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# Colonizing Populations of *Candida albicans* Are Clonal in Origin but Undergo Microevolution through C1 Fragment Reorganization as Demonstrated by DNA Fingerprinting and C1 Sequencing

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**The genetic homogeneity of nine commensal and infecting populations of *Candida albicans* has been assessed by fingerprinting multiple isolates from each population by Southern blot hybridization first with the Ca3 probe and then with the 0.98-kb C1 fragment of the Ca3 probe. The isolates from each population were highly related, demonstrating the clonal origin of each population, but each population contained minor variants, demonstrating microevolution. Variation in each case was limited to bands of the Ca3 fingerprint pattern which hybridized with the 0.98-kb C1 fragment. The C1 fragment was therefore sequenced and demonstrated to contain an RPS repetitive element. The C1 fragment also contained part or all of a true end of the RPS element. These results, therefore, demonstrate that most colonizing *C. albicans* populations in nonimmunosuppressed patients are clonal, that microevolution can be detected in every colonizing population by C1 hybridization, and that C1 contains the repeat RPS element.**

*Candida albicans* lives as a commensal in the majority of healthy individuals (13, 21), but it is capable of causing superficial and systemic infections (3, 13). In spite of its pervasiveness as a commensal and prominence as a major fungal pathogen, very little is known about the genetic homogeneity, evolution, and maintenance or replacement of strains during commensalism and infections. With the recent development of DNA fingerprinting techniques which allow assessment of strain relatedness at the genetic level, these epidemiological questions can now be examined. By using fingerprinting techniques, there have been a number of studies which have monitored the genotype of *C. albicans* through recurrent infections (5, 6, 11, 12, 20, 22, 25). The following three scenarios have emerged: (i) maintenance of the same strain with no genetic change by the criteria used in the study; (ii) maintenance of the same strain, but with minor genetic changes; and (iii) replacement of strains. The first two scenarios appear to be the most prevalent in recurrent vaginal infections (10a), but the genetic homogeneity of commensal and infecting populations has not been adequately explored to make definitive conclusions about how a clonal population evolves or is replaced during continuous commensal carriage, in the transition from a commensal to pathogenic state or in recurrent infections.

We have, therefore, employed the complex fingerprinting probe Ca3 (15, 18, 24) and a 0.98-kb *Sac*I-*Eco*RI subfragment of the 2.6-kb *Eco*RI fragment of the Ca3 probe (1), referred to as the C1 fragment, to assess the genetic homogeneity of *C. albicans* populations colonizing the oral cavities and vaginal canals of healthy individuals and vaginitis patients. Isolates from the oral cavity, vulva, or vaginal canal were directly plated at low density on nutrient agar so that individual cells generated clonal colonies. Between 9 and 14 colonies from each isolate were then fingerprinted sequentially with the Ca3 probe and the C1 fragment, and the hybridization patterns were compared with a computer-assisted system for the level of genetic relatedness between clones. The results of this study demonstrate that all of the nine analyzed populations were clonal in origin, but in every case there was genetic diversity in the population resulting from the reorganization of sequences in the genome which were homologous to the C1 fragment. We, therefore, analyzed the DNA sequence of the C1 fragment. Nucleotide sequence analysis revealed strong homology between a major portion of the C1 fragment and the RPS family of repetitive elements of *C. albicans* (4, 8) and possibly identified an end of an RPS element. These results demonstrate that the majority of commensal and infecting populations are clonal in origin but undergo microevolution which can be discriminated through the frequent reorganization of regions of the genome homologous to the C1 fragment of the Ca3 probe, which results in minor genetic variants in every population.

## MATERIALS AND METHODS

**Sampling and stock maintenance.** Healthy patients were carefully examined and demonstrated to exhibit no signs of oral inflammation or vulvovaginitis. Vaginitis patients were carefully examined and demonstrated to exhibit symptoms of vulvovaginitis, including inflamed vulva and/or vaginal mucosa and a heavy discharge. Specimens were collected by gently swabbing the vulva, vaginal wall, or the back of the tongue with a sterile Culturette (Becton Dickinson, Cockeysville, Md.). The Culturette tip was inserted into 0.5 ml of sterile water in a sterile microcentrifuge tube and mixed vigorously with a vortexer. A 0.1-ml portion of the wash was plated on each of three agar plates containing Lee's medium (10) supplemented with 70 μg of arginine per ml and 0.1 μM $ZnSO_4$ (2). The plates were then incubated at 25°C for 7 days. Nine to 14 colonies from each isolate were then individually streaked onto an agar slant in a capped test tube and stored for subsequent use. Each colony was checked microscopically for the presence of budding yeast cells.

**Southern blot hybridization and DNA fingerprint analysis.** Cells from a single colony were inoculated into 2 ml of YPD medium (2% glucose, 2% Bacto Peptone, 1% yeast extract) in a sterile test tube and grown overnight at 30°C. DNA was prepared from each clone by the methods of Scherer and Stevens (17), digested with *Eco*RI, and electrophoresed in a 0.8% agarose gel overnight at 35 V. DNA was transferred to nitrocellulose membrane by capillary transfer (16). The Southern blot was then hybridized with radiolabeled Ca3 probe (15, 24) as

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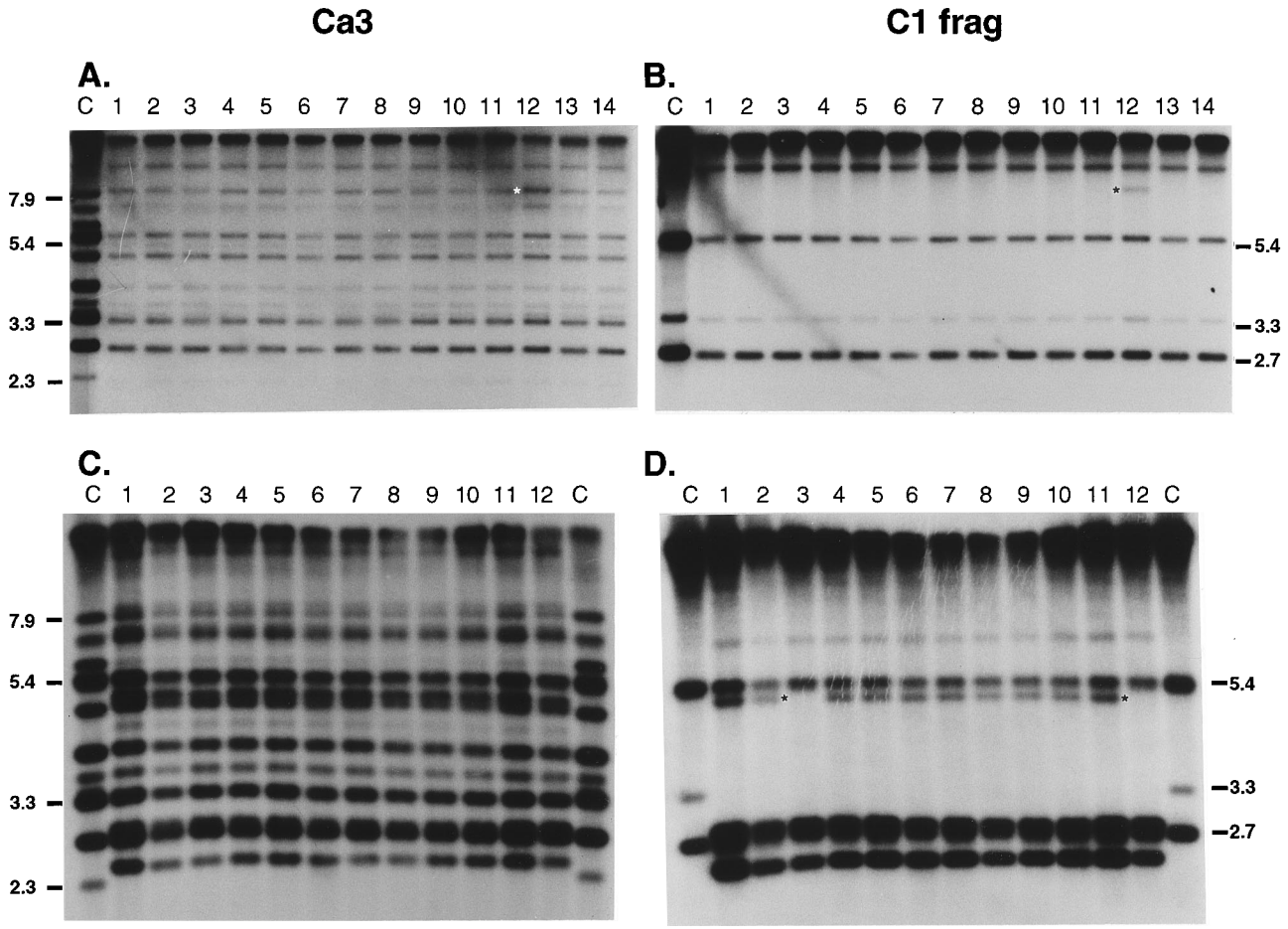


FIG. 1. DNA fingerprinting patterns of isolates from two colonizing populations which show no differences in band positions in the Ca3 patterns but clear differences in the C1 fragment patterns. (A and B) Ca3 and C1 fragment hybridization patterns of 14 isolates from the commensal population carried on the vulva of patient P2. (C and D) Ca3 and C1 fragment (C1 frag) hybridization patterns of 12 isolates from the infecting population colonizing the vaginal canal of patient P6. DNA from each isolate was digested with *Eco*RI, separated on agarose gels, hybridized with radioactive Ca3 on Southern blots, stripped, and rehybridized with the radioactive C1 fragment. Molecular sizes (in kilobases) of key bands are indicated to the left and right of the gels. Stars indicate the positions of added or deleted bands in the minor patterns. C lanes contain *Eco*RI-digested DNA of the laboratory strain 3153A used as a standard for fingerprinting (19).

previously described (19) and exposed to X-OMAT film (Eastman Kodak, Rochester, N.Y.). Blots were then stripped by shaking in water at 80°C for 15 min, checked for any residual signal, and then rehybridized with a radiolabeled subfragment of the Ca3 probe (1). Autoradiogram patterns were digitized into the Dendron program (7, 18–20, 23) with a Scanjet II cx scanner (Hewlett-Packard, Palo Alto, Calif.). Each lane was scanned, and bands were automatically detected and assigned intensity values from 0 for no band to 3 for the most intense band. Similarity coefficients ($S_{AB}$) were then computed for every pair of strains on the basis of band position and intensity by the following formula:

$$S_{AB} = \frac{\sum_{i=1}^{k}(a_i + b_i - |a_i - b_i|)}{\sum_{i=1}^{k}(a_i + b_i)}$$

where $a_i$ and $b_i$ are the intensities of band i in patterns A and B, respectively, and $k$ is the number of bands. Dendrograms were then generated on the basis of $S_{AB}$ values. Strains collected and analyzed in previous studies were recalled from an evolving database in the Dendron system at the University of Iowa.

**Sequencing of the C1 fragment of the Ca3 probe.** The Ca3 probe (15, 24) was cleaved with *Eco*RI, and the 2.6-kb C fragment (1) was gel purified by using a Spin-X column (Costar, Cambridge, Mass.). The C fragment was then subcloned into the *Eco*RI site of pGEM3Z (Promega, Madison, Wis.) to yield plasmid pC18. pC18 was cleaved with *Sac*I, releasing a 1.6-kb subfragment of the C fragment. The plasmid, still containing a 0.98-kb subfragment of the C fragment, was religated to yield plasmid pC1-5, and the insert was designated fragment C1. The 1.6-kb *Sac*I fragment was subcloned into pGEM3Z to yield plasmid pC2-1.6, and the insert was designated fragment C2. Subfragment C1 was sequenced with the Sequenase kit (Promega, Madison, Wis.) essentially as described by the manufacturer except that 1.2 U of Klenow polymerase were added to each reaction and the reactions were incubated for 30 min at 42°C to remove base stacking artifacts (14).

## RESULTS

**Commensal and infecting populations are clonal on the basis of Ca3 hybridization patterns.** To assess the genetic relatedness of the clonal isolates from each of the nine colonizing populations of *C. albicans*, every isolate was fingerprinted by Southern blot hybridization with the complex probe Ca3. For each tested population, the Ca3 hybridization patterns of the individual isolates were highly similar. Figure 1A and C shows two examples of Ca3 hybridization patterns of isolates which are apparently identical. In Fig. 1A, patterns are shown for 14 isolates from the commensal population of healthy individual P2. Only one difference was suggested in isolate 12 by an increase in the intensity of the 7.9-kb band (indicated by a white star). In Fig. 1C, patterns are shown for 12 isolates from the infecting population of vaginitis patient P6. In this set of patterns, there were no obvious differences.

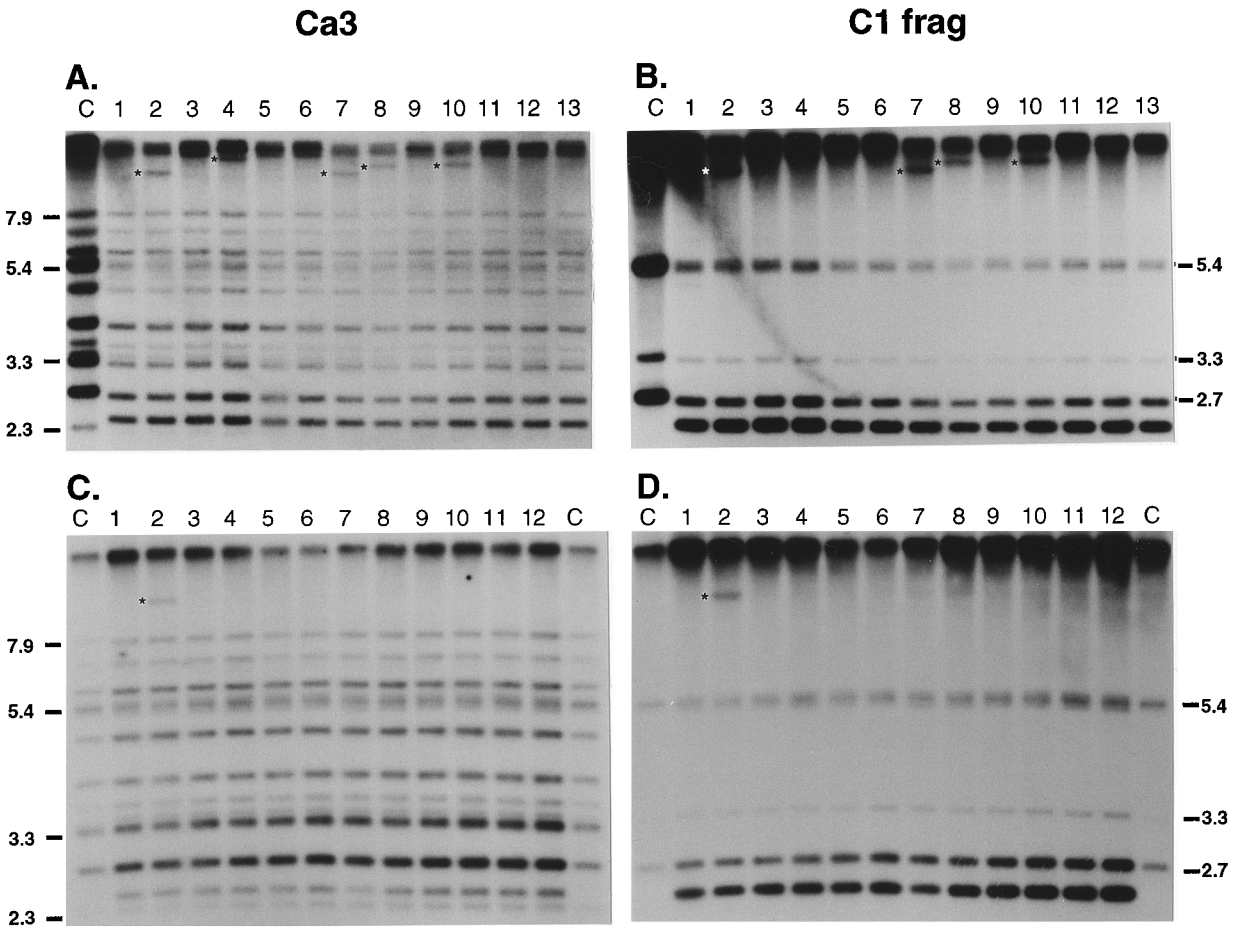


FIG. 2. DNA fingerprinting patterns of isolates from colonizing populations which show differences in band positions in both the Ca3 and C1 fragment (C1 frag) patterns. (A and B) Ca3 and C1 fragment hybridization patterns of 13 clonal isolates from the commensal population carried on the vulva of patient P3. (C and D) Ca3 and the C1 fragment hybridization patterns of 12 clonal isolates from the infecting population colonizing the vaginal wall of patient P9. The details are the same as those outlined in the legend to Fig. 1.

Of the nine sets of isolates analyzed, three (P4, P6, and P7) had no detectable differences and one (P2) had one potential difference in the intensity of a single band of a single isolate (Table 1).

The remaining groups of isolates (P1, P3, P5, P8, and P9) each contained one or more isolates with one or, at most, three high-molecular-weight band variations. In Fig. 2A, the Ca3 hybridization patterns are shown for 13 isolates from the commensal population of healthy individual P3. Isolates 2, 4, 7, 8, and 10 contained one high-molecular-weight band (noted by small black stars) not present in the major Ca3 pattern but contained no other pattern variations. In Fig. 2C, the Ca3 hybridization patterns for 12 isolates from the infecting population of vaginitis patient P9 are shown. In this set, only isolate 2 varied from the major pattern by one high-molecular-weight band (indicated by a black star).

The high level of similarity of the Ca3 patterns suggests that the isolates of each population originated from a single progenitor. This hypothesis is supported by the degree of clustering of each group of isolates in a dendrogram constructed from the $S_{AB}$s computed between all pairs of the combined 106 isolates in the nine populations (Fig. 3). For three of these clusters (P4, P6, and P7), the mean $S_{AB}$ of isolates in the cluster was 1.0. The lowest mean $S_{AB}$ between isolates of a population was 0.97 for P8. Even in this case, no isolate from any other population mixed with isolates from this population. The average $S_{AB}$ between the nine clusters, treated as individual groups, was $0.76 \pm 0.06$ ($n = 36$). This value is close to the average $S_{AB}$ of $0.69 \pm 0.11$ computed for 46 unrelated test strains originally fingerprinted with the Ca3 probe (19). These results demonstrate the high level of genetic relatedness of isolates within a single colonizing population and the low level of relatedness between the nine analyzed populations.

The clonal origin of the individuals within each of the nine commensal and infecting populations was most strikingly demonstrated by generating mixed dendrograms based upon $S_{AB}$s computed between the isolates of a population and unrelated strains retrieved from the data bank of the Dendron system at the University of Iowa. Figure 4 shows a dendrogram constructed from the $S_{AB}$s computed for 60 unrelated strains from the data bank, the 14 isolates of P2, and the 13 isolates of P3. In this dendrogram, the isolates of the P2 population and those of the P3 population remain intact in clusters (Fig. 4). There is, in fact, no penetration of a random strain into either of the two clusters (Fig. 4). The average $S_{AB}$ for the P2 cluster was $0.997 \pm 0.008$, and the average $S_{AB}$ for the P3 cluster was $0.986 \pm 0.013$, while the average $S_{AB}$ for the entire dendrogram (excluding duplications such as the ones in the P2 and P3 clusters) was $0.679 \pm 0.114$.

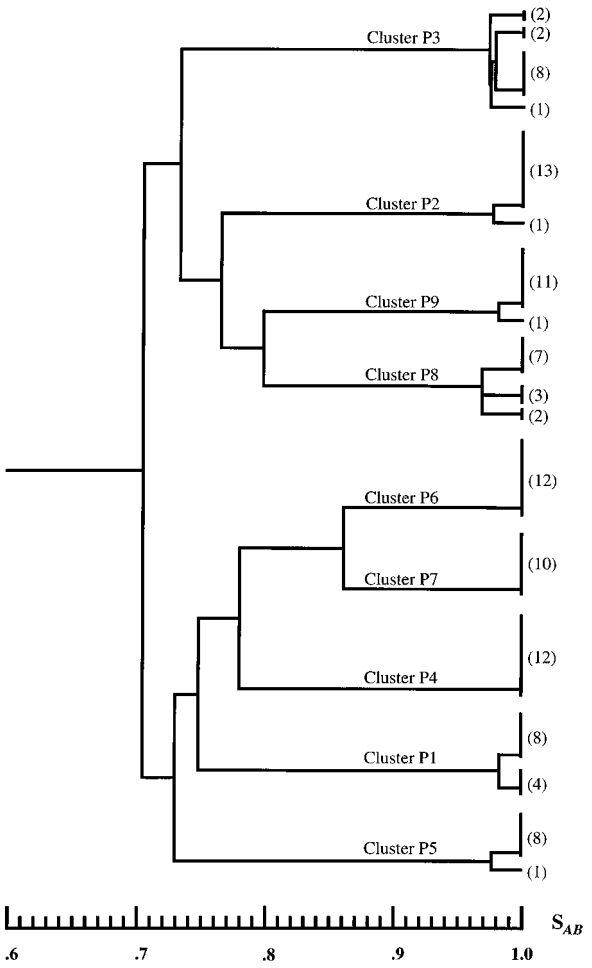


FIG. 3. Dendrogram generated from the $S_{AB}$ values computed from the Ca3 patterns for all clonal isolates in this study. Note that isolates from each patient clustered into a group of highly related strains. The lowest $S_{AB}$ values were between subgroups of P8. The average $S_{AB}$ between clusters, treated as units, was $0.72 \pm 0.003$ ($n = 36$). Three clusters (P4, P6, and P7) contained isolates with indistinguishable Ca3 patterns, but in all of these cases, differences were resolved by C1 fragment fingerprinting. The numbers in parentheses refer to the number of isolates in each subgroup.

**The variation between isolates from the same colonizing population is restricted to bands hybridizing to the C1 fragment.** It was previously demonstrated that differences between the Ca3 hybridization patterns of highly related isolates were usually restricted to the high-molecular-weight bands which hybridize exclusively to the 2.6-kb *Eco*RI C fragment of the Ca3 probe (1, 20). The C fragment was further separated into a 0.98- and 1.6-kb fragment, referred to as the C1 and C2 fragments, respectively, and six isolates of the P3 population, which exhibited variability in the high-molecular-weight bands of the Ca3 hybridization patterns (Fig. 2A) were sequentially hybridized with Ca3, C1, and C2 (Fig. 5). Both the C1 and C2 fragments hybridized with the variable high-molecular-weight bands, but the hybridization signal with C1 was much stronger (Fig. 5B and C). The C1 fragment was, therefore, subsequently employed as a probe to test whether the pattern differences observed between isolates from the nine colonizing populations were restricted to genomic sequences which hybridized to the C fragment. Of the six populations with pattern variation in the Ca3 pattern (P1, P2, P3, P5, P8, and P9), all modifications were restricted to bands which hybridized to the C1 fragment. For example, the single change in the intensity of the 7.9-kb band on the Ca3 hybridization pattern of isolate 12 of P2 (Fig. 1A) proved to represent the addition of a 7.9-kb fragment hybridizing with the C1 fragment (Fig. 1B), and the high-molecular-weight variable bands in the Ca3 hybridization pattern of clones 2, 4, 7, 8, and 10 of P3 (Fig. 2A) also proved to represent bands hybridizing with the C1 fragment (Fig. 2B).

When blots of the three populations with no apparent modifications in the Ca3 pattern were stripped and rehybridized with the C1 fragment, band differences were observed in every population. In these three cases, the differences were camouflaged by fragments of equal molecular weight which hybridized with other portions of the complex Ca3 probe (1). For example, no obvious band differences between the Ca3 patterns of the 12 clones of P6 were evident (Fig. 1C), but two patterns emerged after hybridization with the C1 fragment. The two patterns were distinguishable by the presence or absence of a single band just below the well-characterized 5.4-kb band (Fig. 1D). While the majority of isolates of P6 contained this fragment, clones 3 and 12 did not (Fig. 1D).

**Variation in C1 fragment patterns usually involves a single band.** All of the nine tested populations contained at least one isolate with a modified pattern (i.e., a pattern which varied from the major pattern). The proportion of isolates exhibiting minor patterns varied between 7 and 42% of the population and averaged 19% ± 14% (Table 1). The number of different minor patterns (i.e., minor patterns which varied from each other) varied between one and three and averaged 1.4 ± 0.7 (Table 1). No minor pattern in the nine analyzed populations, which included a total of 106 clones containing 21 variants, involved an increase or decrease of more than one C fragment band (Table 1).

**Sequence analysis of the C1 fragment.** A partial restriction map of the C fragment is presented in Fig. 6A, and the nucleotide sequence of the 0.98-kb C1 fragment is presented in Fig. 6B. The C1 sequence consists of 982 bp, and the region between bp 126 and 982 is 98.6% identical to the sequence spanning 1257 and 2114 bp of the repetitive sequence RPS1 of *C. albicans* (8). This region of the C1 fragment contains a 28- to 29-bp element repeated three times (a, b, and c in Fig. 6B) and previously referred to by Iwaguchi et al. (8) as COM29. The first COM29 element, a, is immediately flanked by the 5′ end of a *Pst*I site (bp 267 to 982), and the third COM29 element, c, is immediately flanked at its 3′ end by an *Eco*RI site (bp 977 to 982) (Fig. 6B). The region of the C1 subfragment between bp 46 and 125 is 47.6% homologous to the sequence between bp 1178 and 1252 of RPS1 (8). However, the region between bp 1 and 45 of the C1 fragment shares no homology to any of the five characterized RPS elements (4, 8).

To test whether the faint hybridization signal between fragment C2 and the high-molecular-weight variable bands of the Ca3 pattern (Fig. 5C) reflects the presence of an RPS sequence in fragment C2, we hybridized unlabeled C1 and C2 with labeled C2 at high stringency (Fig. 7). There was no detectable hybridization between C1 and C2. The lack of homology and the fact that the C1 fragment is contiguous with the C2 fragment suggest that fragment C1 contains at least a portion of the true end of an RPS element. The faint hybridization signal between the variable high-molecular-weight bands of the Ca3 pattern and fragment C2 (Fig. 5C) suggests that C2 may contain a small portion of that end.

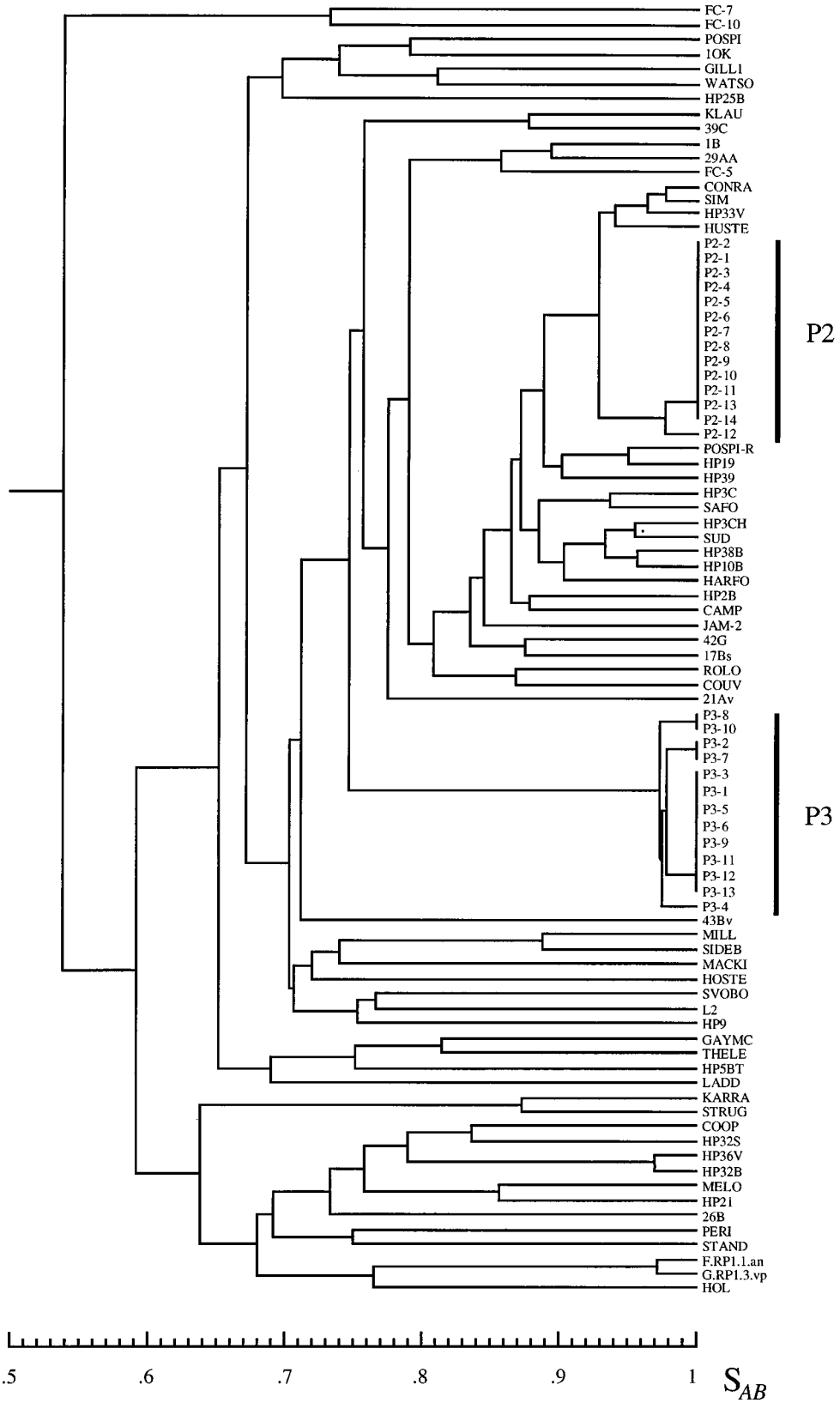


FIG. 4. Dendrogram comparing the genetic relatedness of P2 and P3 isolates with 60 previously analyzed, unrelated strains drawn from the Dendron data bank. The dendrogram is based on $S_{AB}$s computed between the Ca3 patterns of all listed strains. Note that in spite of the diversity of strains, the clonal isolates of P2 and P3 still cluster with no penetration by a data bank strain.

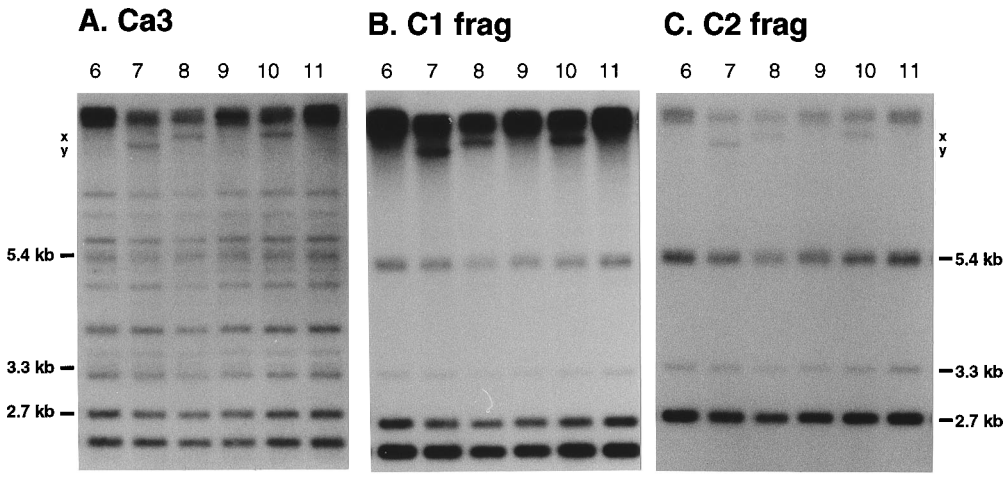


FIG. 5. DNA fingerprinting patterns of six isolates of the P3 population with the probes Ca3, the 0.98-kb C1 fragment of Ca3, and the 1.6-kb C2 fragment of Ca3. The isolate numbers correspond to those in the more complete analysis shown in Fig. 2A. The two high-molecular-weight bands which show variability are labeled x and y.

## DISCUSSION

**Commensal and infecting populations are primarily clonal.** By employing DNA fingerprinting with the Ca3 probe to assess the genetic relatedness of strains from different body locations (23) and in sequential vaginal infections of the same individuals (20), it has been demonstrated that strains of *C. albicans* undergo microevolution at sites of carriage and through recurrent episodes of infection. The modifications in genotype were observed primarily in *Eco*RI genomic fragments which hybridized to the C fragment of the Ca3 probe (1). Although these studies demonstrated genetic change in space and time, they did not address the genetic homogeneity of a commensal or infecting population at a single anatomical locale and at a single time point. Here, we have analyzed the genetic homogeneity of two commensal populations from the vulvovaginal region, one commensal population from the mouth, and six infecting populations from the vulvovaginal region. For each population, cells were cloned directly from the site of colonization. Individual isolates were generated from single cells separated at the time of isolation. Each analyzed population, therefore, was not subjected to passage in vitro prior to cloning, which runs the risk of enriching for a particular genotype with a selective growth advantage under the in vitro conditions employed and, consequently, of changing the original proportion of genotypes in the population.

The individual isolates from each population analyzed in this study proved to be highly related according to a measure of genetic relatedness provided by the computation of similarity coefficients (19). We previously demonstrated that the mean $S_{AB}$ value (± standard deviation [SD]) for 46 unrelated strains of *C. albicans* fingerprinted with the Ca3 probe was 0.69 ± 0.11, and a repeat analysis of 62 unrelated strains in this study resulted in a mean $S_{AB}$ value (± SD) of 0.68 ± 0.11. These mean $S_{AB}$s and their SDs provide a measure of unrelatedness. The lowest $S_{AB}$ between isolates in any one of the nine populations analyzed here was 0.97, suggesting that the isolates

TABLE 1. Genetic heterogeneity of the nine analyzed populations of *C. albicans*

| Patient | Interpreted state of yeast | Body location | No. of clones analyzed[a] | Ca3[b] | | C1 fragment[b] | | No. of minor patterns[c] | Band differences in minor patterns[d] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | % of isolates with the predominant pattern | % of isolates with a minor pattern | % of isolates with the predominant pattern | % of isolates with a minor pattern | | |
| P1 | Commensal | Back of tongue | 12 | 67 | 33 | 67 | 33 | 1 | 1(+/−) |
| P2 | Commensal | Vulva | 14 | 93 | 7 | 93 | 7 | 1 | 1(+) |
| P3 | Commensal | Vulva | 13 | 62 | 38 | 62 | 38 | 3 | 1(+), 1(+), 1(+) |
| P4 | Pathogenic | Vulva | 12 | 100 | 0 | 92 | 8 | 1 | 1(+) |
| P5 | Pathogenic | Vulva | 9 | 89 | 11 | 89 | 11 | 1 | 1(−) |
| P6 | Pathogenic | Vaginal wall | 12 | 100 | 0 | 83 | 17 | 1 | 1(−) |
| P7 | Pathogenic | Vaginal wall | 10 | 100 | 0 | 90 | 10 | 1 | 1(−) |
| P8 | Pathogenic | Vaginal wall | 12 | 58 | 42 | 58 | 42 | 2 | 1(+), 1(+) |
| P9 | Pathogenic | Vaginal wall | 12 | 92 | 8 | 92 | 8 | 1 | 1(+) |
| Mean (± SD) | | | 12 ± 2 | 85 ± 17 | 15 ± 17 | 81 ± 14 | 19 ± 14 | 1.4 ± 0.7 | |

[a] Clones were obtained directly from sites of carriage and infection (see Materials and Methods).
[b] The proportion of predominant and minor patterns were interpreted on the basis of both band position and band intensity.
[c] The number of minor patterns represents the number of distinguishable patterns other than the predominant pattern.
[d] Each variant pattern is assessed according to the number of bands differing from the predominant pattern and whether there is a band addition (+), a band loss (−), or an addition and loss (+/−).

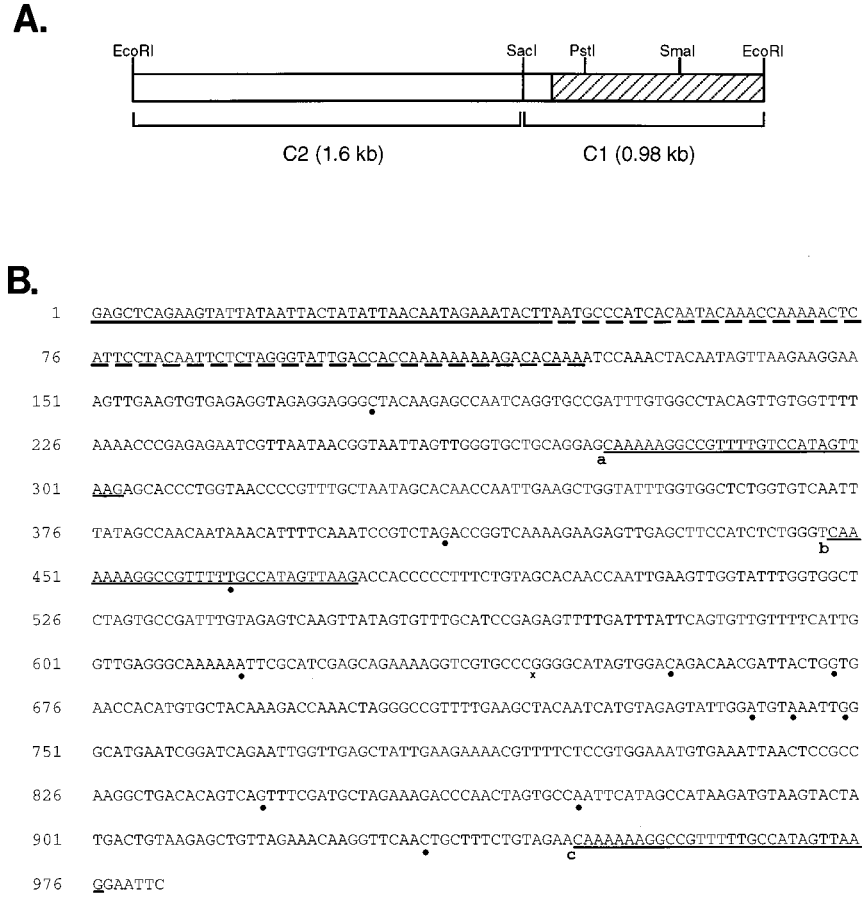


FIG. 6. Partial restriction map of the C fragment (A) and the nucleotide sequence of the 0.98-kb C1 fragment (B). (A) The hatched region of the C1 fragment represents the portion which is 98.6% homologous to the RPS1 element. The *Sac*I site separates the C fragment into a 0.98-kb C1 fragment and a 1.6-kb C2 fragment. (B) The base sequence of the entire C1 fragment. The sequence between bp 125 and 982 is 98.6% homologous to the sequence between bp 1257 and 2114 of the RPS1 element (8). The underlined sequence, between bp 1 and 45 exhibits no significant homology to the RPS1 element, while the dashed underlined sequence between bp 46 and 124 exhibits 47.6% homology to the sequence between bp 1178 and 1252 of the RPS1 element. Base differences between the sequence from bp 125 to 982 and the RPS1 element are indicated by dots. A deletion is noted by an x. Three 28- to 29-bp repetitive elements referred to as COM29 (8) are denoted by the letters a, b, and c and are underlined.

from every analyzed population evolved from a single progenitor. However, even though the nine colonizing populations analyzed here were all clonal, there have been genetic analyses demonstrating that colonizing *Candida* populations can be heterogeneous for species. Heterogeneity has been demonstrated primarily in populations colonizing immunocompromised individuals such as bone marrow transplant patients (25) and AIDS patients (26). It has also been demonstrated in recurrent vaginal infections that one infecting strain can be replaced with a second, unrelated strain and that a mixed infection precedes complete replacement (20).

**Commensal and infecting populations undergo microevolution.** Although the isolates from each of the nine populations analyzed were genetically similar, each population contained genetic variants (i.e., minor genotypes) which differed by one band in the Ca3 and/or C1 fragment hybridization pattern. The proportion of isolates in the population with minor genotypes varied between 7 and 42% and averaged 19% ± 14%. Therefore, on average, there was a major genotype which accounted for 81% of the individual cells in each of the commensal or infecting populations analyzed here. The average number of different minor genotypes in a population was 1.4. This value suggests that cells in a population not exhibiting the major genotype were heterogeneous. In every population tested, minor variants differed from the major genotype as well as from each other by usually one high-molecular-weight band in the Ca3 pattern. The absence of changes in the more invariant bands of the Ca3 pattern and the limited differences between minor and major genotypes in a population suggest that the reorganizational event leading to the minor band difference was a recent one and that these populations do not consist of highly diverging subpopulations which have continued to diverge over a long period of time. It is impossible to know from a single population at a single time point whether a minor genotype has evolved from a major genotype in a population or vice versa. It is also impossible to know without demonstrating phenotypic differences between major and minor genotypes whether enrichment of a variant phenotype results from an adaptive advantage or whether neutral changes simply enrich through chance in small populations (9).

**Genetic variation in populations is due primarily to reorganization of genomic sequences homologous to the C1 fragment of the Ca3 probe.** It was previously demonstrated that minor variations in the Ca3 hybridization patterns of isolates from different anatomical locations of the same individuals (23), from the same oral cavity (7) and from recurrent vaginal infections (20) were restricted primarily to high-molecular-weight bands. Dissection of the Ca3 probe subsequently dem-

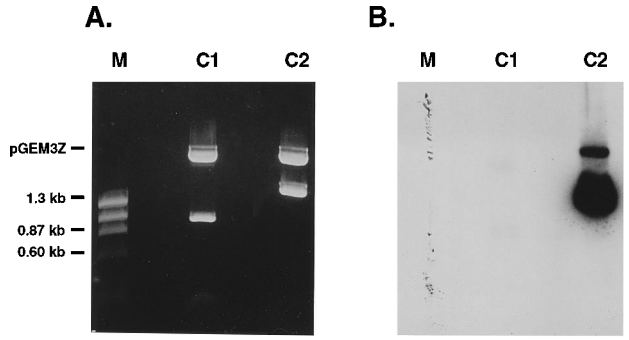


FIG. 7. Absence of cross-hybridization between the C1 and C2 fragment. The pGEM3Z plasmid containing the 0.98-kb C1 fragment was digested with *Eco*RI and *Sac*I, and the pGEM3Z plasmid containing the 1.6-kb C2 fragment was digested with *Eco*RI to release the respective fragments. The digests were separated on 1% agarose gels, and Southern blots were hybridized with randomly primed 1.6-kb C2 fragment. (A) Ethidium bromide-stained gel; (B) Southern blot hybridization with the C2 fragment. Note that the C1 fragment did not hybridize with the C2 fragment. M lanes contain molecular size markers.

onstrated that the variable high-molecular-weight bands hybridized exclusively to a 2.6-kb *Eco*RI fragment of the Ca3 probe designated the C fragment (1). Here we have demonstrated that all variants in the colonizing populations analyzed were the result of reorganization of genomic sequences homologous to the C1 subfragment of the C fragment. In a large-scale analysis of sequential isolates from 30 patients with recurrent vaginitis, variations in pattern were again found to occur primarily in these high-molecular-weight fragments (10a).

The changes observed in the C1 fragment hybridization pattern were usually restricted to high-molecular-weight bands. Several low-molecular-weight bands of the Ca3 pattern which hybridize to the C1 fragment (e.g., at 2.7 and 5.4 kb) are far more invariant. When lower-molecular-weight fragments which hybridize to the C1 fragment did vary between clones, the differences were sometimes camouflaged because they comigrated with invariant bands which hybridized to other portions of the Ca3 probe. Although the evidence presented here demonstrates that the C1 fragment of the Ca3 probe is an excellent indicator of microevolution in a population, using the C1 fragment as a probe is not sufficient for analyzing genetic relatedness for several reasons. First, because it is hypervariable, it may be a better indicator of short-range rather than long-range changes. This is especially true if rules governing reorganization lead to pattern convergence, a possibility which is now being tested in vitro in long-term culturing experiments. Second, because the pattern generated by C1 fragment hybridization is limited, it does not provide enough information to compute a similarity coefficient at lower levels of genetic relatedness. The complete Ca3 probe provides the complexity necessary for such a single computation by virtue of its combination of relatively invariant sequences, allelic sequences, and hypervariable sequences (1, 19). Third, some strains, like the laboratory strain 3153A, do not contain the hypervariable high-molecular-weight bands which hybridize to the C1 fragment (1), but select bands in the remaining Ca3 hybridization patterns are variable enough to allow clear genetic distinction between 3153A and other strains of *C. albicans*. These considerations suggest that serial fingerprinting with the Ca3 probe and the C1 fragment provides a far more powerful method of genetic analysis in epidemiological studies than fingerprinting with either probe alone. In addition, the information now being accumulated for the frequency of variability for the individual bands of the complete Ca3 pattern will facilitate the development of a similarity coefficient in which each band in the pattern is weighted according to the frequency of variability, thus providing a more accurate measure of genetic relatedness.

**The 0.98-kb C1 fragment of the Ca3 probe is partially homologous to the RPS family and may contain a genomic RPS end.** We previously reported the sequence of the 3.0-kb B fragment of the Ca3 probe, which is responsible for a major portion of the Ca3 pattern (1). The B fragment pattern includes both invariant bands and low-variability bands, and although the B fragment contains a number of internal repeat sequences, it exhibits no obvious features which could be interpreted in the context of genomic reorganization and no base sequence homology with any known sequences in data banks (1). The C1 fragment, in contrast, hybridizes to hypervariable regions of the *C. albicans* genome, and for that reason it more likely harbors recombinational sequences. The nucleotide sequence analysis revealed that the C1 fragment is partially homologous to the previously described RPS repetitive elements (4, 8). The RPS family includes tandem multimers containing a repeat of 172 bp, the *alt* element (4). RPS sequences have been demonstrated on all separable chromosomes but chromosome 4 (4), the same chromosome demonstrated to lack hybridization with the C fragment (1).

In a recent comparison of RPS1, the first characterized RPS sequence (8), and a number of additional RPS sequences (4), the RPS element was defined as the sequence between two *Pst*I sites that generate a unit length sequence. However, the ends of the multiple array of RPS elements were not defined with respect to their genomic locations, because the analyzed sequences were probably contained within tandem repeats. The 3′ end of the 0.98-kb C1 fragment, which represents the 3′ end of the Ca3 probe, lies within an RPS element. However, the 5′ end represents a unique sequence contiguous with the 1.6-kb C2 fragment. The C2 fragment has no significant homology with the C1 fragment and is in turn contiguous with the rest of the Ca3 probe (1). These results suggest that a true end of the RPS repeat element resides partially or completely at the 5′ end of the C1 fragment. We are currently sequencing an original fragment which hybridized to the C1 fragment and a naturally reorganized version of that fragment in order to understand how hypervariable genomic sequences rearrange during microevolution in a colonizing population of *C. albicans*.

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