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Phenotypic Switching and Mating Type Switching of *Candida glabrata* at Sites of Colonization

Paula J. Brockert,[1] Salil A. Lachke,[1] Thyagarajan Srikantha,[1] Claude Pujol,[1] Rudolph Galask,[2] and David R. Soll[1]*

Department of Biological Sciences, The University of Iowa,[1] and Department of Obstetrics and Gynecology, The University of Iowa Hospitals and Clinics,[2] Iowa City, Iowa 52242

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Candida glabrata **switches spontaneously at high frequency among the following four graded phenotypes discriminated on agar containing 1 mM $CuSO_4$: white, light brown, dark brown (DB), and very dark brown.** ***C. glabrata*** **also contains three mating type loci with a configuration similar to that of the** ***Saccharomyces cerevisiae*** **mating type cassette system, suggesting it may also undergo cassette switching at the expression locus** ***MTL1*****. To analyze both reversible, high-frequency phenotypic switching and mating type switching at sites of colonization, primary samples from the oral cavities and vaginal canals of three patients suffering from** ***C. glabrata*** **vaginitis were clonally plated on agar containing $CuSO_4$. It was demonstrated that (i) in each vaginitis patient, there was only one colonizing strain; (ii) an individual could have vaginal colonization without oral colonization; (iii) phenotypic switching occurred at sites of colonization; (iv) the DB phenotype predominated at the site of infection in all three patients; (v) genetically unrelated strains switched in similar, but not identical, fashions and caused vaginal infection; (vi) different switch phenotypes of the same strain could simultaneously dominate different body locations in the same host; (vii) pathogenesis could be caused by cells in different mating type classes; and (viii) mating type switching demonstrated at both the genetic and transcription levels occurred in one host.**

The extraordinary success of *Candida albicans* as both a commensal and an opportunistic pathogen is due in part to the phenotypic plasticity it derives from three developmental programs, the bud-hypha transition (9, 15, 21), spontaneous high-frequency phenotypic switching (23, 24, 26), and a recently discovered mating system (4, 29). Until recently, however, the second most successful *Candida* pathogen, *Candida glabrata*, seemed devoid of these three developmental programs. Like *C. albicans*, *C. glabrata* is a relatively successful commensal (15), particularly in the elderly (13), and causes recurrent vaginitis (2) and bloodstream infections (16). If the developmental programs of *C. albicans* indeed play critical roles in commensalism and pathogenesis, how could *C. glabrata* mimic *C. albicans* without these programs? The answer that has emerged in the past few years is that *C. glabrata* does in fact possess similar developmental programs. Although *C. glabrata* does not form compartmentalized hyphae, it does grow in the pseudohyphal growth form (1, 7) and extends tubes up to four cell diameters in length (7). It also undergoes two forms of high-frequency phenotypic switching. First, it switches among the following four colony phenotypes, colored in a graded fashion on $CuSO_4$-containing agar: white (Wh), light brown (LB), dark brown (DB), and very dark brown (vDB) (7, 8). These switch phenotypes also show gradation in the frequency of switching, coloration on phloxine B-containing agar, expression of the metallothionein gene *MT-II* (7, 8), and expression of the mating type gene *MTL*α1 (31). This graded, high-frequency switching system is referred to as the "core" switching system. *C. glabrata* also switches reversibly and at high frequency between the smooth colony phenotypes of the core switching system and an irregular wrinkle phenotype (7). Finally, *C. glabrata* has recently been demonstrated to possess three mating type-like loci (*MTL1*, *MTL2*, and *MTL3*) with a configuration similar to that of *Saccharomyces cerevisiae* (31, 32). Of the three mating type-like loci, two (*MTL2* and *MTL3*) appear to contain silent **a** and α cassettes, respectively, and the third (*MTL1*), the putative expression locus, contains either an **a** or an α cassette that defines the mating type of the cell (i.e., either **a** or α). In *S. cerevisiae*, mating type switching involves a change in the expression cassette from **a** to α or α to **a** by gene conversion with a copy of the alternate silent donor cassette (3).

Here, we have examined whether core switching and mating type switching occurred at sites of carriage in three patients presenting with vaginal *C. glabrata* infections. In the reported studies, we tested (i) whether these patients were colonized in both the oral cavity and vagina, (ii) whether the same strain colonized both anatomical cavities, (iii) whether high-frequency phenotypic switching occurred at sites of colonization, (iv) whether a particular switch phenotype dominated sites of infection, (v) whether a single mating type dominated sites of infection, and (vi) whether mating type switching occurred in the host. The results demonstrate that switching occurs at sites of infection, that different switch phenotypes of the same strain can prevail in different anatomical locations in the same host, and that mating type switching can occur in the host.

* Corresponding author. Mailing address: Department of Biological Sciences, 302 BBE, The University of Iowa, Iowa City, IA 52242, Phone: (319) 335-1117. Fax: (319) 335-2772. E-mail: david-soll@uiowa.edu.

MATERIALS AND METHODS

Isolation and maintenance of ***C. glabrata*** **strains.** *C. glabrata* isolates were individually obtained from underneath the tongues, the inner cheeks, the vaginal

TABLE 1. Switch phenotypes of primary colonies and switching frequencies computed from secondary platings

Patient	Anatomical location[a]	Total no. of primary colonies[b]	Proportion of colony phenotypes	No. of secondary colonies plated[c]	Frequency of variant colonies	Variant colony phenotype(s)[e]
P1	T	30	100% DB	3,214	6.7×10^{-3}	vDB, LB
	VP	872	86% DB	1,542	1.1×10^{-2}	vDB, LB
			14% vDB	2,142	8.7×10^{-4}	LB
	VW	595	86% DB	2,796	2.6×10^{-2}	vDB, LB
			14% vDB	3,292	6.2×10^{-4}	LB
P2	C	0				
	T	0				
	VP	372	100% DB	2,438	4.3×10^{-2}	vDB
	VW	179	100% DB	2,591	3.2×10^{-2}	vDB, LB
P3	C	111	95% Wh[d]	4,142	3.9×10^{-3}	DB
			5% DB	1,973	2.3×10^{-3}	Wh, LB
	T	120	93% Wh	2,515	3.4×10^{-3}	DB
			7% DB	2,990	1.2×10^{-3}	Wh
	VP	581	3% Wh	2,991	4.4×10^{-3}	DB
			97% DB	1,784	5.2×10^{-3}	Wh
	VW	480	2% Wh	2,004	4.3×10^{-3}	DB
			98% DB	1,701	1.6×10^{-3}	Wh

[a] C, cheek; T, under tongue; VP, vaginal pool; VW, vaginal wall.
[b] Total number of primary colonies represents the total number on four agar plates.
[c] The cells from two primary colonies of each phenotype were mixed prior to plating.
[d] Wh could be subdivided into large (Wh1) and small (Wh2) colonies that were heritable for size. For simplicity, we have combined these categories. In both cases, they switched to DB.
[e] Where more than one variant colony phenotype was observed, the order reflects descending frequencies.

canals, and the vaginal pools of three vaginitis patients, using sterile cotton swabs (Culturette; Becton Dickinson and Co., Sparks, Md.). The three patients exhibited symptoms in the vagina but no signs of infection in the oral cavity. Each of the three patients had previously been diagnosed with *C. glabrata* infections based on the sugar assimilation patterns of the sampled yeast. For assessing the intensity of colonization and for distinguishing switch phenotypes, a strategy similar to one previously described for *C. albicans* (27) was used. Each cotton swab, containing a primary sample, was agitated in sterile water to release yeast cells. These samples were then clonally plated on YPD agar (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) plates containing 1 mM $CuSO_4$ (8). The plates were incubated at 25°C for 7 days, and the total number of colonies, as well as the proportions exhibiting the different switch phenotypes (Wh, LB, DB, and vDB), were scored. Isolates were verified as *C. glabrata* first by testing cells from primary colonies with the Vitek YBC system (bioMerieux Vitek Inc., Hazelwood, Mo.) and then by DNA fingerprinting them using Southern blot hybridization with the species-specific probe Cg6 (12). "Colonization" refers to the presence of yeast regardless of the disease state. "Carriage" refers to commensal colonization. "Infection" refers to colonization causing vaginitis symptoms.

Measurements of phenotypic switching. Measurements of the frequency of phenotypic switching of cells from primary colonies were obtained as previously described (8). Cells from a primary colony visually homogeneous for a single switch phenotype were inoculated into liquid YPD medium containing 1 mM $CuSO_4$ (YPD-$CuSO_4$) and grown at 25°C until they reached a concentration of $\sim 5 \times 10^6$ cells per ml. The cells were then diluted and evenly distributed on agar plates containing YPD-$CuSO_4$ medium at a density of 50 to 70 CFU per plate. The plates were incubated at 25°C for 5 to 7 days and then scored for the proportions of the different switch phenotypes.

DNA fingerprinting and computer-assisted analysis. DNA fingerprinting was performed according to methods previously described (12, 17, 25). Total genomic DNA from each *C. glabrata* isolate was isolated using the methods of Scherer and Stevens (18). Three micrograms of DNA was then digested overnight with *Eco*RI (4 U/μg of DNA) for 16 h at 37°C, and the resulting DNA fragments were separated in a 0.65% agarose gel at 45 mV. Digested genomic DNA of the *C. glabrata* reference strain 7549 was run in the outer two lanes of each gel to facilitate computer-assisted analysis of test strains run in the inner lanes (25). The DNA was transferred to a nylon membrane (Hybond N^+; Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England) by capillary blotting, hybridized with randomly primed [^{32}P]dCTP-labeled Cg6 probe, and autoradiographed (12).

Gel patterns were analyzed according to methods previously described (25, 28). Autoradiograms were digitized into the data file of the DENDRON software program, version 2.0, using a Scanjet II cx flatbed scanner (Hewlett-Packard, Palo Alto, Calif.). Distortions in the gels were removed with the unwarping option of DENDRON, and the lanes and bands were identified automatically. Southern blot hybridization patterns were compared using a similarity coefficient (S_{AB}) based on the band position for every pair of patterns (samples) according to the following formula: $S_{AB} = 2E/(2E + a + b)$, where E is the number of bands shared by strains A and B, a is the number of bands unique to A, and b is the number of bands unique to B (25). An S_{AB} of 1.0 represents identical patterns, and an S_{AB} of 0.00 represents patterns with no common bands. Intermediate S_{AB}s in the range of 0.01 to 0.99 represent patterns with increasing proportions of common bands. Dendrograms based on S_{AB} values were automatically generated by the DENDRON program based on the unweighted-pair-group method (20). For comparisons with collections of previously DNA-fingerprinted isolates, S_{AB}s were computed from DENDRON band data files (25).

Determination of mating type class at the genetic and transcriptional levels. Southern analysis employing specific probes was used to distinguish the three main mating type classes of *C. glabrata*: class I (**a**aα), which contains *MTL***a** genes at the *MTL1* locus; class II (**a**$\alpha\alpha$), which contains *MTL*α genes at the *MTL1* locus; and class III (**a**$\alpha\alpha$), which contains *MTL***a** genes at the *MTL1* locus (31). Genomic DNA of each *C. glabrata* isolate was digested with *Xba*I at 37°C overnight. The resulting fragments were separated in a 0.8% agarose gel, transferred to a nitrocellulose membrane (Hybond N^+), and hybridized with one of the following three radiolabeled probes: FuncP2, an antisense oligonucleotide which binds to the *MTL*α2 open reading frame (ORF), which will be referred to as the *MTL*α2 probe; a 395-bp PCR product spanning the 3′ flanking region of *MTL1***a**2, which will be referred to as the *MTL***a**2 probe; and a 300-bp PCR product unique to the *MTL***a**1 ORF, which will be referred to as the *MTL***a**1 probe (31). Northern blot analyses were performed according to methods previously described (31).

RESULTS

Colonization of the oral cavity and vaginal canal. To assess yeast colonization, the cells in primary samples collected on cotton swabs were suspended in buffer and distributed evenly over the surface of an agar plate in order to discriminate individual colonies. In patient P1, both the oral cavity and the vaginal canal were colonized (Table 1). Colonization of the vagina was more intense than that of the oral cavity, consistent

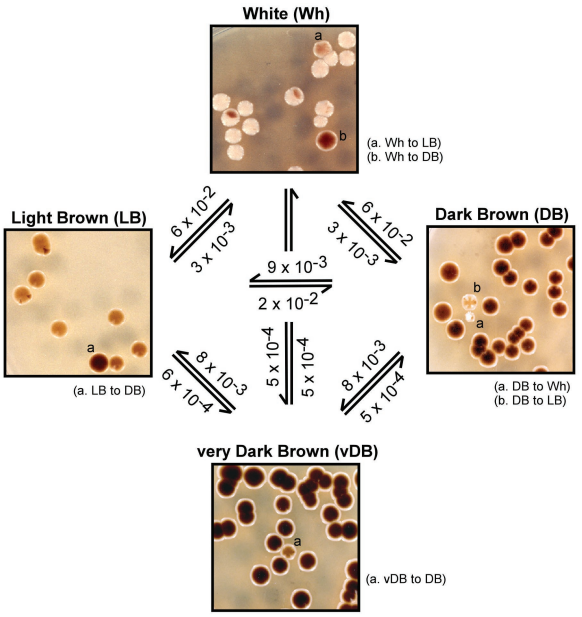

FIG. 1. Core switching system in *C. glabrata*. Cells switch spontaneously among Wh, LB, DB, and vDB phenotypes discriminated on agar containing 1 mM $CuSO_4$. In each box, low-magnification pictures of the dominant phenotypes are presented with examples of switches (a and b in parentheses). The frequencies of variant colony phenotypes are noted on the arrows pointing to those variant phenotypes.

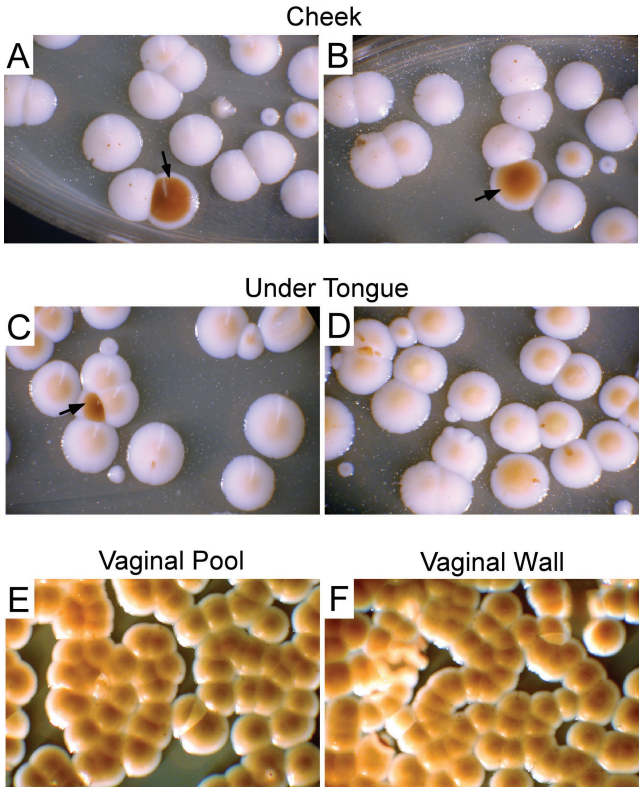

FIG. 2. For patient P3, the Wh phenotype dominated in the oral cavity (cheek and under tongue), while the DB phenotype dominated in the vaginal canal (vaginal wall and vaginal pool). The low-magnification images are of primary colonies plated directly from the original samples (swabs). (A and B) Primary colonies of cheek sample (the arrows point to minority DB colonies). (C and D) Primary colonies of under-tongue sample (the arrow points to a DB colony). (E) Primary colonies of vaginal-pool sample. (F) Primary colonies of vaginal wall sample.

with the vagina as the diagnosed site of infection. In patient P2, only the vagina was colonized (Table 1). The samples from the oral cavity contained no culturable *Candida* sp. cells (Table 1). In patient P3, both the oral cavity and the vaginal canal were colonized, as in the case of patient P1 (Table 1). Colonization of the vaginal canal again was more intense than that of the oral cavity, consistent with the vagina as the diagnosed site of infection. These results demonstrate that women suffering from a vaginal *C. glabrata* infection may or may not have concurrent oral carriage.

Switch phenotypes in primary cultures. In vitro, most strains of *C. glabrata* switch among Wh, LB, DB, and vDB colony phenotypes, discriminated on agar containing 1 mM $CuSO_4$ (Fig. 1) (7, 8). As demonstrated in the examples in Fig. 1, plating cells from a clonal colony exhibiting a single switch phenotype yields a majority of colonies with the original switch phenotype and a minority with alternative (variant) phenotypes, as shown by the frequencies of variant phenotypes presented along the arrows of interconversion.

(i) Patient P1. In the case of patient P1, 100% of the colonies in the primary culture from the cheek exhibited the DB phenotype (Table 1). In marked contrast, 86% of the colonies in the primary cultures from both the vaginal pool and the vaginal wall exhibited the DB phenotype (Table 1). In each of the last two cultures, 14% exhibited the vDB phenotype. Based upon the frequency of variants in the vaginal samples, one would have expected to find four vDB colonies in the oral sample if the switching frequencies in the populations were equivalent. Using Fisher's test, the difference between the proportions of phenotypes in the oral cavity and vagina of patient P1 proved significant ($P = 0.012$). The similarity in the proportions of DB to vDB colonies in the vaginal-pool and vaginal-wall samples (Table 1) attests to the accuracy of the assay.

(ii) Patient P2. In the case of patient P2, only the vagina was colonized. In both the vaginal-pool and vaginal-wall samples, 100% of the primary colonies exhibited the DB phenotype (Table 1).

(iii) Patient P3. In the case of patient P3, a dramatic difference was observed between the proportions of switch phenotypes in oral and vaginal isolates. Ninety-five and 93% of the primary colonies of the cheek and under-tongue samples, respectively, exhibited the Wh phenotype, and 5 and 7%, respectively, exhibited the DB phenotype (Table 1). Pictures of primary cultures from the cheek sample (Fig. 2A and B) and from the under-tongue sample (Fig. 2C and D) are presented. In marked contrast, only 3 and 2% of the primary colonies of the vaginal-pool and vaginal-wall samples, respectively, exhibited the Wh phenotype, while 97 and 98%, respectively, exhibited the DB phenotype (Table 1). Using Fisher's test, the difference between the proportions of phenotypes in the oral cavity and the vagina of patient P3 proved highly significant ($P = 2 \times 10^{-100}$). Pictures of primary cultures from the vaginal pool and vaginal wall are presented in Fig. 2E and F, respectively. Hence, while the dominant phenotype in the oral cavity of

patient P3 was Wh, the dominant phenotype in the vagina was DB.

The results of the analysis of primary colony phenotypes revealed several characteristics of *C. glabrata* colonization. First, colonizing populations can be phenotypically homogeneous or heterogeneous. Second, the DB phenotype dominated the site of infection (the vagina) in all three patients, although DB could also be found in the oral cavity. Third, the oral cavity may or may not be colonized in *C. glabrata* vaginitis patients. Fourth, different switch phenotypes can dominate the oral cavity and vagina of the same patient.

Only one strain colonizes each patient. We assumed in the plating experiments that the cells colonizing the oral cavity and the vagina in the same patient represented a single strain and hence that cells exhibiting different switch phenotypes in the same anatomical location or the same host represented the same strain. To test this assumption, cells from primary colonies sampled from different body locations of the same patient and cells from different colony phenotypes were DNA fingerprinted by Southern blot hybridization with the complex species-specific probe Cg6 (12).

(i) Patient P1. Cells originating from a primary DB colony isolated from under the tongue, from primary DB and vDB colonies isolated from the vaginal pool, and from primary DB and vDB colonies isolated from the vaginal wall of patient P1 were DNA fingerprinted. All of the isolates exhibited similar patterns, but there were minor differences (Fig. 3A). The hybridization pattern of the DB isolate from under the tongue (DB-T) exhibited two major band differences from the patterns of the vaginal-wall and vaginal-pool isolates (Fig. 3A). However, when the isolates from patient P1 were compared with the patterns of the isolates from patients P2 and P3 in a dendrogram based on similarity coefficients, the oral and vaginal isolates of patient P1, including that of DB-T, clustered (Fig. 4), and when compared with the patterns of an additional collection of unrelated *C. glabrata* isolates, all of the patient P1 isolates still clustered as a single genetically related group (Fig. 5). No isolates from the general collection penetrated this group (Fig. 5). These results demonstrate that the isolates from the oral cavity and vagina of patient P1 represent the same strain of *C. glabrata*. These results also demonstrate that the major switch phenotypes DB and vDB in the primary samples represented the same strain. Finally, these results demonstrate that the strain colonizing patient P1 had undergone microevolution.

(ii) Patient P2. Cells originating from a primary DB colony from the vaginal pool and a secondary vDB colony emanating from a primary DB colony from the vaginal pool, as well as from a primary DB colony from the vaginal wall and a secondary vDB colony emanating from a primary DB colony from the vaginal wall, were DNA fingerprinted. The hybridization patterns of three of the four isolates (DB-W, vDB-W, and vDB-P) were identical, while that of the fourth (DB-P) differed by a single band change (Fig. 3B). When compared to the patterns of isolates from the other two patients (Fig. 4) and when further compared to the patterns of the additional collection of unrelated isolates (Fig. 5), the P2 isolates clustered into a group not penetrated by any other isolate in the collections, demonstrating that the DB isolates from the vagina represented a single strain. The results also revealed microevolution in the infecting population.

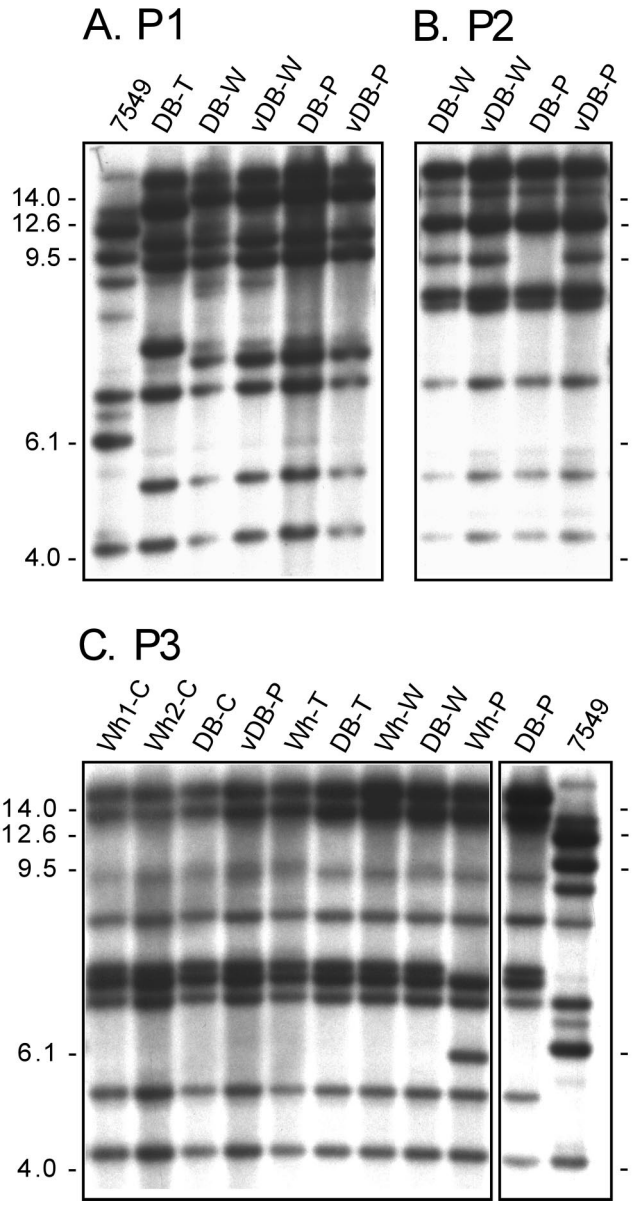

FIG. 3. Southern blot hybridization with the DNA-fingerprinting probe Cg6 demonstrated that only one strain of *C. glabrata* colonized each patient, that the same strain colonized both the oral cavity and the vaginal canal of each patient, and that the switch phenotypes represented the same strain in each individual. 7549 is a reference strain for normalization in computer-assisted analyses. T, under-tongue sample; C, cheek sample; W, vaginal-wall sample; P, vaginal-pool sample. Wh1 and Wh2 are two independent primary Wh colonies from the same patient. The molecular masses on the left of the blots are in kilodaltons. Note the examples of microevolution in DB-T of patient P1, DB-P of patient P2, and Wh-P of patient P3.

(iii) Patient P3. Seven of eight primary Wh and DB isolates from the cheek (Wh1-C, Wh2-C, and DB-C), under the tongue (Wh-T and DB-T), the vaginal pool (DB-P), and the vaginal wall (Wh-W and DB-W) exhibited identical DNA fingerprinting patterns (Fig. 3C). One primary isolate (Wh-P) exhibited a

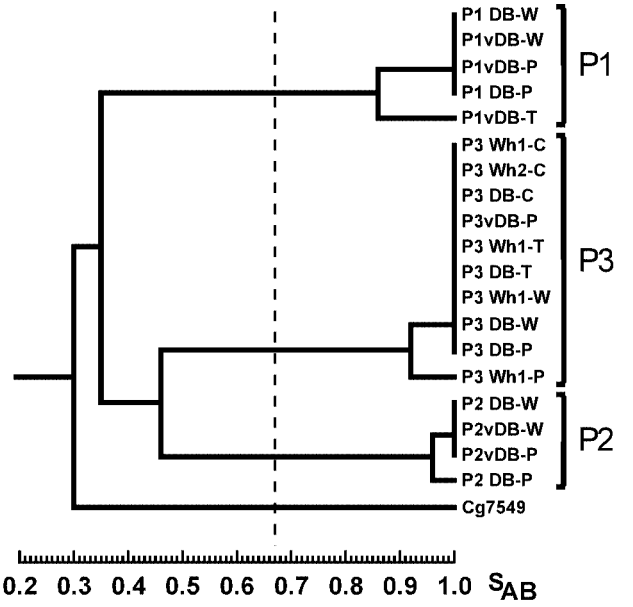

FIG. 4. Dendrogram generated for the DNA-fingerprinted isolates of patients P1, P2, and P3. To generate the dendrogram, the similarity coefficients between all pairs of the DNA-fingerprinted patterns of isolates from patients P1, P2, and P3 were computed. Note that the isolates from each patient form a single cluster, indicating that each patient's collection represented an independent single strain of *C. glabrata*. See the legend to Fig. 3 for an explanation of the isolate names. The dashed line represents the average S_{AB} computed among all isolate pairs.

pattern with two band differences (Fig. 3C). A secondary vDB colony emanating from a primary DB colony of the vaginal pool (vDB-P) had the same DNA fingerprint as the majority of isolates (Fig. 3C). When compared in mixed dendrograms to the patterns of isolates from the other two patients (Fig. 4) and when further compared to the patterns of the additional collection of unrelated isolates (Fig. 5), the P3 isolates coclustered, again demonstrating that they represented the same strain. These results also revealed microevolution.

Together, these results demonstrate that one strain colonized both the oral cavity and the vagina of each of the three patients, that the different colony phenotypes in the same host and in the same anatomical cavity of that host represent switch phenotypes of the same strain, and that there was microevolution in all three colonizing strains. The mixed dendrogram in Fig. 5 further revealed that relatively unrelated isolates of *C. glabrata* from different deep-rooted groups caused vaginal candidiasis and underwent switching between core phenotypes.

Switching characteristics of primary isolates. To confirm that multiple colony phenotypes in the same anatomical location or the same host were in fact the result of switching, we analyzed the switching characteristics of primary isolates. A mixture of cells from two individual primary colonies with the same switch phenotype and from the same anatomical location were plated onto agar containing 1 mM $CuSO_4$, and the frequencies and types of the variant colonies formed were measured. In the case of patient P1, primary DB cells switched primarily to the vDB phenotype at frequencies ranging between 6.7×10^{-3} and 2.6×10^{-2} (Table 1). Primary vDB

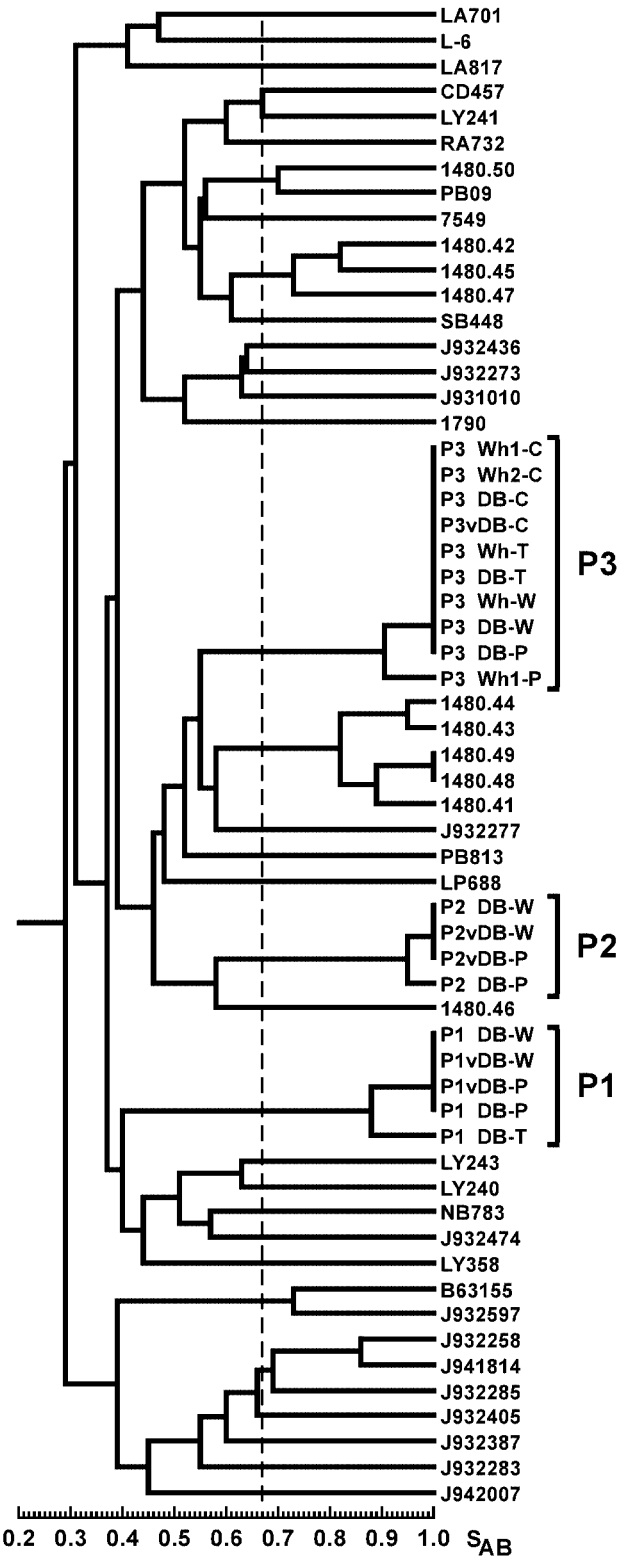

FIG. 5. Dendrogram generated for the DNA-fingerprinted isolates from patients P1, P2, and P3 and a large collection of unrelated *C. glabrata* isolates. Note that even in this larger dendrogram, the clusters of P1, P2, and P3 isolates remain intact, supporting the conclusion that each patient is colonized by a single strain of *C. glabrata*. The dashed line represents the average S_{AB} computed among all isolate pairs for patients 1, 2, and 3.

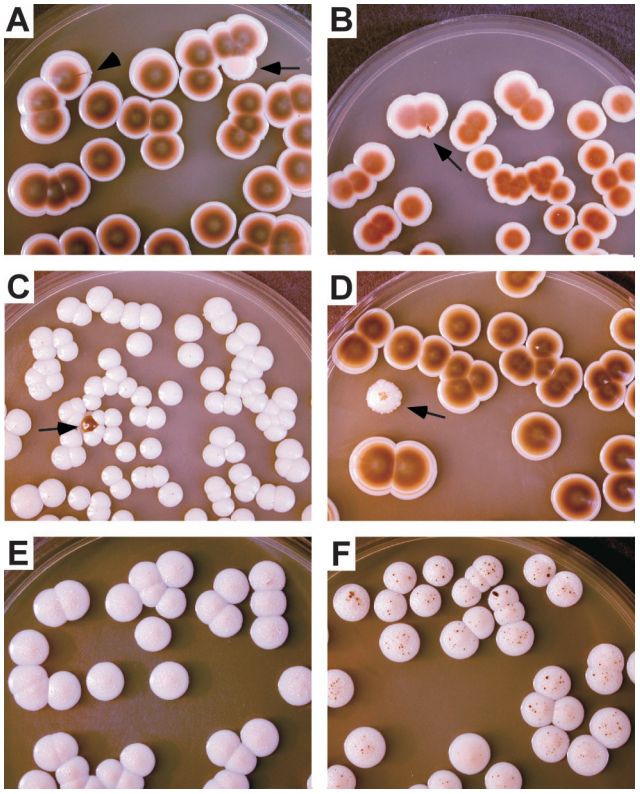

FIG. 6. Examples of in vitro switching. Cells from primary colonies were replated (secondary colonies). (A) Colonies from primary DB-VP colonies of patient P1 (the arrowhead points to switch to vDB sector and the arrow points to switch to LB colony). (B) Colonies from primary DB-VP colonies of patient P2 (the arrow points to switch to vDB sector). (C) Colonies from primary Wh-C colonies of patient P3 (the arrow points to switch to DB colony). (D) Colonies from primary DB-VP colonies of patient P3 (the arrow points to switch to Wh). (E) Colonies from primary Wh-C colonies of patient P3 secondary plating incubated for 6 days (note no visible sectoring). (F) Colonies from primary Wh-C colonies of patient P3 secondary plating incubated for 12 days (note sectoring in every colony).

isolates switched to LB at lower frequencies of 6.2×10^{-4} to 8.7×10^{-4} (Table 1). An example of a switch from vDB to LB is presented in Fig. 6A. In the case of patient P2, primary DB cells switched primarily to vDB at frequencies ranging from 3.2×10^{-2} to 4.3×10^{-2} (Table 1). An example of a vDB sector in a DB colony is presented in Fig. 6B. In the case of patient P3, primary Wh cells switched to DB at frequencies ranging between 3.4×10^{-3} and 4.4×10^{-3}, and primary DB cells switched to Wh at frequencies ranging between 1.2×10^{-3} and 5.2×10^{-3}. Examples of switches from Wh to DB and from DB to Wh for cells from patient P3 are presented in Fig. 6C and D, respectively. To demonstrate that all cells are capable of switching, secondary colonies generated from cells of primary Wh colonies obtained from the under-tongue samples from patient P3 were plated and examined at different times. Early in development (6 days), the colonies were uniformly Wh (Fig. 6E), while later in development (12 days), each colony exhibited multiple sectors of the other variant phenotypes (Fig. 6F). Together, these results demonstrate that cells of all primary phenotypes switched spontaneously to other phenotypes in the core switching system and suggest that the multiple phenotypes in a patient arose through high-frequency, reversible phenotypic switching.

Mating type classes and switching. Recently, Srikantha et al. (31) identified and characterized the mating type loci (*MTL1*, *MTL2*, and *MTL3*) of *C. glabrata*. Based on the *MTL* gene distribution among the three loci in a collection of independent *C. glabrata* isolates, four classes were distinguished (15). The three major classes, I, II, and III, accounted for 97% of all tested isolates. The three classes were readily distinguishable by Southern blot hybridization with an antisense oligonucleotide that hybridizes specifically to the 5′ end of the *MTL*α2 ORF, referred to as the *MTL*α2 probe (Fig. 7A) (31). Each class exhibited a distinct hybridization pattern with the *MTL*α2 probe. For class I cells (reference strain 7549), which contained *MTL***a**1 and *MTL***a**2 at the putative expression locus *MTL1*, the *MTL*α2 probe hybridized only to the *MTL3* band; for class II cells (reference strain PB921), which contained *MTL*α1 and *MTL*α2 in the *MTL1* locus, the *MTL*α2 probe hybridized to the *MTL1* and *MTL3* bands; and for class III cells (reference strain J432285), which contained *MTL***a**1 and *MTL***a**2 at the *MTL1* locus, FuncP2 hybridized to the *MTL3* and *MTL2* bands (31) (Fig. 7A). To assess the mating type class of the strains colonizing patients P1, P2, and P3, Southern blots were probed with the *MTL*α2 probe for the same sets of isolates that had been DNA fingerprinted with the complex probe Cg6 (Fig. 3). Four of the 5 P1 isolates exhibited the class I pattern (Fig. 7B), all 4 of the P2 isolates exhibited the class II pattern (Fig. 7C), and all 10 of the P3 isolates exhibited the class I pattern. Hence, class I cells (patient P1 and P3 strains) contained *MTL***a**1 and *MTL***a**2 in their putative expression locus *MTL1* and were therefore tentatively classified as mating type **a** cells, while class II cells (patient P2 strain) contained *MTL*α1 and *MTL*α2 in their expression locus *MTL1* and were therefore tentatively classified as mating type α cells. These results, therefore, indicate that pathogenesis (vaginal infection) is not limited to a single mating type. The results also demonstrate that in each host, the majority of cells exhibited the same mating type. The single exception was a vDB-P isolate from patient P1 (Fig. 7B). This single isolate exhibited a class II pattern, while the other four isolates from patient P1 exhibited a class I pattern, suggesting a mating type switch through gene conversion at the *MTL1* locus. DNA fingerprinting of vDB-P demonstrated that it was closely related to the other P1 isolates (Fig. 3C, 4, and 5), supporting the conclusion that a mating type switch had occurred in the host at the *MTL1* locus. To verify both the mating type and mating type switching, the Southern blots of the P1 isolates were hybridized first with a probe unique to the *MTL1***a**2 5′ flanking sequence and then with the *MTL***a**1 ORF (31). The probe for the *MTL1* locus hybridized to a single *MTL1* band that was of the same molecular weight in class I and class III isolates but of slightly higher molecular weight in class II isolates due to the fact that the *MTL1*α sequence is 185 bp longer than the *MTL1***a** sequence (Fig. 8A). The four class I isolates from patient P1 exhibited the class I *MTL1***a** band position, while the one class II isolate from patient P1, vDB-P, exhibited the class II *MTL1*α band position (Fig. 8B). The probe for *MTL***a**1 hybridized with the *MTL1* and *MTL2* bands for all class I isolates with similar molecular weights but with only the lower-molecular-weight

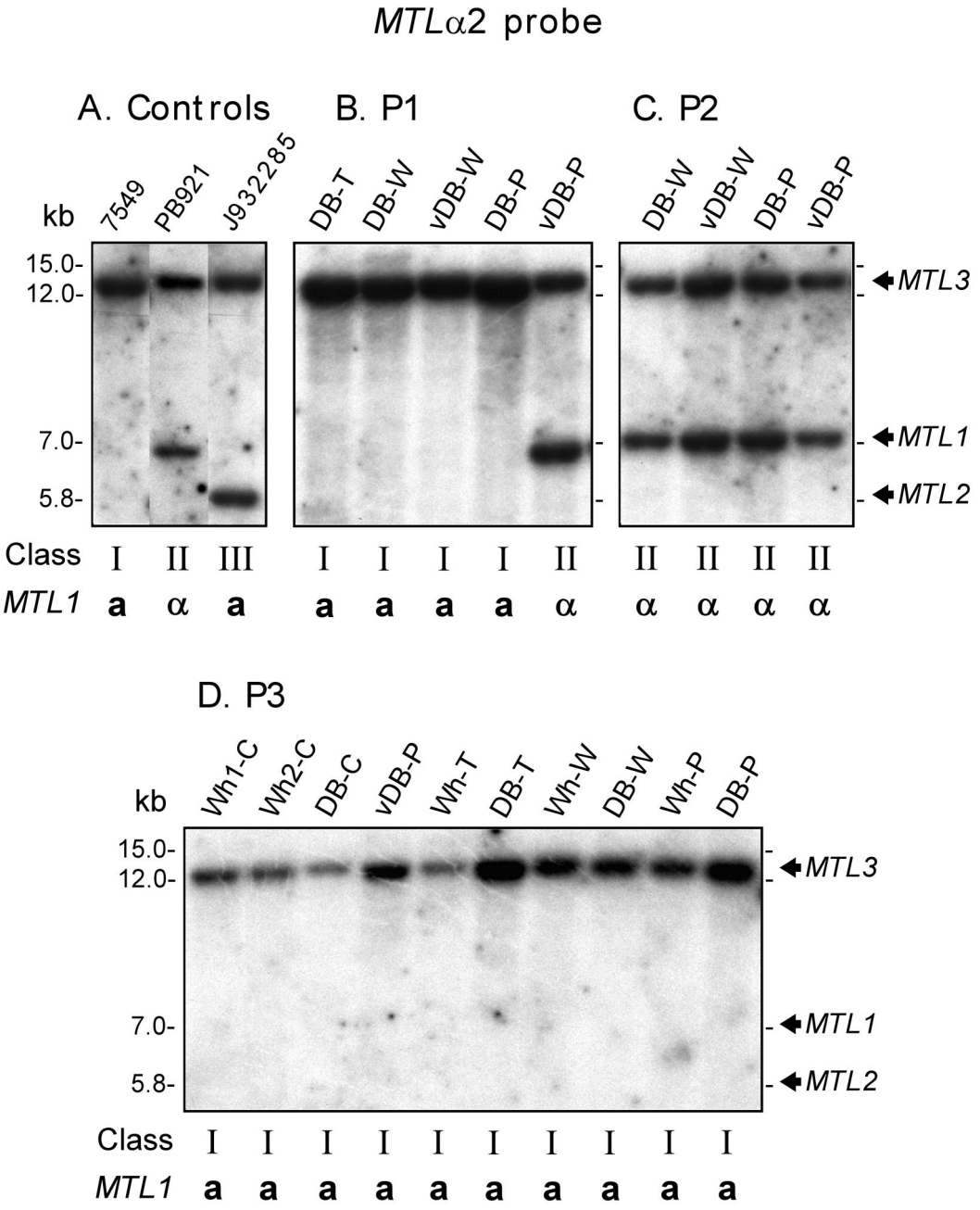

FIG. 7. Southern analysis of mating type classes reveals that infecting strains can be **a** or α and that mating type switching occurred in patient P1. Southern blots of test isolates from each patient were probed with FuncP2, an antisense oligonucleotide that binds to a unique 5′ end sequence of the *MTL*α2 ORF. This *MTL*α2-specific probe discriminates among the three major classes of *C. glabrata*, class I (**a**), class II (α), and class III (**a**) (31). (A) reference strains for the three classes representing the three discriminating patterns. (B) isolates from patient P1. (C) isolates from patient P2. (D) isolates from patient P3. The mating type classes and *MTL1* genotypes are provided below the blots. Note that the prevailing patterns of the isolates from patient P1, P2, and P3 are I (**a**), II (α), and I (**a**), respectively. Note also the single mating type switch from class I to class II in isolate vDB-P of patient P1. In Fig. 3A, it was demonstrated by Cg6 DNA fingerprinting that the five P1 isolates, including vDB-P, represent the same strain. See the legend to Fig. 3 for an explanation of the isolate names. The positions of *MTL1*, *MTL2*, and *MTL3* bands are noted to the right of the blots (31).

MTL2 band for the class II isolate (Fig. 8C), indicating that a mating type switch had occurred at the *MTL1* locus. Because the majority of isolates from patient P1 were class I (**a**) while a minority were class II (α), one might assume that the switch event was from **a** to α, but the reverse could just as well have been the case if **a** had a selective growth advantage.

To test whether the apparent genetic switch from **a** to α at the *MTL1* locus resulted in a change in mating type, the set of P1 isolates was analyzed for *MTL***a** and *MTL*α expression by Northern analysis with an *MTL***a**1-specific probe and an *MTL*α1-specific probe. While the isolates that were **a** at the *MTL1* locus (vDB-T, DB-W, vDB-W, and DB-P) expressed

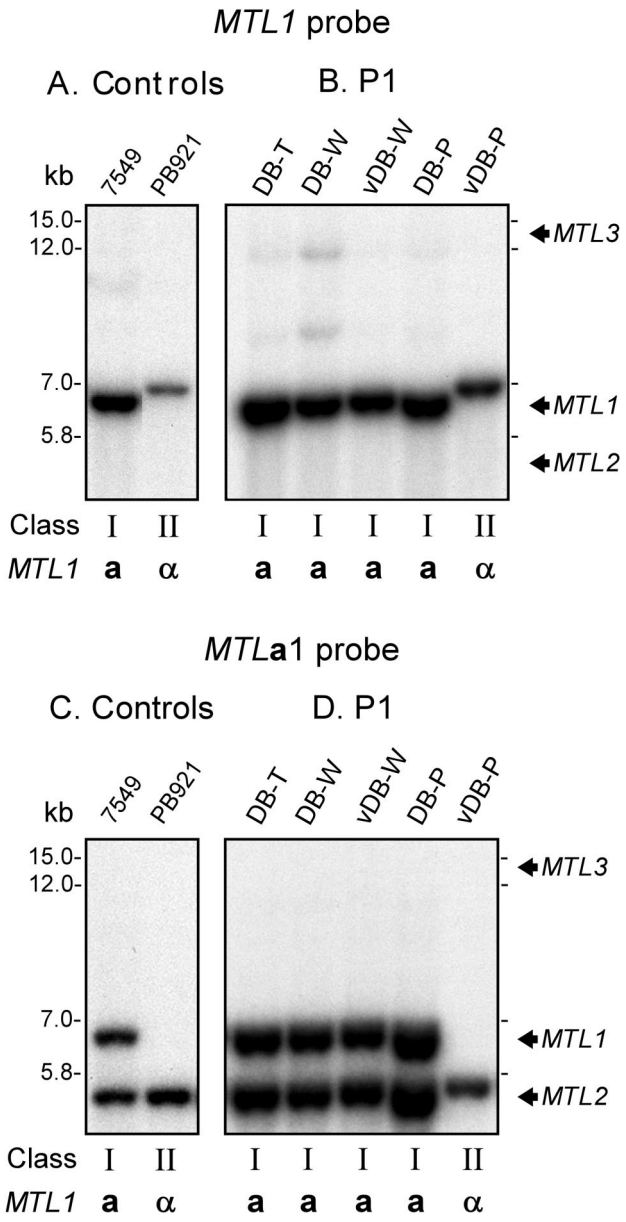

FIG. 8. Southern blot hybridization analyses with probes for the *MTL1* locus and the *MTL***a**1 ORF support the conclusion that a mating type switch is responsible for the vDB-P pattern. (A) Reference strain patterns for class I (**a**) and class II (α) generated by Southern blot hybridization with a probe that specifically binds to the *MTL1***a**2 3′ flanking sequence (15). (B) Southern blot hybridization patterns of primary P1 isolates with the *MTL1***a**2 3′ flanking sequence probe. (C) Reference strain patterns for classes I (**a**) and II (α) generated by Southern blot hybridization with the *MTL***a**1 ORF probe. (D) Southern blot hybridization patterns of primary P1 isolates with the *MTL***a**1 ORF probe. Note that in both cases the isolates DB-T, DB-W, vDB-W, and DB-P exhibit the class I (**a**) pattern while vDB-P exhibits the class II (α) pattern. See the legend to Fig. 3 for explanations of isolate names.

*MTL***a**1 and not *MTL*α1, the one isolate that was α at the *MTL1* locus (vDB-P) expressed *MTL*α1 and not *MTL***a**1 (Fig. 9). These results demonstrate that the genetic switch at the *MTL1* locus in the P1 strain resulted in a change in mating type expression.

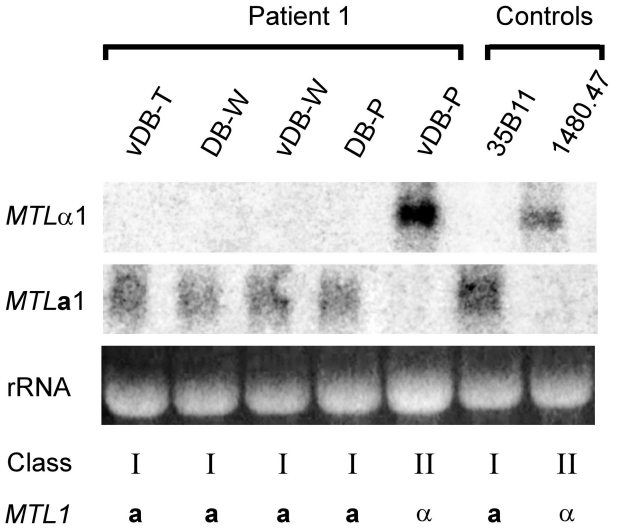

FIG. 9. Northern blot analyses with probes for *MTL***a**1 and *MTL*α1 transcripts reveal that the genetic switch from **a** to α at the *MTL1* locus in vDB-P was accompanied by a switch from *MTL***a**1 to *MTL*α1 expression. The expression patterns of the α strain 35B11 and the **a** strain 1480.47 are presented at the end of the gel for reference. While isolates vDB-T, DB-W, vDB-W, and DP-P, all **a** at the *MTL1* locus, express *MTL***a**1 and not *MTL*α1, isolate vDB-P, which is α at the *MTL1* locus, expresses *MTL*α1 and not *MTL***a**1. See the legend to Fig. 3 for an explanation of the isolate names.

To test whether mating type switching also occurred in vitro, cells from a secondary DB colony of the P1 isolate DB-P (class I; **aa**α) were clonally plated. Fifty of these colonies were then analyzed by Southern blot hybridization with FuncP2. All exhibited the class I pattern of the original DB-P isolate (data not shown). Cells of the DB-P isolate were then mass cultured through 200 generations in four separate flasks; the cells were pooled every 12 generations and analyzed by Southern blot hybridization with the *MTL*α1 probe. The gels were exposed for various lengths of time to monitor the appearance of the *MTL*α1 hybridization band of class II (Fig. 7A), which would indicate a mating type switch from *MTL1***a** to *MTL1*α at the *MTL1* locus. The sensitivity of the overexposed gels would have identified one class II cell per 100 to 500 class I cells. No *MTL1*α band was observed (data not shown), suggesting an extremely low in vitro frequency of switching from class I to class II in DB-P cells.

DISCUSSION

The results presented here first suggest that only one strain of *C. glabrata* colonized each of the three vaginitis patients, as has been demonstrated to be the case for *C. albicans* in vaginitis patients colonized by that species (10, 11). In two of the patients (P1 and P3), the same strain colonized both the oral cavity and the vaginal canal. The higher level of *C. glabrata* in the vaginal canal in both patients was consistent with the clinical diagnosis of the vagina as the site of infection. One patient (P2) had no oral carriage, even though she had a high level of vaginal colonization, suggesting that in some vaginitis patients, the oral cavity may not support, or may suppress, carriage. Similar differences between oral and vaginal colonization of *C.*

albicans in the same host have been demonstrated both in healthy individuals (30) and in vaginitis patients (27).

Here, we provide evidence that the oral cavity and the vaginal canal can be colonized by different proportions of switch phenotypes of the same strain. In the case of patient P1, the primary sample from the oral cavity was composed entirely of cells exhibiting the DB phenotype, while the primary samples from the vaginal wall and vaginal pool were composed of 86% DB and 14% vDB. This difference was significant. If one applied to an initial homogeneous population of 100 DB cells of patient P1 a simple model based on the relatively high in vitro frequency of DB switching to vDB and the relatively low in vitro frequency of switching from vDB to DB, the population would reach the proportions observed in the primary vaginal isolates after only eight cell doublings and would continue to decrease in favor of vDB cells. The fact that we did not observe a single vDB colony in the primary plating of the oral-cavity sample from patient P1, and only 14% in the primary plating of the vaginal sample, indicates a strong selection bias for DB in the vaginal canal and an even stronger bias in the oral cavity or inhibition of switching from DB to vDB in both locations.

Because DB cells from patient P2 switched to vDB at relatively high frequencies in vitro, one would have expected the vaginal population of this patient to contain vDB cells. The primary vaginal samples from patient P2, however, were devoid of vDB cells. Similarly, the in vitro frequency of switching of DB cells from patient P3 to Wh was similar to the in vitro frequency of switching of Wh cells to DB, yet the oral cavity was dominated by the Wh phenotype while the vaginal canal was dominated by the DB phenotype. These results clearly demonstrate that the in vivo situation does not reflect in vitro frequencies of switching.

The results obtained with patient P3 samples suggest for the first time that both the oral cavity and the vaginal canal can select for different switch phenotypes of the same strain. There are several possible ways this could be achieved. First, selection could be through the differential inhibition of cell multiplication. The oral cavity would selectively inhibit DB cell multiplication, while the vaginal canal would selectively inhibit Wh cell multiplication. Second, selection could be the result of the nutrient compositions of the fluids in the two locations. While the oral cavity would provide nutrients that selectively support Wh cell multiplication, the vagina would selectively provide nutrients that support DB cell multiplication. In vitro analyses have revealed no differences in the growth rates of the core switch phenotypes in a synthetic medium (8), but observations of the nutrient requirements of the white and opaque phenotypes of *C. albicans* suggest that growth in rich synthetic medium may be irrelevant to the in vivo situation. Although white- and opaque-phase *C. albicans* cells exhibit similar growth rates in synthetic medium, they exhibit profound differences in their sugar assimilation patterns (22). Similar differences may prove to exist for the core switch phenotypes of *C. glabrata*. Third, selection could be due to differences in adhesion or the capacity to form biofilms on the surfaces of the different cavities. In *C. albicans*, it has been demonstrated that while white-phase cells do not colonize skin, opaque-phase cells are highly proficient (6). Hence, there is precedent for selective colonization of switch phenotypes. Finally, selection could be due to the in vivo inhibition of switching in one direction (e.g., inhibition of switching from DB to vDB). In *C. albicans*, a variety of environmental conditions have been identified that affect the frequency of switching, including temperature (19), oxidants (5), culture age (26), and low doses of UV (14). Particular anatomical locations may selectively or generally inhibit switching or induce switching in one direction. Experiments to identify the mechanisms for the phenotypic selection of switch phenotypes of *C. glabrata* in the oral cavity and vaginal canal are now in progress. In addition, we are pursuing the possibility that the DB phenotype, which dominated the site of infection in each of the three vaginitis patients, may be a more pathogenic phenotype, although it should be noted that DB did not cause infections in the oral cavity.

Finally, our results demonstrate that both class I and class II cells, representing mating type **a** and α cells, respectively, can cause similar vaginal infections and that one mating type dominates in each colonizing strain. More interestingly, the results from patient P1 revealed a mixture of mating type **a** (class I; **aa**α) and mating type α (class II; **a**αα) cells in the vagina. Southern analysis revealed that the differences in the distribution of *MTL* genes at the *MTL1* locus of the mating type **a** and α isolates was consistent with conserved gene replacement at the *MTL1* locus in a single strain. Northern analysis demonstrated that this genetic change resulted in a change from *MTL***a** to *MTL*α expression. DNA fingerprinting of the strains verified that the *MTL***a** and *MTL*α isolates from patient P1 represented the same strain. These results demonstrate for the first time a mating type switch at the *MTL1* locus. Furthermore, these results demonstrate that mating type switching occurs at the site of colonization. Remarkably, mass culturing of the mating type **a** isolate (class I; **aa**α) through 200 generations in vitro did not result in a switch to mating type α (class II; **a**αα), suggesting either that the strain colonizing patient P1 had undergone far more than 200 generations in vivo for a switch to have occurred or that mating type switching occurs more frequently in vivo.

In conclusion, our analysis of the colonizing populations of *C. glabrata* in three vaginitis patients has revealed both high-frequency phenotypic switching and mating type switching in the host. Our results also suggest either that these processes do not occur at the same frequencies in vivo as they do in vitro or that host environments select for particular switch phenotypes or mating types. Finally, our results demonstrate for the first time that different phenotypes of the core switching system of a single strain can dominate different anatomical locations of the same host.

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