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Genetic Dissimilarity of Commensal Strains of *Candida* spp. Carried in Different Anatomical Locations of the Same Healthy Women

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***Candida* spp. carriage and strain relatedness were assessed in 52 healthy women at 17 anatomical locations by using an isolation procedure which assesses carriage intensity and by using a computer-assisted DNA fingerprinting system which computes genetic similarity between strains on the basis of the patterns of Southern blots probed with the moderately repetitive sequence Ca3. *Candida* spp. were cultured from 73% of the test individuals, most frequently from the oral (56%), vulvovaginal (40%), and anorectal (24%) regions. Half of the test individuals with *Candida* spp. carried the organism simultaneously in more than one of the three general areas of carriage. Isolates from different body locations of the same individual were either completely unrelated, identical, or highly similar but nonidentical. In 11 cases in which *Candida* spp. were simultaneously isolated from the oral cavity and vaginal canal, seven pairs of isolates were genetically unrelated and four pairs were similar but nonidentical. In the latter cases, the isolate pairs each appear to have arisen by genetic divergence from a single progenitor. A comparison of the genetic relatedness of isolates from different individuals further uncovered a single strain which was vaginospecific in the Iowa City, Iowa area and reduced genetic diversity among vulvovaginal strains compared with those isolated from other body locations. These results suggest that strains adapt to different anatomical locations and, conversely, that in a healthy individual there is anatomical selection of vaginotropic, anotropic, and orotropic strains of *Candida* spp.**

It has been generally assumed that the infecting strain in most cases of vaginal candidiasis originates from the commensal strain inhabiting the vaginal canal, oral cavity, or gastrointestinal tract prior to infection. We have, therefore, tended to consider vaginal candidiasis more a problem of the physiological state of the host (5, 7, 12, 20, 29) and less a problem of strain specificity. In early studies employing the resistogram method for comparing strain relatedness, these assumptions were reinforced by results which suggested that each patient carried one biotypically homogeneous strain in different anatomical locations (8, 28). However, in a recent study by Odds and coworkers (11), it was demonstrated that in 58% of the cases in which vaginal and oral isolates of *Candida* spp. were obtained simultaneously from the same asymptomatic patients, each pair of isolates exhibited different biotypes. These results suggested that in the healthy female, different strains may be carried in different anatomical locations. Unfortunately, biotyping is based on phenotype rather than genotype (9, 22, 23), and therefore it runs the risk of grouping isolates which are phenotypically similar but genotypically dissimilar and separating isolates which are phenotypically dissimilar but genotypically similar. This problem is compounded by high-frequency switching (18, 21, 24), which can reversibly alter several biotyping parameters, including patterns of sugar assimilation (22), susceptibility to antifungal drugs (17, 24, 25), and the environmental constraints on hypha formation (1). Therefore, we have examined *Candida* spp. carriage and the relatedness of isolates from different anatomical locations of 52 asymptomatic women by using isolation procedures which assess carriage intensity (25, 26, 27) and by using computer-assisted DNA fingerprinting techniques which compute genetic similarity on the basis of the banding patterns of Southern blots of *Eco*RI-digested DNA probed with the moderately repetitive sequence Ca3 (13, 16, 22, 23). In addition to information on the frequency and intensity of commensal carriage in 17 anatomical locations, the data demonstrate that healthy women frequently carry genetically distinct strains in different anatomical locations. Isolates from different body locations of the same individual were in many cases either completely unrelated (sometimes they were different species) or highly similar but nonidentical. In these latter cases, it is likely that the genetically distinct isolates arose by genetic divergence from a single progenitor. A comparison of genetic relatedness of isolates from different individuals further uncovered a single strain which was vaginospecific in the Iowa City, Iowa area and demonstrated reduced genetic diversity among vulvovaginal isolates compared with isolates from other body locations. Together, these data suggest for the first time at a genetic rather than phenotypic level of analysis that the vaginal, oral, and anorectal environments select for vaginotropic, orotropic, and anotropic strains, respectively (i.e., for strains that have a selective advantage at one specific body location) and that strain specificity may indeed play a role in vulvovaginal, oral, and anorectal carriage.

MATERIALS AND METHODS

Patient pool and sampling procedure. Fifty-two healthy women were recruited from the general university community of Iowa City. Women who had suffered from one or more episodes of vaginal or oral candidiasis in the 6-month period preceding sampling were omitted from the study, as were women with symptoms of vaginal infection. The mean age of the sampled population was 28 years (± standard deviation [SD], 11 years). No known sister-sister, mother-sister, or cohabitation relationships were identified in subjects with carriage. Sampling was performed in the Obstetrics and Gynecology Clinic at the University of Iowa hospitals and clinics by the same physician to ensure sam-

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pling uniformity. Test individuals were requested to refrain from bathing, brushing their teeth, or using deodorant for the 24-h period preceding sampling. Samples were taken from the following anatomical locations: ear (e), inner cheek (ch), under tongue (ut), back of tongue (bt), under arm (ua), nipple (n), under breast (ub), navel (na), pubic hair line (phl), groin (g), vulva (vu), vaginal wall (vw), vaginal pool (vp), anus (an), rectum (stool) (st), under knee (uk), and between toes (bt). Samples are presented in the text according to patient code (hp1 to hp52) and body location. For example, hp2vw refers to the vaginal wall isolate of patient hp2. In sampling all anatomical locations except the rectum, a Culturette (Marion Scientific, Kansas City, Mo.) was moistened with the encapsulated modified Stuart's transport medium according to the manufacturer's directions and gently rubbed across the sampling surface three times or, in the case of the vaginal pool, passed through the vaginal fluid in the posterior fornix pool. In the case of rectal samples, a sterile gloved finger was rubbed across the rectal wall, and the gloved finger was swabbed with a Culturette. Culturettes were immediately reinserted into plastic encasings after sampling. All anatomical sites were checked by the physician for pathology. In four cases, women were excluded from the study because of vaginal or oral infections. Culturettes were transported at room temperature in insulated boxes to the microbiology laboratory within 1.5 h of sampling and immediately plated.

Plating of samples. Each culturette tip was immersed in 0.5 ml of sterile water and rigorously mixed, and 0.1 ml of the sample suspension was spread on each of three agar plates (100 by 15 mm, 2% agar) containing the amino acid-rich, defined medium of Lee et al. (6) supplemented with 70 μg of arginine per ml and 9 μM zinc (3). Cultures were incubated for 7 days at 25°C and then assessed for the number of *Candida* colonies per three plates. The relative level of *Candida* carriage at a particular body location was categorized as (i) negative (0 colonies per three plates), (ii) very low (1 to 4 colonies per three plates), (iii) low (5 to 20 colonies per three plates), (iv) medium (21 to 200 colonies per three plates), and (v) high (over 200 colonies per three plates). The relative intensity of carriage was quantified by scoring very low (VL), low (L), medium (M), and high (H) carriage as 1, 2, 3, and 4, respectively. Representative clones from the original cultures were streaked on agar slants in air-tight tubes and stored at room temperature for later use.

DNA fingerprinting, calculation of S_{AB}, and genesis of dendrograms. The methods of Schmid et al. (16) were employed for DNA fingerprinting. In brief, cells from each storage streak were inoculated into 20 ml of YPD medium (2% dextrose, 2% Bacto-Peptone, and 1% yeast extract) (2) in 125-ml Erlenmeyer flasks and rotated at 200 rpm at 37°C. Cells were harvested at early stationary phase, and DNA was prepared according to the methods of Scherer and Stevens (14). DNA was measured by ethidium bromide dot quantitation, digested with *Eco*RI enzyme, and separated on a 0.8% (wt/vol) agarose gel. DNA was then transferred to nitrocellulose membrane and hybridized with the nick-translated probe λCa3 (13, 16, 22, 23, 25, 26, 27). Hybridization membranes were washed and pressed against XAR-S film (Eastman Kodak Co., Rochester, N.Y.) with a Cronex Lightning-Plus (Du Pont Co., Wilmington, Del.) intensifying screen in a light-proof chamber.

Southern blot patterns of different isolates were compared with results obtained from the Dendron program, described in detail in a previous communication (16). Band intensities were scored as 0 (no band), 1 (low intensity), 2 (medium intensity) and 3 (high intensity) (16). The similarity coefficient (S_{AB}) between the patterns of two isolates, A and B, was calculated by the following formula:

$$S_{AB} = \sum_{i=1}^{k} [a_i + b_i - | a_i - b_i |] / \sum_{i=1}^{k} (a_i + b_i),$$

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. If the banding patterns of A and B are identical (i.e., all bands are the same sizes and intensities), the S_{AB} will be 1.0; if the banding patterns are totally dissimilar (i.e., no bands are the same size), the S_{AB} will be 0.0. In a previous assessment of the reproducibility of this typing method (16), we found that S_{AB}s computed for repeat patterns of the same strains were 0.96 ± 0.02, providing us with a practical estimate of identicalness. However, highly similar but nonidentical patterns differing by more than 1 intensity unit at one or more bands of equal molecular weight, or differing in the position of a low-intensity band, can have S_{AB}s of ≥0.96. The average S_{AB} value for unrelated strains of *Candida albicans* has been estimated to be roughly 0.69 (16). Dendrograms based on S_{AB} values were then generated through the Dendron program (16) by the unweighted pair group method (19). Clustering patterns in dendrograms were confirmed by creating several dendrograms after randomizing the order of strains.

Species typing by sugar assimilation patterns. Select isolates were typed for species by sugar assimilation patterns with a commercial kit (API 20C) purchased from Analytab Products (Plainview, N.Y.). In all cases, typing was performed twice.

RESULTS

Carriage in 17 body locations. Seventy-three percent of the 52 subjects carried *Candida* spp. in one or more body locations (Table 1). *Candida* spp. were not cultured from the ear, underarm, or nipple. They were cultured only once from under the breast (hp39), the navel (hp23), and the back of the knee (hp27). In the latter two cases, the intensity of carriage was very low.

Forty percent of subjects carried *Candida* spp. in one or more genital locations (vulva, vaginal pool, vaginal wall) (Table 1). The average intensity scores for vaginal wall, vaginal pool, and vulva were 3.2, 2.9, and 2.7, respectively. These scores were higher than those for all other tested anatomical locations (see bottom line in Table 1). Since the vaginal wall and pool are contiguous, one would expect simultaneous carriage, and this was true in 13 of 18 cases. In the majority of cases in which carriage was demonstrated for the pool or wall, but not both, the levels were low. In 31% of tested subjects, *Candida* spp. were cultured from the vulva. In three cases (hp7, hp19, hp24), *Candida* spp. were cultured from the vulva at reasonably high levels (H, M, and L, respectively) but were not cultured from the vaginal wall or pool, and in two cases (hp21, hp52) *Candida* spp. were cultured from the vaginal wall or pool but not from the vulva, again at reasonably high levels (H and M, respectively) (Table 1). In five cases, *Candida* spp. were cultured from the groin (Table 1). In four of these five cases, carriage in the groin was accompanied by carriage of equal or greater intensity in both the vaginal canal and vulvar region, suggesting that the latter two sites were the primary sites of carriage. Carriage at the pubic hair line was demonstrated in

TABLE 1. *Candida* carriage in 52 healthy, predominantly young women[a]

Individual	Carriage in:											Oral carriage	Anorectal carriage	Vulvo-vaginal carriage	Any carriage
	Vaginal wall	Vaginal pool	Vulva	Groin	Anus	Rectum (stool)	Under breast	Inner cheek	Under tongue	Back of tongue	Between toes				
hp2	H	H	ND[b]		VL	ND			L	M	ND	+	+	+	+
hp3			ND			ND		VL			ND	+	−	−	+
hp4			ND			ND				L	ND	+	−	−	+
hp5						ND				VL	ND	+	−	−	+
hp6					L	ND		VL	L		ND	+	+	−	+
hp7			H[c]		H[c]	ND					ND	−	+	+	+
hp9						ND			VL	L	ND	+	−	−	+
hp10						ND				VL	ND	+	−	−	+
hp11	H	H	H		H	ND					ND	−	+	+	+
hp12					VL	ND		L		M	ND	+	+	−	+
hp13	L	L	L			ND				L	ND	+	−	+	+
hp15			ND			ND				L	ND	+	−	−	+
hp17					ND	ND		VL	VL	VL		+	ND	−	+
hp19			M		ND	ND				L		+	ND	+	+
hp20		VL										−	−	+	+
hp21	H	H										−	−	+	+
hp22	H	H	H		L	VL		L	VL	L		+	+	+	+
hp24			L									−	−	+	+
hp26									VL			+	−	−	+
hp27	H	H	H	H	M	VL		L			ND	+	+	+	+
hp28	L					M		M	VL	H		+	+	+	+
hp29	M	VL	VL					H	VL	L		+	−	+	+
hp31		M	VL							L		+	−	+	+
hp33	H	H	H	VL	VL	VL		H	VL	L		+	+	+	+
hp34										M		+	−	−	+
hp36	H	L	M					M	L	M	VL	+	−	+	+
hp37		VL						VL		VL		+	−	+	+
hp39	H	M	L				H	VL	VL	VL	L	+	−	+	+
hp41				VL				VL	L	L		+	−	−	+
hp42	VL		VL	VL							VL	−	−	+	+
hp43					VL	VL		VL				+	+	−	+
hp44								VL	VL	L		+	−	−	+
hp47										VL		+	−	−	+
hp48					VL			VL	VL	VL		+	+	−	+
hp49	H	H	L	VL	VL			VL	VL	VL		+	+	+	+
hp50	VL	L	H									−	−	+	+
hp52	M	M										−	−	+	+
Percentage	29	31	31	10	22	15	2	33	29	46	8	56	24	40	73[d]
Intensity score[e]	3.2	2.9	2.7	1.6	1.9	1.4		1.8	1.3	1.9	1.3				

[a] Data are presented only for the 37 women with carriage at 1 or more of the 11 tested body locations. Fourteen women had no detectable carriage. No *Candida* isolates were obtained from underarm, nipple, and ear. *Candida* spp. were isolated in only one case from the navel, under the breast, and the back of the knee and in only two cases from the pubic hair line. These body locations were not included in the table. Colonization levels (VL, L, M, H) are explained in Materials and Methods; blank space indicates that carriage was not detected.

[b] ND, not determined.

[c] Reculture negative; original colonies small.

[d] This computation includes hp23, who carried *Candida* spp. in the navel at a VL level.

[e] The intensity score for carriage was calculated only when three or more individuals carried *Candida* spp. in a particular anatomical location. Intensity was scored as VL, 1.0; L, 2.0; M, 3.0; H, 4.0.

only two test individuals, and in both cases, the intensity of carriage was VL and accompanied by carriage at high intensity in vulvovaginal regions.

Twenty-two percent of test subjects carried *Candida* spp. in the anus, and 15% carried *Candida* spp. in the rectum (stool) (note that only 33 of the 52 test individuals were sampled for rectal carriage; Table 1). The average intensities of carriage were 1.9 and 1.4, respectively, significantly lower than the average intensities in the vulvovaginal regions (Table 1).

Fifty-six percent of subjects carried *Candida* spp. in the oral cavity (Table 1). Of the three oral locations sampled (inner cheek, back of tongue, and under tongue), carriage was most frequent in the region in back of the tongue. The average intensity of carriage for all three locations was relatively low (1.8 for the inner cheek, 1.3 for under the tongue, and 1.9 for the back of the tongue), although intensity was high in a few individual cases (e.g., hp28 and hp29).

Candida spp. were cultured simultaneously from at least two of the three major areas of colonization (vulvovaginal, anorectal, and oral) in 37% of subjects and from all three areas in 12% of subjects (Table 1). Half of all individuals with any carriage exhibited carriage in two or more areas. The same proportion of subjects with or without vulvovaginal carriage also exhibited oral carriage, but three times as many subjects with vulvovaginal carriage also exhibited anorectal carriage than did subjects without vulvovaginal carriage

TABLE 2. Relationship of carriage in one region to carriage in another region

Region	% Carriage[a]		
	Anorectal	Oral	Vaginal
Vulvovaginal			
Carriage	42 (8/19)	62 (13/21)	
No carriage	13 (4/31)	52 (16/31)	
Anorectal			
Carriage		83 (10/12)	67 (8/12)
No carriage		45 (17/38)	32 (12/38)
Oral			
Carriage	37 (10/27)		48 (14/29)
No carriage	9 (2/23)		35 (8/23)

[a] The number of positive tests per test group is presented in parentheses.

(Table 2). Twice as many subjects with anorectal carriage exhibited vulvovaginal and oral carriage than did subjects without anorectal carriage (Table 2). The same proportion of subjects with or without oral carriage exhibited vulvovaginal carriage, but four times as many subjects with oral carriage exhibited anorectal carriage than did subjects without oral carriage (Table 2).

Fingerprinting with the moderately repetitive sequence Ca3. A representative Southern blot for 12 *C. albicans* isolates and the reference strain 3153A is presented in Fig. 1. The Southern blot hybridization pattern of each strain contains between 10 and 20 bands of varying intensity in the size range of 1.7 to 19 kb. In Fig. 2, a dendrogram based on S_{AB} values has been generated for 50 fingerprinted *C. albicans* isolates obtained from 21 subjects. Five additional fingerprinted isolates (hp2vw, hp2vu, hp13bt, hp21vp, and hp48an) yielded no significant hybridization signal when probed with Ca3 and, therefore, were not included. These isolates also typed as species other than *C. albicans* by sugar assimilation patterns. The majority of isolates with S_{AB}s of 0.95 or greater in the dendrogram in Fig. 2 are from the same individuals. Figure 1 contains the following four examples of isolates from different anatomical locations of the same individuals with S_{AB}s greater than 0.95: (i) hp11an and hp11vw, (ii) hp13vu and hp13vw, (iii) hp22st and hp22bt, and (iv) hp33bt and hp33an. The patterns of the first two pairs were identical both for band position and relative intensity, even when gel loading was obviously not identical (e.g., hp11an and hp11vw); patterns of the remaining two pairs differed by only one and two faint bands, respectively. The high level of similarity between isolates from different body locations of the same individual was also evident when the average S_{AB} for pairs of isolates from different body locations of the same individual was compared with the average S_{AB} for pairs of isolates from the same body location of different individuals. In the former case, the average S_{AB} was 0.87 ± 0.15 (mean ± SD, n = 15 strains). In the latter cases, the average S_{AB} was 0.67 ± 0.11 (n = 18) for isolates from the oral cavity, 0.59 ± 0.17 (n = 10) for isolates from

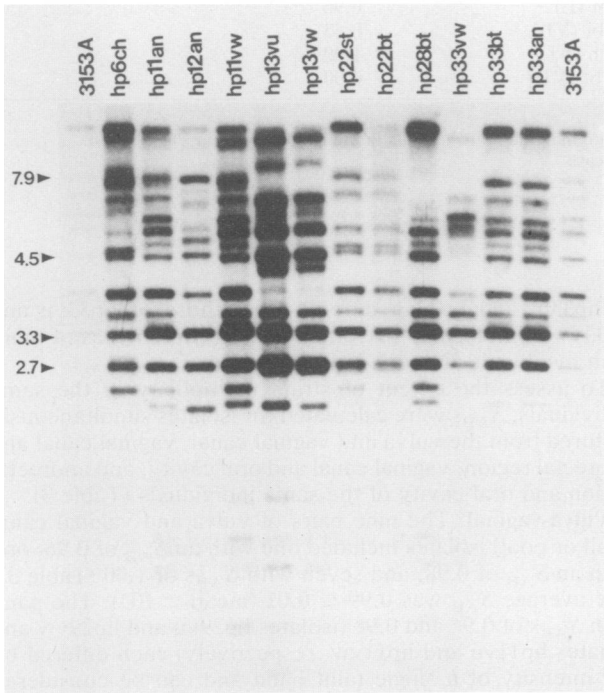

FIG. 1. Southern blot hybridization patterns of *Eco*RI-digested whole cell DNA probed with the moderately repetitive sequence Ca3. 3153A is the common laboratory reference strain. The nomenclature of *C. albicans* isolates is explained in Materials and Methods. Molecular masses in kilobases are noted to the left of the blot.

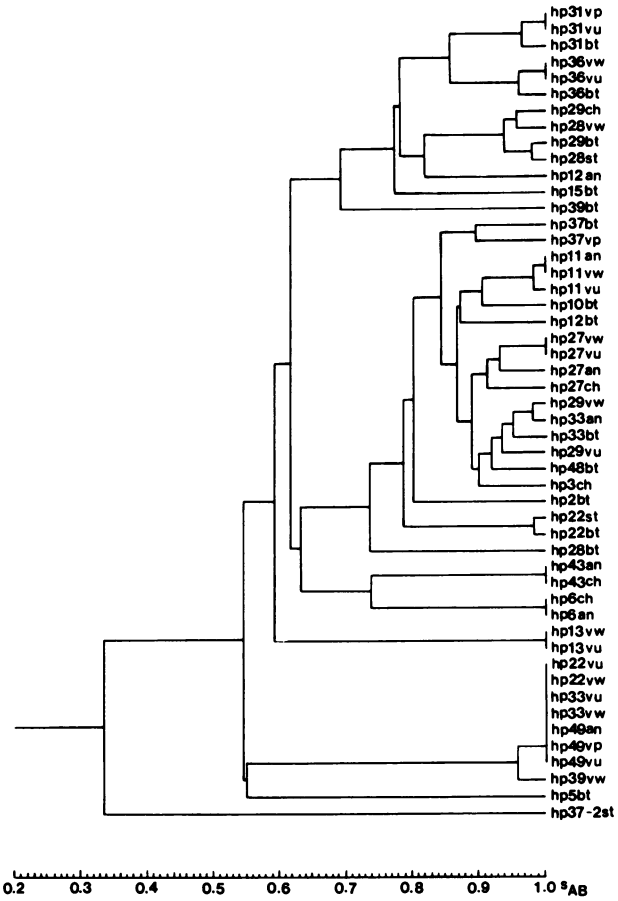

FIG. 2. A composite dendrogram based on the S_{AB}s of *C. albicans* isolates from 21 test individuals fingerprinted with the moderately repetitive sequence Ca3. Five fingerprinted isolates exhibited no signal with the probe Ca3 and were therefore not included in this dendrogram. An explanation of dendrogram genesis is provided in Materials and Methods.

TABLE 3. S_{AB} values for pairs of strains from different anatomical locations of the same individual

Location	Individual	Strains[a]	S_{AB}[b]	Mean ± SD (*n*)
Vulva-vaginal canal	hp11	vu (H), vw (H)	0.98	0.99 ± 0.01 (9)
	hp13	vu (L), vw (L)	1.00	
	hp22	vu (H), vw (H)	1.00	
	hp27	vu (H), vw (H)	1.00	
	hp29	vu (VL), vw (M)	0.96	
	hp31	vu (VL), vp (M)	1.00	
	hp33	vu (H), vw (H)	1.00	
	hp36	vu (M), vw (H)	1.00	
	hp49	vu (L), vw (H)	1.00	
Vaginal canal-anorectal	hp11	vw (H), an (H)	1.00	0.80 ± 0.22 (7)
	hp22	vw (H), an (L)	0.65	
	hp27	vw (H), an (M)	0.93	
	hp28	vw (L), st (M)	0.96	
	hp33	vw (H), an (VL)	0.65	
	hp37[c]	vp (VL), st-2 (—)[f]	0.44	
	hp49	vp (H), an (VL)	1.00	
Vaginal canal-oral	hp2	vw (H),[d] bt (M)	0.00	0.62 ± 0.34 (11),
	hp13	vw (L), bt (L)[d]	0.00	0.76 ± 0.17[e] (9)
	hp22	vw (H), bt (L)	0.67	
	hp27	vw (H), ch (L)	0.91	
	hp28	vw (L), bt (H)	0.67	
	hp29	vw (M), ch (H)	0.65	
	hp31	vp (M), bt (L)	0.96	
	hp33	vw (H), bt (L)	0.63	
	hp36	vw (H), bt (M)	0.96	
	hp37	vp (VL), bt (VL)	0.90	
	hp39	vw (H), bt (VL)	0.50	
Anorectal-oral	hp6	an (L), ch (VL)	1.00	0.73 ± 0.35 (9),
	hp12	an (VL), bt (M)	0.74	0.80 ± 0.20[e] (8)
	hp22	st (VL), bt (L)	0.98	
	hp27	an (M), ch (L)	0.91	
	hp28	st (M), bt (H)	0.68	
	hp33	an (VL), bt (L)	0.96	
	hp37[c]	st-2 (—),[f] bt (VL)	0.33	
	hp43	an (VL), ch (VL)	1.00	
	hp48	an (VL),[d] bt (VL)	0.00	

[a] Carriage intensity is given in parentheses.
[b] The S_{AB} values were calculated for pairs of strains and are therefore more accurate than the branch points in the dendrograms in Fig. 2 and 4.
[c] Stool sample was taken 2 weeks later than the back-of-tongue sample in this one case.
[d] Strains are not *C. alibcans* and therefore do not hybridize with Ca3.
[e] Calculated only for *C. albicans* strains.
[f] —, intensity was not determined.

the anorectal region, 0.65 ± 0.17 (n = 12) for isolates from the vaginal canal, and 0.64 ± 0.17 (n = 9) for isolates from the vulvar region. A t test was used to demonstrate that the average S_{AB} value for multiple isolates from the same individual was significantly higher than the average S_{AB} values for isolates from the same body locations of individual patients (P <0.01 to <0.001).

Genetically distinct strains are carried by the same individual in different anatomical locations. Although the majority of genetically similar or identical isolates in the dendrogram in Fig. 2 were obtained from different body locations of the same individuals, there were many examples of unrelated isolates from the same individual. For example, the oral and anal isolates from test individual hp33 (hp33bt and hp33an) exhibited an S_{AB} of 0.95 in the dendrogram in Fig. 2 and similar Southern blot hybridization patterns in Fig. 1. However, they differed from the vulvar and vaginal isolates of hp33. The S_{AB} for hp33vu and hp33vw was 1.0, but the S_{AB}s for hp33vw and hp33bt and for hp33vu and hp33an were 0.63 and 0.65, respectively (Table 3). The Southern blot patterns of hp33bt and hp33an differed from those of hp33vu and hp33vw in molecular size and/or relative intensities of more than five bands above 3.45 kb (Fig. 1).

To assess the extent of strain multiplicity in the same individuals, S_{AB}s were calculated for isolates simultaneously cultured from the vulva and vaginal canal, vaginal canal and anorectal region, vaginal canal and oral cavity, and anorectal region and oral cavity of the same individuals (Table 3).

Vulva-vaginal. The nine pairs of vulva and vaginal canal (wall or pool) isolates included one with an S_{AB} of 0.96, one with an S_{AB} of 0.98, and seven with S_{AB}s of 1.00 (Table 3). The average S_{AB} was 0.99 ± 0.01 (mean ± SD). The pairs with S_{AB}s of 0.96 and 0.98 (isolates hp29vu and hp29vw and isolates hp11vu and hp11vw, respectively) each differed by the intensity of a single faint band and can be considered identical within the limits of resolution (16). Examples of identical Southern blot hybridization patterns for vulva and vaginal wall isolates are presented in Fig. 1 (hp13vu and hp13vw) and Fig. 3 (hp27vu and hp27vw).

Vaginal-anorectal. The seven pairs of vaginal and anorec-

tal isolates included three with S_{AB}s of 0.65 or less, one with an S_{AB} of 0.93, and three with S_{AB}s above 0.95 (Table 3). The three pairs with S_{AB}s of 0.65 or less exhibited very different Southern blot hybridization patterns, and an example of this is presented in Fig. 1 (hp33vw and hp33an). The patterns of the pair with an S_{AB} value of 0.93 (hp27vw and hp27an) differed in the position of two bands (indicated by arrows in Fig. 3, panel hp27). The positions and intensities of all other bands in the patterns were identical. The S_{AB} for isolates hp28vw and hp28st was 0.96. Since the patterns differed by a moderately intense 16-kb band exclusive to the hp28st pattern, the two isolates must be considered highly similar but nonidentical. The Southern blot hybridization patterns of the remaining two pairs were identical, and an example of this is presented in Fig. 1 (hp11an and hp11vw). Therefore, of the seven pairs of vaginal and anorectal isolates simultaneously cultured from the same individuals, three were unrelated, two were similar but nonidentical, and two were identical.

Vaginal-oral. The 11 pairs of vaginal and oral (back of tongue, cheek) isolates included 2 with S_{AB}s of 0.00, 5 with S_{AB}s between 0.50 and 0.67, 2 with S_{AB}s of 0.90 and 0.91, and 2 with S_{AB}s of 0.96 (Table 3). In the two pairs with S_{AB}s of 0.00, one isolate of each pair (hp2vw and hp13bt) exhibited no significant hybridization with Ca3 and did not type as *C. albicans* by sugar assimilation pattern. In the five pairs with S_{AB}s ranging between 0.50 and 0.67, the Southern blot hybridization patterns of each pair were quite dissimilar (e.g., hp33vw and hp33bt in Fig. 1 and hp29vw and hp29ch in Fig. 3). In the four remaining pairs, with S_{AB} values of 0.90, 0.91, 0.96, and 0.96, the patterns were highly similar but nonidentical. The two pairs with S_{AB}s of 0.96 differed by the positions of at least one band of low to moderate intensity. The patterns of the latter two pairs (isolates hp31bt and hp31vp and isolates hp36bt and hp36vw) are presented in Fig. 3. Therefore, of the 11 pairs of vaginal and oral isolates simultaneously cultured from the same individuals, 7 were unrelated and 4 were similar but nonidentical.

Anorectal-oral. The nine pairs of anorectal and oral isolates included one with an S_{AB} of 0.00, three with S_{AB}s between 0.33 and 0.74, one with an S_{AB} of 0.91, two with S_{AB}s of 0.96 and 0.98, and two with S_{AB}s of 1.00 (Table 3). In the pair with an S_{AB} of 0.00 (hp48an and hp48bt), the anorectal isolate exhibited no hybridization with Ca3 and did not type as *C. albicans* by sugar assimilation pattern. Four pairs had dissimilar Southern blot hybridization patterns (S_{AB}s of 0.74 or less). hp27an and hp27ch were similar but differed by the presence or absence of two moderately intense bands and one faint band (S_{AB} = 0.91). The two pairs with S_{AB}s of 0.96 and 0.98 (isolates hp33an and hp33bt and isolates hp22st and hp22bt; Fig. 1) differed by only 1 unit in the relative intensity of one faint band each and were considered identical within the limits of resolution. The remaining two pairs (isolates hp6an and hp6ch and isolates hp43an and hp43ch) with S_{AB}s of 1.00 had identical patterns, by definition (Fig. 1). Therefore, of the nine pairs of anorectal and oral isolates simultaneously cultured from the same individuals, four were unrelated, one was similar but nonidentical, and four were identical.

Oral-vulvovaginal-anorectal. In five cases, *C. albicans* was simultaneously cultured from oral, vulvovaginal, and anorectal locations (Table 4). In two cases, hp22 and hp33, the oral and anorectal isolates were identical within the limits of resolution, while the vulvovaginal strain in each case was completely dissimilar; in one case, hp28, the vulvovaginal and anorectal isolates were highly similar, while the oral isolate in each case was dissimilar; and in one case, hp37, the oral and vulvovaginal isolates were highly similar, but the anorectal isolate was dissimilar. In only one case, hp27, the oral, vulvovaginal, and anorectal strains were all highly similar (Table 4). However, they were nonidentical, each possessing one moderately intense band that the other two did not possess.

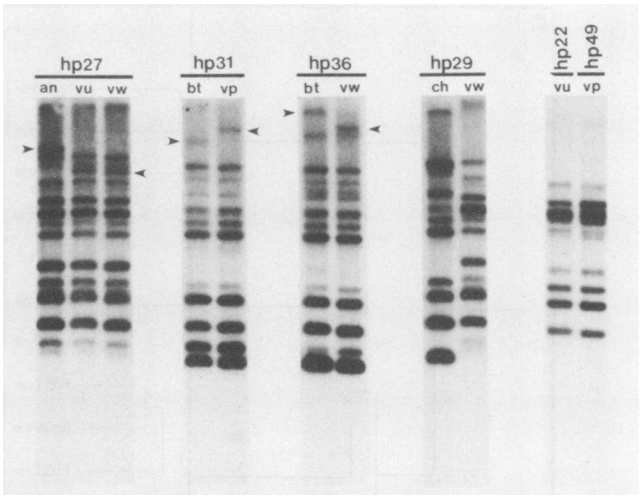

FIG. 3. Southern blot hybridization patterns of *Eco*RI-digested DNA probed with Ca3 for three sets of isolates from the same individuals which were highly similar but nonidentical (hp27an versus hp27vu or hp27vw; hp31bt versus hp31vp; hp36bt versus hp36vw), one set of strains from the same individual which were unrelated (hp29ch versus hp29vw), and one set of strains from different individuals which were identical (hp22vu versus hp49vp). Arrows point to bands which differ in location in the similar but nonidentical isolates from hp27, hp31, and hp36.

Similarity of isolates from the same anatomical locations of different individuals. In a previous analysis of fingerprinting with the Ca3 probe, we found that the average S_{AB} of a set of 46 unrelated tester strains was 0.69 ± 0.11 (mean ± SD) and that the average S_{AB} for the same strains in repeat blots was 0.96 ± 0.02. For the present study, we have arbitrarily selected an S_{AB} value of 0.85, the average S_{AB} for unrelated tester strains plus 1.5 times the SD, as a threshold for identifying clusters of genetically similar isolates. The largest cluster is located in the center of the dendrogram in Fig. 2 and includes 17 isolates from nine different individuals, beginning with hp37bt and ending with hp3ch. Seven mem-

TABLE 4. S_{AB} values for sets of simultaneous isolates from the three major anatomical regions of five subjects[a]

Individual	S_{AB}		
	Vulvovaginal-anorectal	Vulvovaginal-oral	Anorectal-oral
hp22	0.65 (vw, st)	0.67 (vw, bt)	0.98 (st, bt)
hp27	0.93 (vw, an)	0.91 (vw, ch)	0.91 (an, ch)
hp28	0.96 (vw, st)	0.67 (vw, bt)	0.68 (st, bt)
hp33	0.65 (vw, an)	0.63 (vw, bt)	0.96 (an, bt)
hp37	0.44 (vp, st)	0.90 (vp, bt)	0.33 (st, bt)

[a] S_{AB}s were calculated for pairs of strains and are therefore more accurate than the branch points in the dendrograms in Fig. 2 and 4. The isolate pairs which were compared for S_{AB} are presented in parentheses.

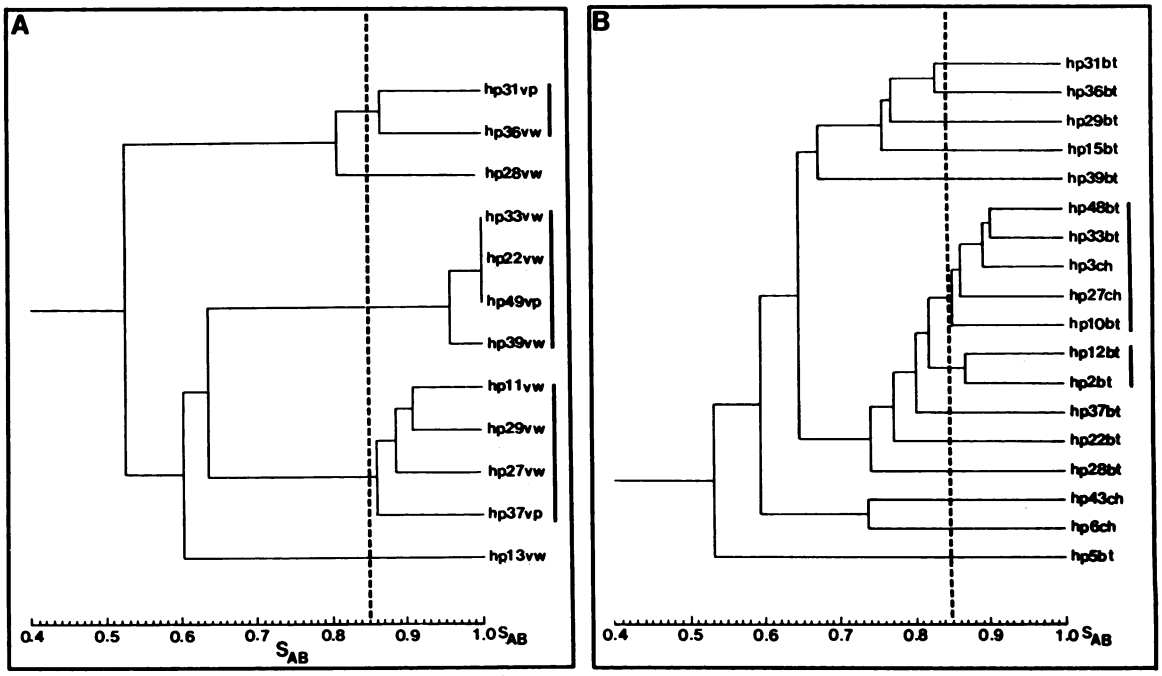

FIG. 4. Dendrograms independently generated for vaginal isolates, each from 1 of 12 test individuals (A), and for oral isolates, each from 1 of 18 test individuals (B). The vertical dashed line marks the position of $S_{AB} = 0.85$, the arbitrary threshold for clustering. Heavy vertical lines to the right of each dendrogram mark the positions of clusters.

bers of this cluster were cultured from oral sites, seven were cultured from vulvovaginal sites, and three were cultured from anorectal sites. Therefore, this major cluster includes isolates from each of the three major anatomical locations in roughly the same proportions observed in the entire collection of isolates. However, the second largest cluster, located close to the bottom of the dendrogram, includes eight isolates from four different individuals, and except for one anal isolate, members of this cluster were found solely in vulvovaginal locations. A z test was performed to test whether random sampling could account for the isolation of this strain in 7 of the 21 fingerprinted vulvovaginal isolates but in only 1 of the 29 fingerprinted anorectal and oral strains. The null hypothesis that the strain occurs in both groups with the same frequency was rejected ($P < 0.01$).

To obtain a better assessment of strain similarity as a function of anatomical location, individual dendrograms were generated for 12 isolates which were each cultured from the vulvovaginal region of a different individual (Fig. 4A) and for 18 isolates which were each cultured from the oral region of a different individual (Fig. 4B). A marked difference was observed in the degree of clustering. Ten of the 12 vulvovaginal isolates (83%) grouped into three clusters containing 2, 4, and 4 isolates, respectively (Fig. 4A). In contrast, 7 of the 18 oral isolates (39%) grouped into two clusters containing 5 and 2 strains, respectively (Fig. 4B). A similar degree of clustering was observed among anorectal isolates (data not shown). Isolates found in the vulvovaginal region are therefore derived from a much more limited number of groups (i.e., genetically less diverse) than isolates found in oral and anorectal regions. Although the vulvovaginal strains exhibited a higher degree of clustering than the oral strains, two of the three vulvovaginal clusters were related to oral clusters. Vulvovaginal isolates in the cluster which included hp11vw, hp29vw, hp27vw, and hp37vp (Fig. 4A) were members of the large central cluster in the middle of the composite dendrogram in Fig. 2, which also included the isolates in the major oral cluster in the dendrogram in Fig. 4B (hp48bt, hp33bt, hp3ch, hp27ch, and hp10bt). A second small vulvovaginal cluster which included hp31vp and hp36vw coclustered with their oral counterparts hp31bt and hp36bt (Fig. 4A and B). Therefore, the isolates in these two vulvovaginal clusters were not members of genetically distinct vaginotropic groups. However, isolates in the third cluster, which included hp33vw, hp22vw, hp49vp, and hp39vw, did appear to represent a genetically distinct vaginotropic group. Isolates of this genetically identical cluster were obtained almost exclusively from vaginal locations (Fig. 2). No isolate in this cluster was obtained from the oral cavity, and only one isolate was obtained from the anorectal region. In this latter case, the vulvovaginal region carried the same strain, and while the intensity of anal carriage was VL, the intensities of vaginal wall, vaginal pool, and vulva carriage were H, suggesting that the vulvovaginal area was the primary site of carriage. The subjects were unrelated and did not cohabit. Again, by using a z test to test whether random sampling could account for the isolation of this strain in 4 of 12 vaginal isolates but 0 of 18 oral isolates, the null hypothesis that the strain occurs in both groups with the same frequency was rejected ($P < 0.01$).

DISCUSSION

Isolates from different anatomical locations of the same individuals. Fingerprinting with the moderately repetitive genomic sequence Ca3 (13) provides a very effective method for assessing the genetic similarity of independent isolates of *C. albicans* (16). In a previous analysis of *Candida* colonization of a single-recurrence patient, we demonstrated that the same two strains persisted in their separate anatomical

locations (oral cavity versus vulvovaginal region) through three sequential episodes of vaginal candidiasis (25). Scherer and Stevens (15) noticed that isolates from different anatomical locations of two of three tested patients exhibited small differences in Southern blot hybridization patterns generated with the probe 27a. Here, we have assessed the genetic similarity of isolates from different body locations by calculating S_{AB}s (16) from Southern blot hybridization patterns generated with the probe Ca3. We found that the patterns of nine pairs of isolates from the vulva and the vaginal canals of the same women were identical. This is not an unexpected finding, given the anatomical contiguity of the two sampling sites, and these results provide us with a reference for the range of S_{AB}s for pairs of genetically identical isolates simultaneously cultured from the same individuals. In marked contrast to the pairs of vulva and vaginal canal isolates, a significant proportion of isolate pairs simultaneously cultured from the vaginal and anorectal regions, the vaginal and oral regions, and the anorectal and oral regions of the same individuals were completely unrelated. Three of 7 pairs from the vaginal and anorectal areas, 7 of 11 pairs from the vaginal and oral areas, and 4 of 9 pairs from the anorectal and oral areas were either unrelated *C. albicans* strains or different species.

Perhaps our most surprising result was that several of the remaining pairs were highly similar but nonidentical. This applied to 2 of the 7 pairs of isolates from the vaginal and anorectal areas, 4 of the 11 pairs of isolates from the vaginal and oral areas, and 1 of the 9 pairs of isolates from the anorectal and oral areas. In the most interesting situation, isolates from the vaginal, anorectal, and oral regions of one individual (hp27) were all similar but nonidentical. That these small differences in patterns did not represent artifacts of culturing or DNA digestion was demonstrated by the identical patterns obtained for the nine pairs of vulva and vaginal canal isolates.

Commensal strains in different body locations which are genetically similar but nonidentical may be the result of parallel colonization by similar strains or divergence of a single strain. The former possibility seems less likely given the general diversity of strains (16) and given the observation that 7 of the 11 strains which were simultaneously carried in the oral and vulvovaginal areas were totally dissimilar, while the remaining 4 strains were highly related (i.e., there were no S_{AB}s between 0.67 and 0.91). It seems more likely that similar but nonidentical isolates from different anatomical locations of the same individual have evolved from a single progenitor strain which has adapted to the disparate anatomical locations and is in the process of diverging genetically (4). It does not seem unreasonable to suggest that the phenotypic diversity necessary for a single strain to rapidly adapt to disparate anatomical locations is provided by high-frequency phenotypic switching (21, 22, 23).

The genetic similarity of isolates in the same anatomical locations of different individuals. Strains from the same anatomical location of different individuals, with one exception, did not group into anatomically specific clusters in the composite dendrogram in Fig. 2. Indeed, the major cluster in the center of this dendrogram included vulvovaginal, anorectal, and oral isolates. When independent dendrograms were generated for vulvovaginal and oral isolates, a higher degree of clustering was observed among the vulvovaginal isolates. However, isolates in the major vulvovaginal cluster coclustered in the composite dendrogram in Fig. 2 with isolates of the major oral cluster. Therefore, the major cluster in the center of the composite dendrogram in Fig. 2 appears to represent a highly adaptable group of genetically similar strains.

One extraordinary cluster of highly similar isolates from four test individuals appeared to be specific to the vulvovaginal region. Isolates in this cluster were cultured from the vulva and vaginal canal of three individuals and from the vaginal canal and anus of a fourth individual. In the latter case, the intensity of anal carriage was very low while the intensity of carriage in the vulvovaginal area was high, and no carriage was measured in the rectum, suggesting that primary carriage was vulvovaginal. Related isolates were not found in the oral cavity of these four individuals or in any body location of the remaining 48 women in the study. Our laboratory has now fingerprinted well over 200 isolates from the United States and Great Britain and analyzed the fingerprints with the Dendron software package. Although we previously identified a cluster of related strains in a group of male AIDS patients from Leicester, England, who were socially but not sexually interactive (10), we have never before identified a cluster of strains so highly similar from four socially and sexually noninteractive test individuals (unpublished observation). In the combined dendrogram of the 200 *C. albicans* isolates, this vulvovaginospecific cluster remained uniquely dissimilar to all other strains.

Anatomically tropic strains. The fact that different genetically nonidentical strains simultaneously inhabit different anatomical locations of the same individual suggests that there is strain adaptation and that vaginotropic, orotropic, and perhaps anorectotropic strains indeed exist. The identification of a single vulvovaginotropic strain in four unrelated females in the Iowa City area supports this conclusion. The results presented here argue strongly against the common assumption that a single commensal strain is carried by healthy individuals and complicates the assumption that a single commensal strain is the source of subsequent infection. We must now ask which commensal strain is responsible for subsequent infection, if, in fact, commensal strains are always responsible, and we must wonder whether the infecting strain is genetically similar but nonidentical to the original commensal strain. We must also consider the origin of infecting strains in women who appear to be relatively free of *Candida* spp. in the healthy state (roughly one-fourth of all women tested in this study). With the capacity to fingerprint strains and assess genetic similarity through the calculation of similarity coefficients, these questions can now be answered.

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