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Genetic Similarity and Phenotypic Diversity of Commensal and Pathogenic Strains of *Candida albicans* Isolated from the Oral Cavity

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Colony phenotype and genetic similarity were assessed within and between groups of commensal and pathogenic strains of *Candida albicans* collected from the oral cavities of individuals in a single geographical locale. Thirty-eight percent of pathogenic isolates contained predominant or minor variant colony morphologies (other than smooth) when samples from the sites of infection were cultured on plates, while 16% of commensal isolates contained minor variant colony morphologies when samples from the sites of carriage were cultured. The genetic similarities of isolates within and between groups were assessed by DNA fingerprinting by using Southern blot hybridization with the fingerprinting probe Ca3 and analysis with the computer-assisted, automated Dendron system. Both the commensal and the pathogenic groups contained a major cluster of genetically similar *C. albicans* isolates representing 31 and 33% of the strains in the respective groups. When a combined dendrogram of both commensal and pathogenic isolates was generated, the major clusters of genetically similar isolates in each group mixed into one large cluster. Minor clusters in the individual dendrograms also mixed. These results suggest common clonal origins for commensal and pathogenic strains in the same geographical locale.

Candida albicans resides in the oral cavities of a majority of healthy individuals as a commensal organism, causing no apparent morbidity (8, 23). However, under a number of predisposing conditions, the level of *C. albicans* increases dramatically, resulting in tissue penetration and inflammation of the oral mucosa. Despite its prevalence as a commensal organism and the frequency of oral candidiasis, we still have not answered some of the most fundamental questions related to commensal carriage, infection, or the transition from the commensal to the pathogenic state (21, 22). For instance, it is generally assumed that because the organism so commonly inhabits the oral cavities of healthy individuals, the commensal strain is the source of subsequent infection. This assumption, in turn, suggests that all or most commensal *C. albicans* organisms are capable of causing an infection, and all strains, by definition, are opportunistic. However, it is also possible that in the transition from a healthy to an infected mouth, there is strain substitution (18).

In the past, it was difficult to answer this type of basic epidemiological question because strain comparisons were based on biotyping techniques (7, 9, 12), which compared phenotypes rather than genotypes and, therefore, ran the risk of grouping genetically unrelated strains with similar phenotypes and separating related strains with different phenotypes. In recent years, a number of DNA fingerprinting techniques which provide genetic assessments of strain relatedness have been developed (6, 11, 15, 16, 24). One of the most effective of these has been the use of Southern blot hybridization with DNA probes containing moderately repetitive sequences (3, 10, 16, 19, 22, 24). The hybridization patterns generated by this technique are amenable to computer-assisted analysis with the Dendron software program (19, 22). The patterns differ between unrelated strains, are constant over hundreds of generations in a single strain (16, 19), and are complex enough to provide similarity coefficients which reflect genetic relatedness (19, 22). In the present study, we used this DNA fingerprinting technique with a newly developed automated Dendron program to test whether a group of commensal strains is genetically distinct from a group of pathogenic strains of *C. albicans* isolated from the oral cavities of individuals in the same geographical locale. We found no genetic distinctions between commensal and pathogenic strains. Rather, we identified in dendrograms both major and minor clusters of genetically related strains which included both commensal and pathogenic strains suggesting common clonal origins for commensal and pathogenic strains in the Iowa City, Iowa, locale.

MATERIALS AND METHODS

Group selection. Healthy patients from whom samples were positive for *C. albicans* on culture and who conformed to the following restrictions were included in the study: (i) no history of symptoms or diagnosis of oral candidiasis, (ii) no immunologic or other compromising condition, (iii) no recent use of antibiotics or steroids, and (iv) no history of recurrent *C. albicans* infections in other body locations. This group consisted of 12 females and 7 males. The average age was 41.5 ± 17.0 years. Patients with oral candidiasis were included in the study if they displayed symptoms of erythematous or pseudomembranous candidiasis and samples from the patients were positive for *C. albicans* on culture. This group consisted of 16 females and 7 males. The average age

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was 55.8 ± 20.5 years. One male in the latter group was subsequently discovered to be immunocompromised as a result of AIDS. Complete medical histories were evaluated for both groups for inclusion in the study.

Culturing of commensal and pathogenic strains. Five intraoral sites (buccal mucosa, salivary pool, floor of mouth, dorsum of tongue, palate) of healthy individuals and patients with candidiasis were individually swabbed with a wet sterile Culturette (C8852-1; American Scientific Products, McGaw Park, IL.) by previously described methods (23). Each swab was vigorously agitated in 5 ml of sterile water, and 0.1 ml was spread onto each of three agar plates containing the defined amino acid-rich composition of the medium developed by Lee et al. (5) supplemented with 0.1 μM zinc and 70 μg of arginine per ml (2). Colony number and morphology were assessed after 7 to 9 days of incubation at 25°C. The isolates from the 19 healthy individuals and 23 patients with candidiasis were considered in the "basic" groups of commensal and pathogenic strains, respectively, in the present study. These strains were collected over a 1-year period between January 1991 and January 1992. Additional commensal and pathogenic strains isolated from the oral cavities of patients in prior studies in the Iowa City, Iowa, locale were also used when noted in comparisons of strain similarities. These strains were referred to as "additional" groups. The additional commensal and pathogenic strains were collected between 1988 and 1990. The average ages and geographical locales were similar for the patients from which the basic and additional pathogenic strains were collected. However, the patients from whom additional commensal strains were collected were, on average, 15 years younger and had a greater proportion of females than the patients from whom basic commensal strains were collected.

Southern blot hybridization and DNA fingerprint analysis. Strains were fingerprinted with the DNA probe Ca3 by previously described methods (19, 24). In brief, DNA was isolated from cells of each strain by the method of Scherer and Stevens (15). The concentration of DNA in each preparation was assessed by comparing an ethidium bromide-stained sample with a set of ethidium bromide-stained standards of calf thymus DNA. DNA samples were then electrophoresed in a 0.7% agarose gel. Each gel contained 15 lanes, with *Eco*RI-digested DNA of reference strain 3153A in lanes 2 and 15, 12 test strains in lanes 3 through 14, and a 1-kb ladder of molecular mass markers (Bethesda Research Laboratory, Gaithersburg, Md.) in lane 1. Gels were run first at 70 V for 20 min and then at 30 to 40 V until the bromophenol blue marker had traveled 16 cm from the well. Gels were stained with ethidium bromide and were photographed with a UV light source. Gels were then washed, blotted onto a nitrocellulose membrane, hybridized with nick-translated Ca3 probe, and exposed to XAR-S film (Eastman Kodak Co., Rochester, N.Y.) with a Cronex Lightning Plus intensifying screen (Du Pont Co., Wilmington, Del.).

Hybridization patterns were then automatically analyzed with the newly developed automated Dendron program (Solltech Inc., Iowa City, Iowa) (22). Autoradiogram patterns were digitized into the Dendron data base with a Sharp scanner, the digitized images were unwarped (straightened), and lanes were image processed, when necessary, and scanned (22). Bands were automatically identified and intensities were classified between 0 (no band) and 3 (highest intensity). The Dendron program then computed the similarity coefficient (S_{AB}) for every pair of strains on the bases of the positions and intensities of the bands in the hybridization pattern, generating a matrix of similarity coefficients. Dendrograms based on S_{AB}s were then generated for selected strains by using the unweighted group pair method (19, 20). In an analysis of 45 *C. albicans* strains, each strain was fingerprinted twice with the Ca3 probe on separate gels at different times, the mean ± standard deviation S_{AB} for pairs was 0.96 ± 0.03, which is considered a measure of identicalness for strains analyzed in different gels. For 10 fingerprints of the same strain on the same gel, the mean S_{AB} was 1.00 ± 0.00. In an analysis of 30 independently isolated, unrelated *C. albicans* strains randomly selected from the Dendron data base and not part of the present study, the average S_{AB} was 0.69 ± 0.11, which is considered a measure of unrelatedness. Lanes on different gels were "neighbored" (the digitized lane images from different gels were placed next to each other), and band positions were normalized according to the band positions of the 3153A reference patterns bordering each gel by a Dendron subprogram developed for this purpose (17, 23). The digitized lane images from different gels that were placed next to each other were then photographed from the monitor screen.

Biotyping by sugar assimilation. The API 20C kit (bioMerieux, Hazelwood, Mo.) was used to type the species. Isolates from stored slants were streaked onto nutrient agar and were incubated at 25°C for 24 h. Samples were then analyzed according to the manufacturer's prescribed methods.

RESULTS

Colony phenotypes and strain identification of the basic sets of commensal and pathogenic strains. The level of commensal strain carriage ranged between 6 and 342 colonies per 15 plates, with a mean of 52.4 ± 77.0 (Table 1). In contrast, the level of colonization with pathogenic strains ranged between 9 and 7,500 colonies per 15 plates, with a mean of 569 ± 1,594 (Table 2). While only 1 of 19 samples of commensal strains (5%) contained more than 120 colonies per 15 plates, 9 of 23 samples of pathogenic strains (39%) contained more than 120 colonies per 15 plates (Tables 1 and 2). The dominant colony morphology of the 19 samples of commensal strains was smooth white (Fig. 1A; Table 1). In 3 (16%) of the samples of commensal strains (C4, C11, C14), the minor colony morphology was heavily myceliated (Fig. 1B or C; Table 1). The dominant colony morphology in 21 of the 23 samples of pathogenic strains was also smooth white (Table 2). However, the dominant colony morphology in 2 of the 23 samples of pathogenic strains was heavily myceliated (P3, P5). In addition, 6 of the 23 samples of pathogenic strains (26%) contained minor colony phenotypes (Table 2). Samples P7, P13, and P19 contained isolates with a minor wrinkled colony morphology (Fig. 1D), sample P8 contained isolates with a star colony morphology (Fig. 1E), sample P17 contained isolates with minor wrinkled, ring (Fig. 1F), and star colony morphologies, and sample P20 contained isolates with a minor heavily myceliated colony morphology. Therefore, 15% of the commensal isolates had variant colony morphologies, while 34% of pathogenic isolates had variant colony morphologies. This difference was possibly significant, with $P < 0.25$.

Species identification was assessed in two ways. First, *Eco*RI-digested DNAs of individual isolates were electrophoresed and Southern blots were hybridized with the Ca3 probe, which is species specific for *C. albicans* (13). Strains of *C. albicans* exhibited a hybridization pattern containing 15 to 25 bands, with both highly conserved and variant bands (1). Strains of *Candida stellatoidea* exhibit patterns close to

TABLE 1. Colony number, colony phenotypes, and species identification of commensal strains by DNA fingerprinting and sugar assimilation

Sample no.	No. of colonies[a]	Major colony morphology[b]	Minor colony morphology[c]	DNA fingerprint pattern[d]	Sugar assimilation pattern[e]
C1	66	Sm		+	C.a.
C2	36	Sm		+	C.a.
C3	342	Sm		+	C.a.
C4	9	Sm	HM	+(Sm), +(HM)	C.a.(Sm), C.a.(HM)
C5	6	Sm		−	NI
C6	12	Sm		+	C.a.
C7	108	Sm		−	C.p.
C8	75	Sm		+	C.a.
C9	6	Sm		−	C.p.
C10	69	Sm		+	C.a.
C11	60	Sm	HM	+(Sm), +(HM)	C.a.(Sm),C.a.(HM)
C12	21	Sm		−	Sac.
C13	18	Sm		+	C.a.
C14	9	Sm	HM	+(Sm), +(HM)	C.a.(Sm),C.a.(HM)
C15	18	Sm		+	C.a.
C16	24	Sm		+	C.a.
C17	6	Sm		+	C.a.
C18	21	Sm		+	C.a.
C19	90	Sm		+	C.a.

[a] The value is the sum of the number of colonies on 15 plates.
[b] Sm, smooth white phenotype.
[c] In all three samples, (C4, C11, C14), colonies of isolates of the heavily myceliated phenotype (HM) made up less than 20% of all colonies.
[d] Since the Ca3 probe is relatively specific for *C. albicans*, a plus sign represents a standard *C. albicans*-type pattern, and a minus sign represents either no hybridization or two or fewer bands, as is the case for *C. tropicalis* (17).
[e] Sugar assimilation tests were performed twice for each sample. The best identification is presented. C.a., *C. albicans*; C.p., *C. parapsilosis*; Sac., *S. cerevisiae*; NI, not identified by the test; Sm, smooth colony phenotype; HM, heavily myceliated colony phenotype.

those of *C. albicans* strains (4), while other species exhibit patterns ranging from two low-intensity, high-molecular-mass bands, in the case of *Candida tropicalis*, to no bands for less related species (13, 25). Second, isolates were biotyped by their sugar assimilation patterns (9). Isolates from 15 of the 19 samples of commensal strains (79%) typed as *C. albicans* both by hybridization with the Ca3 probe and by sugar assimilation (Table 1). In the three samples of commensal strains in which there were both major and minor colony phenotypes (C4, C11, C14), isolates of both phenotypes typed as *C. albicans* by Ca3 hybridization and sugar assimilation (Table 1). Isolates from 4 of the 19 samples of commensal strains (C5, C7, C9, C12) did not hybridize significantly with Ca3 and were therefore not *C. albicans* (Table 1). By sugar assimilation, two (C7, C9) typed as *Candida parapsilosis*, one (C12) typed as *Saccharomyces cerevisiae*, and one (C5) was unidentifiable.

Isolates from 22 of the 23 samples of pathogenic strains (96%) typed as *C. albicans* by hybridization with Ca3 and sugar assimilation (Table 2). These included isolates from samples P3 and P5, which exhibited a dominant heavily myceliated phenotype. In the cases of samples P1 and P19, both the major and minor phenotypes of the isolates in the samples typed as *C. albicans* by both Ca3 hybridization and sugar assimilation. In the cases of isolates from samples P8 and P13, the major smooth phenotype did not type as *C. albicans* by Ca3 hybridization, while the minor phenotypes did. In both cases, the major phenotype typed as *S. cerevisiae* and the minor phenotype typed as *C. albicans* by sugar assimilation. In the case of isolates from sample P20, both the major and minor phenotypes exhibited reduced hybridization patterns and typed as *C. stellatoidea* by sugar assimilation.

General features of hybridization patterns. The Southern blot hybridization patterns of the *C. albicans* strains probed with nick-translated Ca3 contained between 10 and 15 major bands in the molecular mass range of 2.25 to 19 kb (Fig. 2) and between 3 and 8 minor bands in the molecular mass range of less than 2.25 kb (data not shown in Fig. 2). The patterns of most *C. albicans* strains tested so far show that the strains contain highly conserved bands at 2.68 and 3.30 kb (1). The majority of other bands are moderately variable between strains. One or more bands above 7.9 kb have been found to be highly variable (1, 24). Digestion of whole-cell DNA with *Eco*RI resulted in a very clear and highly reproducible banding pattern. This is evident in the identical patterns of reference strain 3153A, labeled R in Fig. 2. In some cases, the Ca3 probe did not hybridize to the *Eco*RI-digested DNA of an isolate (e.g., Fig. 2, lanes 3 and 12). All of these isolates typed as species other than *C. albicans* by their sugar assimilation patterns (Tables 1 and 2). In some cases, the probe generated a highly reduced pattern (e.g., Fig. 2, lanes 10 and 18). Again, these strains typed as a species other than *C. albicans* by sugar assimilation (Table 2). In rare cases, endonuclease digestion was interfered with in repeat experiments (e.g., Fig. 2, lane 8), making it difficult or impossible to analyze. It has been demonstrated that Ca3 distinguishes between the great majority of independent isolates by one or more bands (19), and this can be verified by a comparison of the independent isolates whose fingerprint patterns are shown in Fig. 2.

Genetic similarity of multiple phenotypes from the same oral cavities. Three sets of samples of commensal strains (C4, C11, C14) exhibited a predominant smooth phenotype (Fig. 1A) and a minor heavily myceliated phenotype (Fig. 1B and C). To test whether the major and minor phenotypes in each sample represented the same or different strains, clones of the smooth and variant phenotypes were fingerprinted with Ca3. In all three cases, clones of both phenotypes typed as *C. albicans* by sugar assimilation (Table 1), and the

TABLE 2. Colony number, colony phenotypes, and species identification of pathogenic strains by DNA fingerprinting and sugar assimilation

Sample no.	No. of colonies[a]	Major colony morphology[b]	Minor colony morphology[c]	DNA fingerprint pattern[d]	Sugar assimilation pattern[e]
P1	225	Sm		+	C.a.
P2	9	Sm		+	C.a.
P3	78	HM		+	C.a.
P4	21	Sm		+	C.a.
P5	24	HM		+	C.a.
P6	12	Sm		+	C.a.
P7	21	Sm	Wr	+ (Sm), + (Wr)	C.a. (Sm), C.a. (Wr)
P8	42	Sm	St	− (Sm), + (St)	Sac. (Sm), C.a. (St)
P9	459	Sm		+	C.a.
P10	1,200	Sm		+	C.a.
P11	51	Sm	+	C.a.	
P12	150	Sm		+	C.a.
P13	126	Sm	Wr	− (Sm), + (Wr)	Sac. (Sm), C.a. (Wr)
P14	36	Sm		+	C.a.
P15	81	Sm		+	C.a.
P16	15	Sm		+	C.a.
P17	2,277	Sm	Wr, R, St	+ (Sm), + (Wr), + (R), + (St)	C.a. (Sm), C.a. (Wr), C.a. (R), C.a. (St)
P18	276	Sm		+	C.a.
P19	72	Sm	Wr	+ (Sm), + (Wr)	C.a. (Sm), C.a. (Wr)
P20	354	Sm	HM	+, un (Sm), + un[c] (HM)	C. stel. (Sm), C. stel. (HM)
P21	36	Sm		+	C.a.
P22	18	Sm		+	C.a.
P23	>7,500	Sm		+	C.a.

[a] The value is the sum of the number of colonies on 15 plates.
[b] Sm, smooth white phenotype; HM, heavily myceliated phenotype.
[c] In all cases, colonies of isolates of minor phenotypes made up less than 25% of all colonies. Wr, wrinkled phenotype; St, star phenotype; R, ring phenotype.
[d] A plus sign represents standard *C. albicans*-like pattern; a minus sign represents either no hybridization or minor hybridization to 2 or less bands; +un represents significant hybridization, but uncharacteristic of standard *C. albicans* strains.
[e] Best identification by sugar assimilation. C.a., *C. albicans*; Sac., *S. cerevisiae*; C. stel., *C. stellatoidea*; Sm, smooth colony phenotype; Wr, wrinkled colony phenotype; St, star colony phenotype; R, ring colony phenotype; HM, heavily myceliated colony phenotype.

fingerprints were either identical (samples C4 and C14) or highly similar (sample C11). In Fig. 3A, the Southern blot hybridization patterns of clones from samples C4 (smooth) and C4 (heavily myceliated), which were on different gels, were neighbored by the Dendron software program (see Materials and Methods) (17, 23). The banding patterns were identical (the very-high-molecular-mass broad band at the top of lane Sm in Fig. 3A proved to be undegraded DNA and not an analyzable hybridization band. In Fig. 3B, the patterns of clones from samples C11 (smooth) and C11 (heavily myceliated) are presented. The patterns are highly similar, differing by the position of only one high-molecular-mass band (noted by an arrow), which has been demonstrated to change size in the same strain at a higher frequency than other bands in the hybridization pattern obtained by Ca3 fingerprinting (1, 23).

Six samples of pathogenic strains contained strains with multiple phenotypes. In each case, the multiple phenotypes were fingerprinted. In four of the six cases (samples P7, P17, P19, and P20), the fingerprint patterns were identical. In Fig. 3C, the patterns are presented for clones of P17 ring, smooth, and wrinkled phenotypes. In Fig. 3D, the reduced patterns of clones from sample P20 (smooth) and P20 (heavily myceliated), which typed as *C. stellatoidea*, were identical. In two of the six cases (samples P8 and P13), strains of the smooth dominant phenotype biotyped as *S. cerevisiae*, and strains of the minor phenotype biotyped as *C. albicans* by sugar assimilation (Table 2). In both cases, the major strain, exhibiting the smooth phenotype, did not hybridize with Ca3, while the strain of the minor phenotype generated a standard *C. albicans* hybridization pattern (Fig. 4A and B).

Genetic relatedness within each group of commensal and pathogenic isolates. A dendrogram based on S_{AB}s was generated for single isolates from the basic group of 19 samples of commensal strains (Fig. 5A). In the case of samples of commensal strains with multiple phenotypes (samples C4, C11, and C14), only the major colony phenotype was used, since strains of the major and minor phenotypes were demonstrated to be genetically identical or highly similar (Table 1; Fig. 3A and B). Four of the 19 isolates (samples C5, C7, C9, and C12) did not hybridize with the Ca3 probe (e.g., C9 in lane 3 of Fig. 2), and in the dendrogram in Fig. 5A, the 4 isolates are connected to all other isolates in the basic group of commensal strains at an S_{AB} of 0.0. By sugar assimilation, the strains in samples C7 and C9 typed as *C. parapsilosis*, the strain in sample C12 typed as *S. cerevisiae*, and the strain in sample C5 was not identifiable (Table 1). The isolate in sample C16 had only three identifiable low-molecular-mass bands because of interference in digestion in repeat analyses. Because the three interpretable bands lined up with common bands in the Ca3 pattern of mainstream strains of *C. albicans*, we believe that this strain is *C. albicans*, and this was reinforced by its sugar assimilation pattern (Table 1). However, in the dendrogram, it connected to all other strains with an S_{AB} of 0.20 because of an incomplete pattern. The remaining 14 isolates were connected in the dendrograms at S_{AB}s of 0.58 or greater. The mean ± standard deviation S_{AB} for these 14 isolates com-

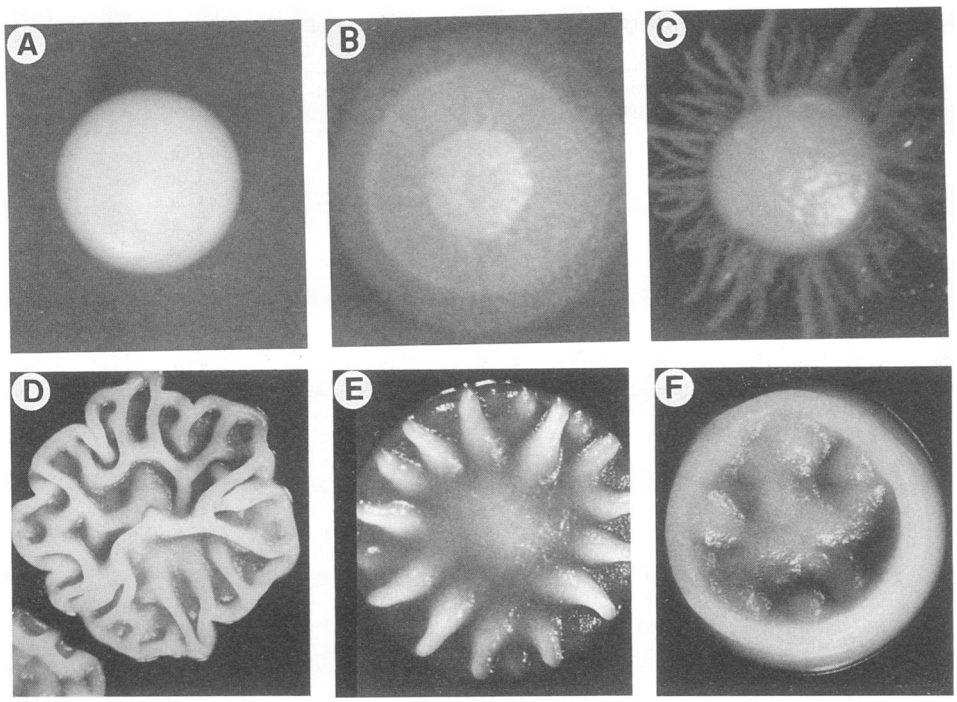

FIG. 1. Examples of the standard smooth and variant colony morphologies observed in commensal or pathogenic strains of *C. albicans* in the present study. (A) Standard smooth colony phenotype; (B) an example of the heavily myceliated colony phenotype (note the central colony dome and mycelial halo in the agar surrounding the dome); (C) another example of the heavily myceliated colony phenotype; (D) an example of the irregular wrinkle colony phenotype; (E) an example of the star colony phenotype; (F) an example of the ring colony phenotype.

puted from the matrix of S_{AB}s was 0.64 ± 0.09. Using 0.80 as an arbitrary threshold for genetic similarity (roughly one-half of randomly selected unrelated *C. albicans* strains in the Dendron data base formed clusters of two or more strains at S_{AB}s of 0.80 or greater in a dendrogram), we found two clusters containing four isolates (cluster a, isolates from samples C10, C13, C18, and C3) and two isolates (cluster b, isolates from samples C4 [smooth colony phenotype] and C2). The remaining eight strains were connected to each other and to all other strains at S_{AB}s of less than 0.80.

To increase the pool of oral commensal isolates, a dendrogram (Fig. 5B) was generated for the 19 isolates in the basic set of commensal strains (Fig. 5A) plus 13 additional oral commensal isolates that were collected on average 2.5 years before the basic set was collected from individuals in the same geographical locale (Iowa City, Iowa). Of the 32 combined isolates, 5 were not *C. albicans* (the 4 in the basic set plus 1 [from sample C24] from the additional set). The mean S_{AB} for the remaining 26 *C. albicans* isolates was 0.67 ± 0.10. Seventeen of the 26 *C. albicans* isolates (63%) clustered into four groups at S_{AB}s of ≥0.80, including one cluster of 10 isolates (cluster a), one cluster of 3 isolates (cluster d), and two clusters of 2 isolates (clusters b and c). The largest of the four clusters, cluster a (Fig. 5B) still contained the four isolates of cluster a in the dendrogram of the basic set (Fig. 5A) plus six isolates of the additional set and, therefore, represented a cluster of genetically similar commensal organisms that persisted in Iowa City over a 3- to 4-year period. This cluster contained 37% of the commensal isolates that typed as *C. albicans* and 31% of all commensal isolates analyzed.

A dendrogram was also generated for the 23 pathogenic isolates of the basic group (Fig. 5C). This group included one isolate from each of the 21 samples with only one colony morphology and two isolates each from samples P8 and P13, representing isolates of the alternative phenotypes. Two of the 25 isolates (smooth colony phenotype isolates from samples P8 and P13) did not hybridize with the Ca3 probe and were therefore not *C. albicans*. These isolates biotyped as *S. cerevisiae*. A smooth colony phenotype strain from sample P20 had only three bands and was connected to the remaining 22 isolates at an S_{AB} of 0.3. This isolate biotyped as *C. stellatoidea*. The remaining 22 isolates were connected at S_{AB}s of 0.60 or greater and typed as *C. albicans* by their sugar assimilation patterns (Table 2). The mean S_{AB} for this subset computed from the matrix of S_{AB}s was 0.66 ± 0.10. Using an S_{AB} of 0.80 as a threshold for relatedness, we found that 10 of the 22 isolates (50%) clustered into two groups, one containing 8 isolates (cluster a) and one containing 2 isolates (cluster c).

To increase the pool of pathogens, a dendrogram (Fig. 5D) was generated for the 25 isolates in the basic pathogenic set plus 8 additional pathogenic isolates collected from the oral cavities of patients in the same geographical locale on average 2.5 years prior to isolation of the basic set of isolates (Fig. 5D). Of the 33 isolates, 3 (from samples P8 and P13, which were of the sw phenotype, and P28) represented species other than *C. albicans*, and 1 (an isolate of the smooth colony phenotype from sample P20) exhibited only three hybridization bands. The mean S_{AB} for the remaining 29 *C. albicans* isolates computed from the matrix of S_{AB}s was 0.67 ± 0.11. Seventeen of the 33 isolates (52%) clustered into three groups with S_{AB}s of ≥0.80, one containing 11 isolates (cluster a), one containing 4 isolates (cluster c), and one containing 2 isolates (cluster b). The largest of the clusters (cluster a) contained the eight isolates in the major

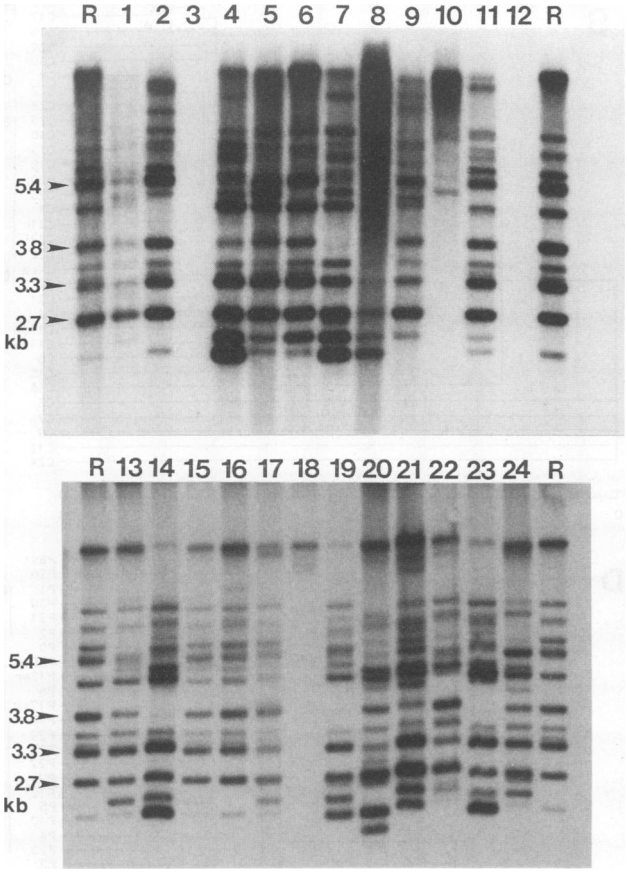

FIG. 2. Two representative southern blots of *Eco*RI-digested DNA of isolates probed with Ca3. In each blot, test strains are bordered by reference strain 3153A, labeled R. The following lanes contain test strains from the indicated samples: 1, C14-HM (HM is heavily myceliated); 2, P3; 3, C9; 4, P9; 5, P17-St (St is star); 6, C10; 7, C17; 8, C16; 9, C1; 10, P20; 11, P23; 12, C24; 13, P12; 14, P19-Sm (Sm is smooth); 15, C3; 16, P4; 17, P10; 18, P20-Sm (Sm is smooth); 19, P1; 20, P11; 21, P14; 22, C8; 23, P19-Wr (Wr is wrinkled); 24, C15.

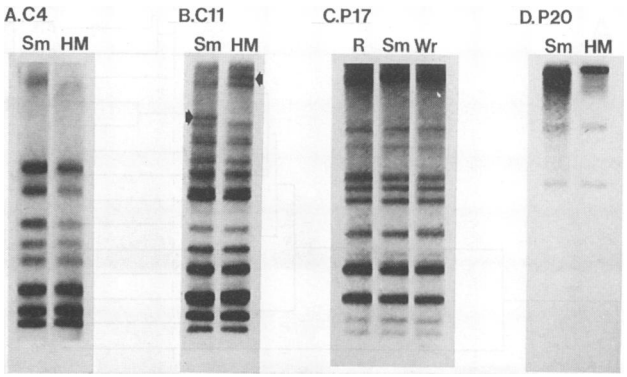

FIG. 3. Identical or highly similar Southern blot hybridization patterns of Ca3-probed *Eco*RI-digested DNAs of clones of multiple colony phenotypes from the same individuals. Since the multiple colony phenotypes of strains from samples C4 and P20 were hybridized on different gels, the lanes were "neighbored" (see text) with the Dendron software neighboring program. Note that the multiple phenotypes from samples C4, P17, and P20 exhibited identical hybridization patterns, while the multiple phenotypes from sample C11 exhibited similar but nonidentical patterns, differing by a single high-molecular-mass band, noted by an arrow. Sm, smooth; HM, heavily myceliated; R, ring; Wr, wrinkled. Examples of the variant phenotypes are presented in Fig. 1.

cluster in the dendrogram of the basic set (Fig. 5C) plus three isolates from the additional set. Cluster a contained 37% of pathogenic isolates that typed as *C. albicans* and 33% of all pathogenic isolates analyzed. The second largest cluster (cluster c) in the combined dendrogram (Fig. 5D) contained the two isolates in the minor cluster of the basic pathogenic set (Fig. 5C).

Genetic relatedness between commensal and pathogenic strains. To assess the genetic similarity of the basic set of 19 commensal isolates and the basic set of 25 pathogenic isolates, a common dendrogram was generated (Fig. 6A). Of the 44 total isolates, 6 were not *C. albicans*, 1 (an isolate of the smooth colony phenotype from sample P20) gave a highly diminished band pattern, and 1 (from sample C16) was *C. albicans* but was uninterpretable. The mean S_{AB} for the remaining 36 *C. albicans* isolates computed from the matrix of S_{AB}s was 0.65 ± 0.10. Of these, 21 (58%) clustered into four groups with S_{AB}s of ≥0.80, with one cluster containing 12 isolates (cluster a), two clusters containing 3 isolates each (clusters b and c), and one cluster containing 2 isolates (cluster d). Cluster a contained all isolates in the major cluster in the dendrogram of the basic set of commensal isolates (Fig. 5A) and the major cluster in the dendrogram of the basic set of pathogenic isolates (Fig. 5C). Finally, a combined dendrogram was generated for all basic and additional commensal and pathogenic isolates (Fig. 6B). The

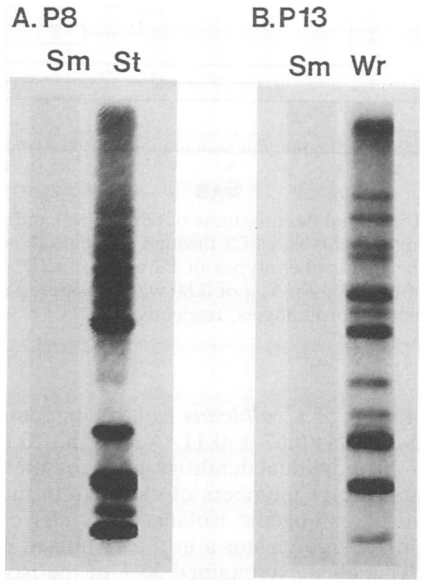

FIG. 4. Dissimilar Southern blot hybridization patterns of Ca3-probed *Eco*RI-digested DNAs of multiple colony phenotypes from the same individuals. For both P8 and P13, the smooth (Sm) samples did not hybridize to Ca3. In both cases, they biotyped as *S. cerevisiae* by their sugar assimilation patterns. In both cases, strains of the variant phenotypes hybridized to Ca3 and biotyped as *C. albicans* by their sugar assimilation patterns. Sm, smooth colony morphology; St, star colony morphology; Wr, wrinkled colony morphology. See Fig. 1 for examples of the different colony morphologies.

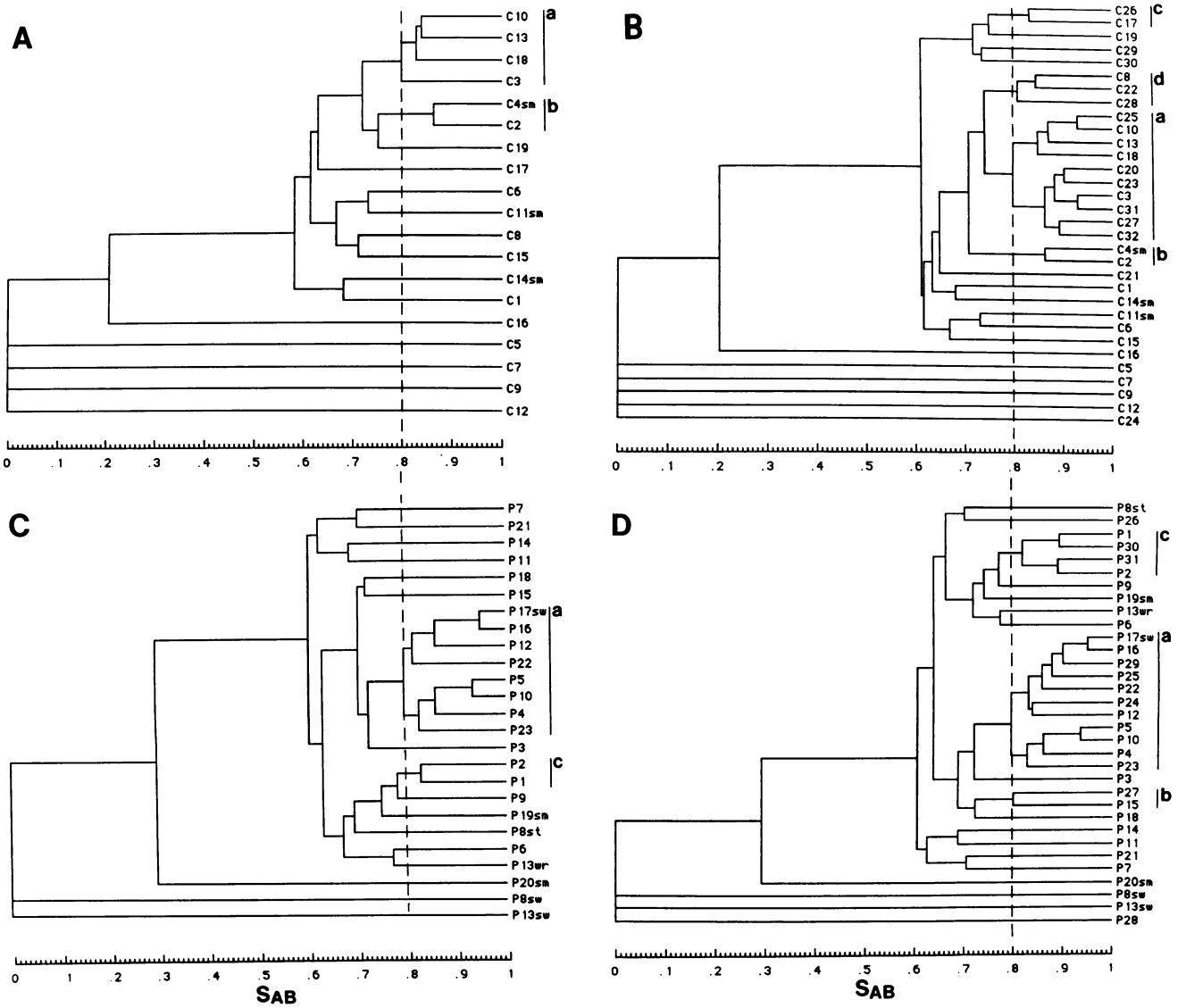

FIG. 5. Individual dendrograms of commensal and pathogenic isolates. (A) Basic group of commensal isolates C1 through C19; (B) basic group of commensal isolates C1 through C19 plus 13 additional oral commensal isolates C20 to C32; (C) pathogenic isolates P1 through P23 (including the minor phenotypes of P8 and P13); (D) basic group of pathogenic isolates P1 through P23 plus 8 additional oral pathogenic isolates (P24 to P31). An S_{AB} of 0.80 was considered the threshold for a significant degree of similarity. Sm, st, and wr, smooth, star, and wrinkled colony morphologies, respectively.

mean S_{AB} for the 55 *C. albicans* isolates computed from the matrix of S_{AB}s was 0.67 ± 0.11. Again, the 10 members of cluster a in the individual dendrogram of commensal isolates (Fig. 5B) and the 11 members of cluster a in the individual dendrogram of pathogenic isolates (Fig. 5D) combined to form the single large cluster a in the combined dendrogram (Fig. 6B). This cluster contained 38% of the isolates which typed as *C. albicans*. When the stringency for relatedness was raised from an S_{AB} of 0.80 to an S_{AB} of 0.85, a value which has been used in previous studies of strain relatedness with the Ca3 probe (17, 18, 23), cluster a subdivided into three major clusters of three or more isolates (Fig. 6B). Each of these clusters contained random mixtures of commensal and pathogenic strains.

Minor clusters in the individual dendrograms also mixed in the combined dendrograms (Fig. 6B). Cluster b in the combined dendrogram (Fig. 6B) contained the two commensal isolates in cluster b of the individual dendrogram in Fig. 5B and the two pathogenic isolates in cluster b of the individual dendrogram in Fig. 5D. Cluster c in the combined dendrogram (Fig. 6B) contained the two commensal isolates in cluster c of the individual dendrogram in Fig. 5B and the four pathogenic isolates of cluster c in the individual dendrogram in Fig. 5D. Finally, cluster d in the combined dendrogram (Fig. 6B) contained the three commensal isolates of cluster d in the individual dendrogram (Fig. 5B) and one new pathogenic isolate.

DISCUSSION

We first examined the phenotypic variability and genetic similarity of a basic set of 19 commensal isolates and a basic

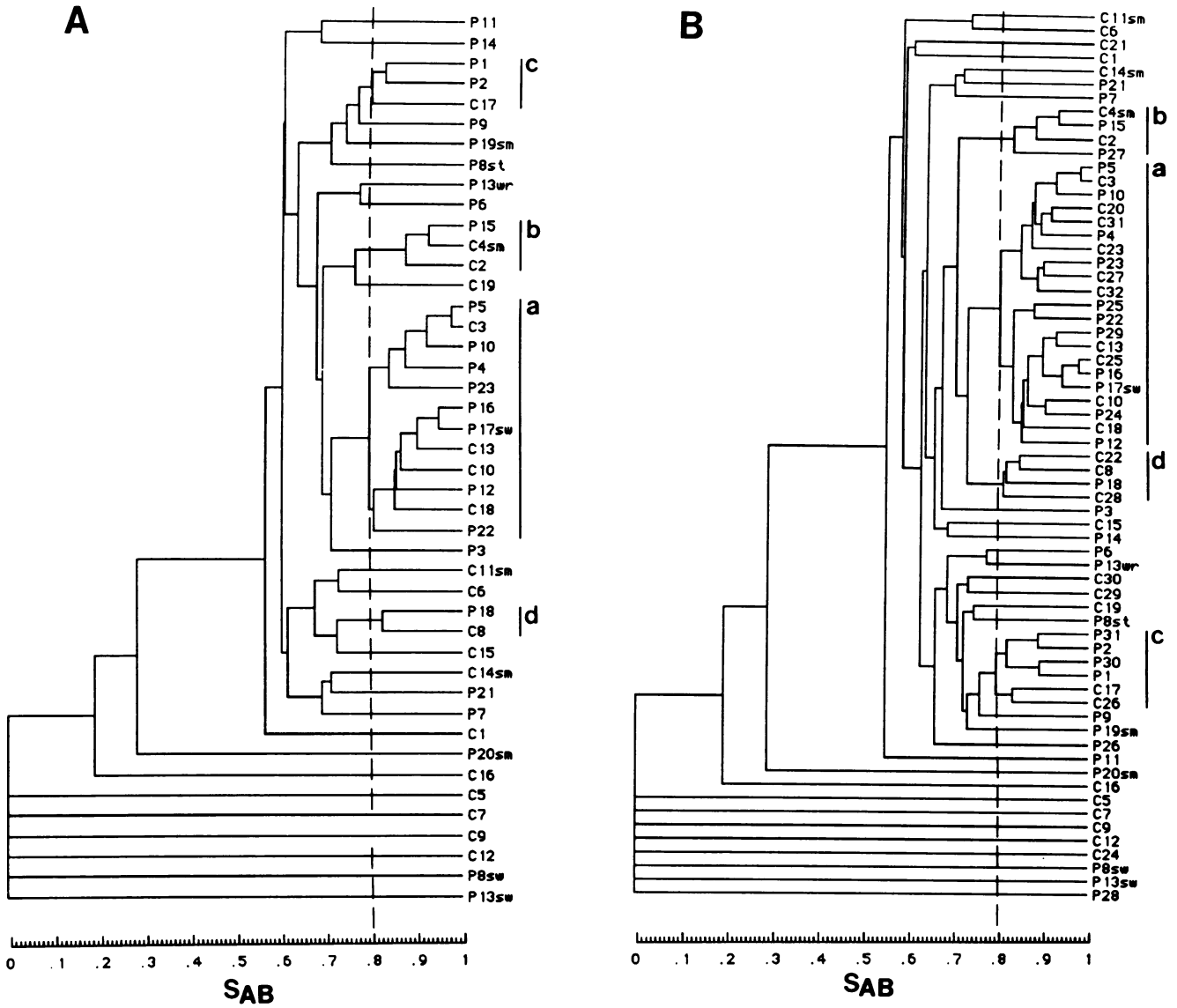

FIG. 6. Combined dendrograms of commensal and pathogenic strains of *C. albicans*. (A) Combined basic sets of commensal and pathogenic strains; (B) combined basic and additional sets of commensal and pathogenic strains. See legend to Fig. 5 for details. Sm, st, wr, smooth, star, and wrinkled colony morphologies, respectively.

set of 23 pathogenic isolates from the oral cavities of individuals visiting the same clinic in Iowa City over the same 1-year time period. We then expanded the analysis of genetic similarity with additional commensal and pathogenic isolates collected from the oral cavities of individuals in the Iowa City locale on average 2.5 years earlier. The study purposefully included individuals from a single geographical locale since recent results demonstrated that the major clusters in a dendrogram based on the Ca3 hybridization patterns of strains from Iowa City, Iowa, did not mix with the major clusters in a dendrogram of strains from Ann Arbor, Mich., in combined dendrograms, suggesting that genetically distinct strains predominated in the different geographical locales (18). Although the basic set of pathogens in the present study were defined as isolates from the oral cavities of individuals who displayed erythematous or pseudomembranous candidiasis, we assumed that the cloned strains were involved in inflammation or tissue destruction in each diagnosed case. It should be kept in mind that the diagnosed symptoms may involve other etiological agents, and that in some cases, *C. albicans* may represent a secondary infection or may not be involved in the infection. Therefore, a repeat of the present study with *C. albicans* isolates from infections such as yeast vaginitis, in which the diagnosis of candidiasis is firmer, is warranted. Such a study is in progress.

Of the 19 samples containing basic commensal strains, all strains in the samples were found to exhibit a predominant uniform smooth colony morphology when samples from sites of carriage were immediately plated. Three contained minor variant phenotypes, but in all cases, minor and major variant phenotypes proved to represent the same strain by DNA fingerprinting. The results were somewhat different for the basic set of 23 pathogenic strains. Twenty-one samples contained strains that exhibited a predominant uniform smooth colony phenotype, while two samples contained

strains that exhibited a predominant variant phenotype when samples from sites of infection were immediately plated. In addition, 6 of the 23 samples contained strains with a minor phenotype, and in 1 of these samples, strains of three different variant phenotypes were identified. In four of these six samples, strains with the major and minor variant phenotypes proved to be the same strain by DNA fingerprinting, but in two samples, the predominant phenotype of the strain was *S. cerevisiae* and the minor phenotype of the strain was *C. albicans*. Therefore, only 16% of samples of commensal strains contained the variant phenotypes (i.e., other than o-smooth), while 35% of pathogenic samples of pathogenic strains contained one or more variant phenotypes. These results suggest that pathogenic strains, on average, exhibit more phenotypic variability at sites of infection than commensal strains at sites of carriage. We assume that the increase in the frequency of variant colony morphologies in pathogenic strains is a result of an increase in the frequency of phenotypic switching (21).

Although the basic group of pathogenic isolates exhibited, on average, more phenotypic variability than commensal isolates, the levels of genetic diversity of each group were surprisingly similar, and the genetic similarity between the two groups was quite high. Of the 19 basic commensal isolates, 4 (21%) were species other than *C. albicans*, and of the 21 basic pathogenic isolates, 3 (13%) were other species. The S_{AB} of the 14 conventional *C. albicans* strains in the basic commensal group was 0.64 ± 0.09, and that of the 22 conventional *C. albicans* isolates in the basic pathogenic group was 0.66 ± 0.10. The S_{AB} of the expanded group of 26 commensal isolates of *C. albicans* was 0.67 ± 0.10, and that of the expanded group of 29 pathogenic isolates of *C. albicans* was 0.67 ± 0.11. Both are extremely close to each other and to the S_{AB} of 0.69 ± 0.03 for 30 unrelated isolates of *C. albicans* randomly selected from the Dendron data base. These results demonstrate that there is no reduction in genetic diversity in pathogenic isolates from the oral cavities of a group of individuals who were predominantly not immunocompromised (22 of 23). This is in contrast to a set of pathogenic isolates from a group of patients with AIDS in Leicester, England (17). This set of isolates exhibited a significant decrease in genetic diversity when compared with the genetic diversity of isolates from healthy individuals from the same geographical locale, suggesting strain replacement in the former group (17).

The similarity between commensal and pathogenic isolates from the oral cavities of individuals in Iowa City was even more evident in combined dendrograms. Isolates in the major cluster of the individual dendrogram of basic commensal strains and isolates in the major cluster of the individual dendrogram of basic pathogenic strains mixed in a combined dendrogram. The same was true when a combined dendrogram was generated for the expanded groups of commensal and pathogenic strains. The major cluster in the latter dendrogram contained 10 commensal and 11 pathogenic isolates, representing 38% of all *C. albicans* isolates. These results suggest a common clonal origin in the major cluster of commensal and pathogenic strains within the geographical locale of Iowa City. In addition, two of the three minor clusters (clusters b and c in Fig. 6B) in individual dendrograms mixed in the combined dendrogram, again supporting the conclusion that highly related groups of commensal and pathogenic strains share common clonal origins.

Since *C. albicans* is a diploid organism with no known sexual cycle (14, 26) and, when tested, has been found to carry balanced lethal alleles (27), it is highly unlikely that meiosis is a source of genetic diversity in a *C. albicans* population. In addition, there has been no indication of a natural transformation system. Therefore, the progeny of a cell represents a completely independent lineage in time and is immediately in direct competition with the progeny of every other cell for commensal and pathogenic niches. New alleles which provide a competitive edge are not shared horizontally in the population through mating or transformation, and more competitive or more adapted clones will continually become enriched in a geographical locale and less competitive clones will disappear. The capacity to assess genetic relatedness by fingerprinting strains with the Ca3 probe provides us with a method for monitoring, over time, the enrichment of competitive strains in a particular geographical locale. The commensal and pathogenic strains in cluster a in the combined dendrogram in Fig. 6B appear to have evolved from a single progenitor which provided its progeny with a distinct advantage over other strains for both commensalism and pathogenesis. The rate at which a particular strain becomes enriched in a geographical locale, like Iowa City, has not yet been assessed, but the capacity to compare every new strain with every previous strain analyzed and stored in the Dendron data base will allow us to obtain a rough estimate. In the present study, the basic sets of commensal and pathogen strains were collected on average 2.5 years after the additional sets were collected. Of the 38 combined basic commensal and pathogenic strains of *C. albicans* analyzed, 32% were in cluster a. Of the 19 additional commensal and pathogenic strains collected earlier, 47% were in cluster a. If a subsequent analysis of commensal and pathogenic strains of the oral cavity demonstrates further diminution of isolates in cluster a, it will indicate that new strains are being enriched in the Iowa City locale; these strains are more competitive than the commensal and pathogenic strains in cluster a. This third analysis is in progress.

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