilliermondii* (total, four isolates).
[e] *C. glabrata.*

mother, and B's father) demonstrated very similar, though not identical, karyotypes in the range smaller than 1.4 Mb. But isolates from other individuals (A, A's mother, D's mother, and E's mother) showed different karyotypes. Four different electrophoretic karyotypes in those areas were distinguished in 65 isolates from patient A and his mother (52 isolates). This method resolved three to six chromosomal bands smaller than 1.4 Mb (Fig. 1b, lane 2, and Fig. 2a). The frequencies of differentnumbers of chromosomal bands in electrophoretic karyotypes are summarized in Table 2. All four types were identified in patient A and his mother. A major type (Fig. 2a, lanes 1, 4, and 5) was detected in 46 isolates from patient A, while no dominant type was found in isolates from his mother. All isolates from patient B and her parents showed very similar karyotypes (Fig. 2b), while those of isolates from patient C were similar to each other but different from those of her mother, which were in turn very similar to each other (Fig. 2c).

Restriction enzyme patterns of *C. albicans* chromosomes. It has been previously demonstrated that isolates collected repeatedly from the same patient usually have an identical

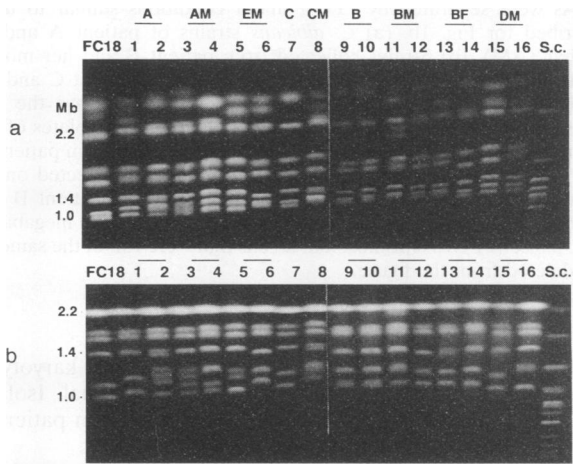

FIG. 1. Examples of chromosome band patterns in *C. albicans* isolates from leukemia patients and their parents. Chromosomal DNAs were separated by PFGE under the following conditions: (a) 300 s for 24 h at 140 V, then 1,000 s for 48 h at 90 V; (b) 100 s for 15 h at 180 V, then 300 s at 140 V for 20 h. The origins of the strains are noted above the lane numbers: A to E, patient designations; M, mother; F, father (e.g., AM, mother of A). The leftmost and rightmost lanes are *C. albicans* FC18 and *S. cerevisiae* x2180-1A, respectively. Numbers on the left margin indicate previously determined sizes (in megabases) of FC18 chromosomal DNAs (7).

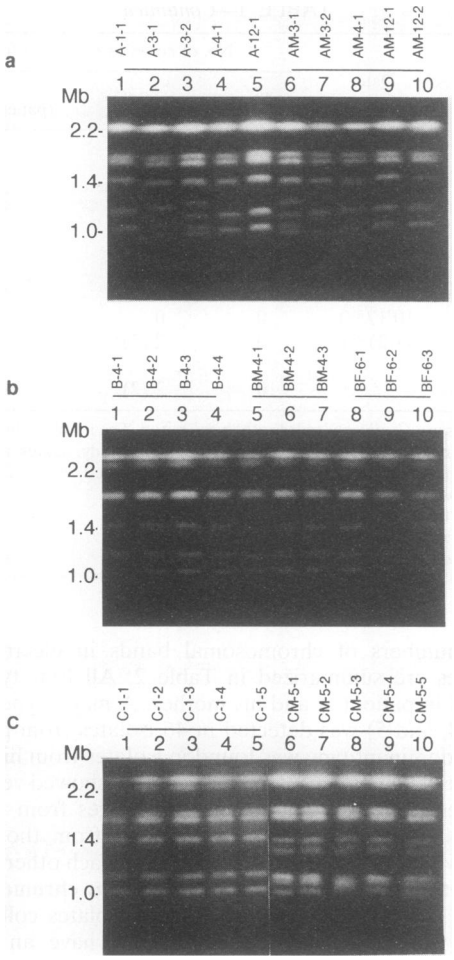

FIG. 2. Electrophoretic karyotype profiles of *C. albicans* strains recurrently isolated from patients and their parents. Chromosomal DNAs were separated by PFGE under conditions similar to those described for Fig. 1b. (a) *C. albicans* strains of patient A and his mother (AM). (b) Strains collected from patient B and her mother (BM) and father (BF). (c) Strains collected from patient C and her mother (CM). The origins of the strains are noted above the lane numbers as in these examples: A-1-1 denotes the first isolates of five colonies collected on the first sampling (29 May 1990) from patient A; BF-6-2 denotes the second isolates of five colonies collected on the sixth sampling (25 January 1991) from the father of patient B (see Table 1). Numbers in the left margins indicate the sizes (in megabases) of *C. albicans* FC18 chromosomal DNAs that were run in the same gel but deleted from the figure.

karyotype (1, 8). In the present study, different karyotypes appeared among isolates from the same individual. Isolates with identical karyotypes were also recovered from patient A and his mother.

To examine the relationship between isolates, we analyzed our samples by RFLP. Digestion of genomic DNA was performed with several types of restriction enzymes, including *Eco*RI, *Sma*I, *Cla*I, and *Sfi*I. Of these, *Sma*I digestion seemed to generate the most distinguishable pattern and was, therefore, selected for further analysis. *Sma*I digestion of chromosomal DNAs of PFGE sample plugs yielded six or seven intense bands between 460 and 245 kb (range A), intensely smeared bands between 245 and 19 kb (range B), and one or two intense bands between 19 and 3.5 kb (range C) (Fig. 3). Very intense bands close to 15 kb were hybridized by an rDNA probe (data not shown). The numbers and sizes of rDNA bands were identical among isolates from the same individual but different from those from other individuals (Fig. 3 and 4). *Sma*I band patterns were more distinctive in the same individual than were the results of karyotype analysis. Isolates from the same individual showed similar band patterns (Fig. 3 and 4), while NUM strains of different individuals in other wards in the same hospital showed different patterns in bands between 460 and 245 kb (range A) and rDNA bands (Fig. 4, lanes 13 to 23), although their electrophoretic karyotypes were similar to each other (9). This was mostly noticeable when bands in range A were compared. Although karyotypes of patient A and his mother were different, *Sma*I band patterns were almost identical to each other (Fig. 2 and 4) except for two isolates having a 400-kb band rather than the 370-kb band found in other isolates (Fig. 4, lanes 7 and 8).

*Sma*I band patterns of patient B and her parents were identical (Fig. 3, lanes 1 to 8), as revealed also by electrophoretic karyotyping (Fig. 2b). On the other hand, *Sma*I band patterns from patient C and her mother were different from each other (Fig. 4, lanes 9 to 16), as revealed also in karyotypes (Fig. 2c). Similar RFLP results were observed with digestion with *Cla*I, *Eco*RI, or *Sfi*I, though their band patterns were less distinctive than *Sma*I patterns (data not shown).

Hybridization of *C. albicans* repetitive element RPS1 to genomic DNA. A species-specific repetitive sequence is a useful probe to examine strain relatedness by hybridization patterns of total *C. albicans* DNA digested by a restriction enzyme. Ca3 and 27A have been used in previous studies as probes for strain delineation (11, 27). Recently, Iwaguchi et al. (13) described a *C. albicans*-specific repetitive sequence, RPS1, with a unit size of approximately 2.1 kb that was partly homologous with Ca3 and 27A. Our preliminary experiments demonstrated that Southern hybridization of RFLP with a probe of RPS1 gave a distinctive band pattern in different strains that were the same among isolates taken repeatedly for 6 months from the same individual (data not shown). Thus, the hybridization pattern with RPS sequencing is a stable, reproducible, and highly sensitive method for strain discrimination.

Gels of *Sma*I digests of various isolates from patients and their mothers and those of patients in other pediatric wards (Fig. 4) were probed with RPS1. RPS1 was hybridized into 8 to 15 bands ranging from 1.9 to 245 kb (Fig. 5). Although RPS1 profiles of isolates from different individuals were different from each other, isolates from the same individual had similar profiles (Fig. 5). The differences in bands of less than 3.5 kb were small among isolates from the same individual (range D in Fig. 5, lanes 1 to 12), but large between different individuals (Fig. 5, lanes 13 to 23). Eleven bands were common to isolates from patient A and his mother (Fig. 5, lanes 1 to 12, marked by arrowheads). A few extra bands also appeared in some isolates (Fig. 5, lanes 4 to 6 and 9). Seven common bands were found in isolates from different individuals (Fig. 5, lanes 13 to 23, marked by arrowheads), and a few extra or deleted bands were found in range C (3.5 to 19.0 kb), depending on isolates. No signal appeared in range A (above 245 kb; data not shown).

Patterns identified by RPS1 hybridization were similar in isolates from patient B and her parents but were different between isolates from patient C and those from her mother (data not shown).

DISCUSSION

Genotyping systems have been recently applied to delineate strains of *Candida* yeasts and examine the relatedness between

TABLE 2. Frequency of chromosomal bands smaller than 1.4 Mb in electrophoretic karyotypes of isolates of *C. albicans* from patient A and his mother

Sample no.	Date[a]	No. of isolates from the indicated source with the indicated no. of bands							
		A				A's mother			
		3	4	5	6	3	4	5	6
1	05/29/90	5	0	0	0	—[b]	—	—	—
2	06/06/90	3	2	0	0	2	2	1	0
3	06/20/90	1	1	0	3	2	1	2	0
4	07/04/90	4	1	0	0	0	0	4	1
5	07/18/90	2	1	1	1	0	1	2	2
6	08/01/90	5	0	0	0	—	—	—	—
7	08/22/90	1	4	0	0	0	2	1	2
8	09/19/90	5	0	0	0	0	2	3	0
9	10/03/90	3	2	0	0	0	3	1	1
10	10/17/90	5	0	0	0	0	2	3	0
11	10/30/90	5	0	0	0	4	0	1	0
12	12/13/90	2	2	1	0	3	2	0	0
13	11/27/91	5	0	0	0	2	0	0	0
Totals		46	13	2	4	13	15	18	6

[a] Dates are given as month/day/year.
[b] —, not determined.

delineated isolates in epidemiological studies of candidiasis. Soll and colleagues (26, 27, 29) assessed genetic similarities between *C. albicans* isolates using DNA fingerprinting techniques and Southern blot hybridization with Ca3, a DNA probe containing moderately repetitive sequences. Magee et al. (16) suggested that a combination of different molecular genotyping methods could eventually differentiate between isolates. These investigators also suggested that the relatedness between different strains depends on the rate at which certain parameters, such as restriction sites, chromosome length, and chromosomal configuration of repeat sequences, change as a function of cell growth.

Recently, the stability of DNA electrophoretic patterns of *C. albicans* strains was tested by Bart-Delabesse et al. (2) in patients with AIDS who had developed resistance to fluconazole. They reported that after approximately 500 generations, progeny karyotypes were not always identical to the parental type, while *Eco*RI and *Hin*fI patterns were identical. Karyotype polymorphism could occur in clonal progenies, not only with a high frequency in rDNA chromosomes (14) but also with a frequency higher than that of RFLP in other chromosomes. These observations suggest that karyotype variation occurring through chromosomal rearrangement may not directly influence RFLP as well as its Southern hybridization pattern. Alter-

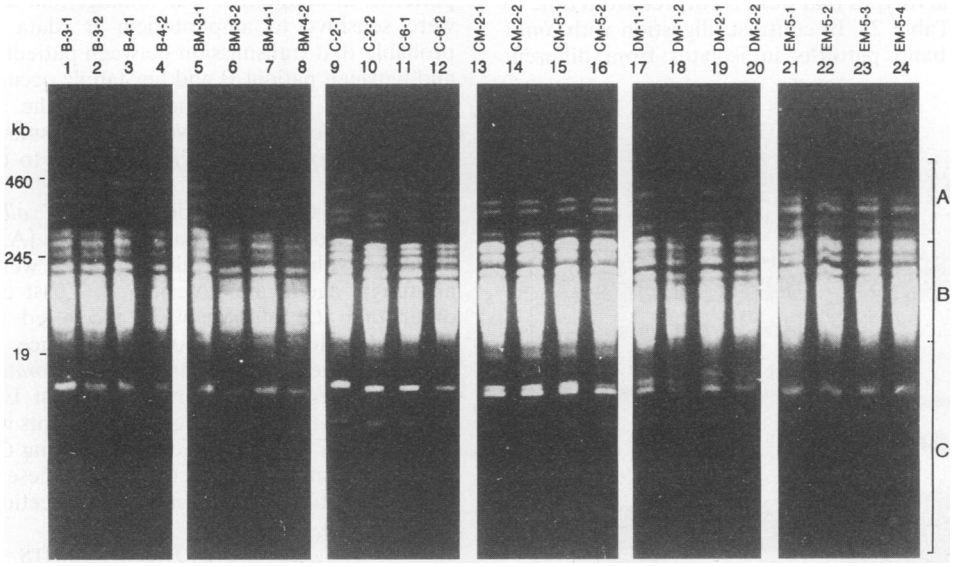

FIG. 3. *Sma*I digestion profiles of *C. albicans* isolates recovered repeatedly from six individuals (B, BM, C, CM, DM, and EM; see the Fig. 1 legend) as marked above the lane numbers. Chromosomal DNAs were digested with *Sma*I and separated by PFGE under conditions of 20 to 100 s (ramping) at 180 V for 15 h. Numbers in the left margin indicate size markers obtained from *S. cerevisiae* chromosomal DNAs and DNA digested by *Eco*T14I (Takara Shuzo Co.) which were run in the same gel but deleted from the figure. The ranges for regions A, B, and C (right margin) are defined in the text as 245 to 460 kb, 19 to 245 kb, and 3.5 to 19 kb, respectively.

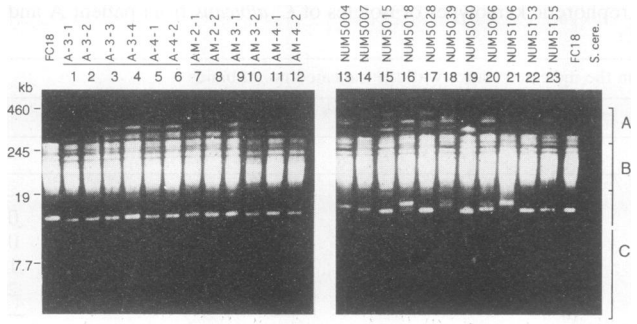

FIG. 4. Comparison of *Sma*I digestion patterns between isolates of *C. albicans*. Chromosomal DNAs from isolates from patient A and his mother (AM) (lanes 1 to 12), strains isolated from individual patients (lanes 13 to 23), and FC18 acting as a reference, were digested with *Sma*I and separated by PFGE under conditions described in the Fig. 3 legend. The origins of different strains are indicated (see the Fig. 1 and 2 legends) above the lane numbers. The strains from individual patients were isolated from throat swabs (lanes 13, 17, and 19), sputum (lanes 14, 18, 21, 22, and 23), and urine (lanes 15, 16, and 20). Numbers on the left margin indicate the sizes (in kilobases) of *S. cerevisiae* chromosomal DNAs and DNA digested by *Eco*T14I (Takara Shuzo Co.). See the Fig. 3 legend for the definitions of regions A, B, and C.

natively, chromosome rearrangement may occur more frequently than changes altering RFLPs. These considerations warrant the following discussion.

In this study we restricted our analysis of strain relatedness to *C. albicans* isolates obtained from three families, those of patients A, B, and C and their parents, using the contour-clamped homogeneous electric field method for electrophoretic karyotyping and RFLP in combination with Southern hybridization and the repetitive sequence RPS1. Electrophoretic karyotypes of strains isolated repeatedly from a single individual reported hitherto generally showed karyotypes that were identical or very similar to each other (1, 8, 19). Our results demonstrated that the same individual always carried yeasts of different karyotypes that were recovered from time to time (Fig. 2a and Table 2). In contrast, digestion with *Sma*I produced distinct band patterns in isolates from different

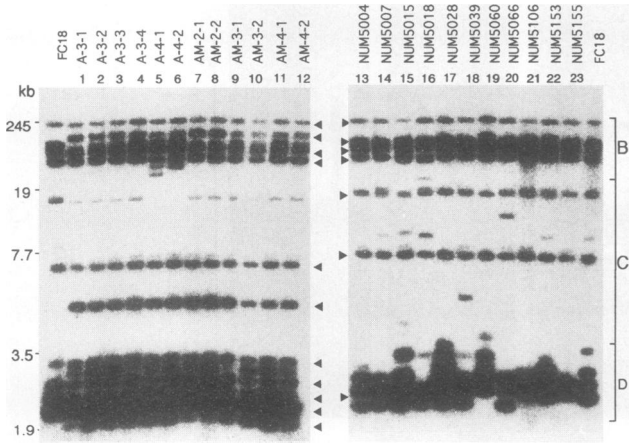

FIG. 5. Southern hybridization profiles of the gel shown in Fig. 4 probed with RPS1. Numbers on the left indicate DNA size markers (in kilobases). The data range is defined on the right as in Fig. 3 for regions B and C, and the range for region D is 1.9 to 3.5 kb.

individuals but identical or very similar patterns in the same individual (Fig. 3). In addition, the pattern of *Sma*I of an individual did not change in isolates taken repeatedly during the period of the study. Thus, the *Sma*I pattern is capable of discriminating between isolates from one individual and those from others.

Isolates from patient C and her mother demonstrated that DNA band patterns of electrophoretic karyotype, *Sma*I digestion, and RPS1 hybridization were different between individuals, indicating that the two different strains in each individual were unrelated to each other or were of different origins. Isolates from patient B and her mother, as demonstrated by three types of genotyping analyses, were identical, being closely related to each other. It is noteworthy that patient B received treatment with an antifungal agent during chemotherapy throughout the course of the study. Yeast cells in this patient were isolated only when she developed signs of infection, such as high fever and an elevated C-reactive protein level. These results suggested the possibility that yeast cells in this patient were colonized through transmission from the mother. The case of patient A was rather intriguing. Recurrent isolates from this patient and his mother showed four patterns in karyotype analysis that were common in both individuals, but only a single pattern in RFLP by *Sma*I digestion and Southern hybridization with RPS1. This finding indicates that the yeast cells are closely related or had a common origin. The appearance of different karyotypes in the same individual was assumed to be due to a minor chromosomal rearrangement that did not influence the *Sma*I digestion pattern. This rearrangement may have occurred by the action of certain agents such as anticancer drugs or may have developed spontaneously in cells colonized in the patient and/or in his mother. Patient A was also under treatment with amphotericin B and always carried *C. albicans* organisms in the oral cavity without any associated generalized symptoms such as high fever or an elevated level of C-reactive protein. It is also noteworthy that his mother also constantly carried a high number of yeast organisms compared with the number carried by other parents (Table 1). On the basis of *Sma*I digestion and hybridization patterns, in addition to our findings that all *Candida* isolates were sensitive to amphotericin B (data not shown), it is probable that transmission between patient A and his mother and between patient B and her family occurred. This is highly conceivable, since the mother was the bedside attending person for her child. However, our results do not exclude a possible endogenous proliferation due to changes in genetic constitution.

Results of this study indicated that *C. albicans* strains were recovered from 60% of patients (three [A, B, and C] of five patients) with leukemia although they were treated with an antifungal agent, amphotericin B. Yeast colonies of species other than *C. albicans* were recovered from patients (as opposed to their parents) only twice: once, at the onset of the study, in patient D, infected with *C. glabrata*, and once, at the end of a therapeutic course, in patient E, infected with *C. guilliermondii*. Yeasts in these two patients were different from those in their mothers, who were carrying *C. albicans*, and we think that failure to detect yeasts in these patients indicates that the antifungal agent prevented infection.

ACKNOWLEDGMENTS

We thank T. Suzuki, Nara Women's University, for reading and critically evaluating the manuscript.

This work was supported by a grant-in-aid to M.D. for scientific research from the Ministry of Education, Science, and Culture in Japan.

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