Usefulness of Multilocus Sequence Typing for Characterization of Clinical Isolates of Candida albicans

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Molecular characterization of Candida albicans is essential for understanding the epidemiology of nosocomial infections caused by this yeast. Here, we investigated the potential value of multilocus sequence typing (MLST) for characterizing epidemiologically related or unrelated C. albicans strains of various clinical origins. Accordingly, we sequenced the internal regions (loci) of six selected housekeeping genes of 40 C. albicans clinical isolates and 2 reference strains. In all, 68 polymorphic nucleotide sites were identified, of which 65 were found to be heterozygous in at least one isolate. Ten to 24 different genotypes were observed at the different loci, resulting, when combined, in 39 unique genotype combinations or diploid sequence types (DSTs). When MLST was applied to 26 epidemiologically unrelated isolates and the 2 reference strains, it allowed the identification of 27 independent DSTs, thus demonstrating a discriminatory power of 99.7. Using multidimensional scaling together with the minimum spanning tree method to analyze interstrain relationships, we identified six groups of genetically related isolates on the basis of bootstrap values of greater than 900. Application of MLST to 14 epidemiologically related isolates showed that those recovered from patients in the same hospital ward during the same 3 months had specific DSTs, although 73% of these isolates were genetically very close. This suggests that MLST can trace minute variations in the sequences of related isolates. Overall, MLST proved to be a highly discriminatory and stable method for unambiguous characterization of C. albicans.

Candida albicans is a commensal yeast which is also a leading cause of nosocomial infections. The number of patients infected by C. albicans has risen sharply over the last two decades, and these infections are associated with high mortality rates despite of the introduction of novel antifungal agents. Prevention of nosocomial infections caused by C. albicans and related species is difficult, because little is yet known about either the dynamics of transmission among hospitalized patients or the characteristics of the infecting strains. During the last 10 years, the sources of the C. albicans isolates responsible for infecting hospital patients have been identified in epidemiological studies by using molecular typing techniques. It is now well accepted that strains which colonize patients prior to the occurrence of a systemic infection are usually those that are at the origin of the infection (17, 20, 29). Colonized patients are the main reservoir of C. albicans in hospitals, and the cross-contamination that occurs between patients (21, 22, 24, 28) suggests that the source of colonization can be either a commensal strain belonging to the patient’s microflora prior to hospitalization or a strain acquired through cross-contamination within the hospital. Longitudinal surveillance studies have shown that several endemic strains were sometimes carried by patients from different wards (17, 21).

However, the typing methods used to characterize C. albicans isolates in epidemiological studies are very diverse. They include restriction fragment length polymorphism analysis (20, 28), Southern blot hybridization with discriminating probes (17, 21, 24), electrophoretic karyotyping (29), and randomly amplified polymorphic DNA analysis (22). Results are laboratory dependent, which precludes interlaboratory comparison of infecting isolates. As no consensual typing method is presently available to characterize C. albicans isolates circulating within or between hospitals, it is not clear whether some strains have a particular ability to cause nosocomial infections or have a local or international distribution. Thus, no global view of the population dynamics and epidemiology of the C. albicans strains that cause nosocomial infections has yet emerged.

These questions are common to the study of nosocomial infections caused by other microorganisms, including bacteria. For bacteria, the multilocus sequence typing (MLST) method has been recently proposed to overcome these flaws (16). MLST is a highly resolutive method based on the analysis of nucleotide polymorphisms of the sequences of approximately 450- to 500-bp internal fragments (loci) of housekeeping genes. For each housekeeping locus, the different sequences present within a bacterial species are assigned as distinct alleles, and for each isolate, the alleles at each of the sequenced loci define an allelic profile or sequence type. Each isolate of a species is therefore unambiguously characterized by a series of integers which correspond to the alleles at the housekeeping loci studied. A major advantage of MLST over other typing methods is that sequence data can be easily compared between laboratories, thus permitting the exchange of molecular typing data via the internet for global epidemiology (16). MLST has been used to characterize many bacterial pathogens, including Neisseria
Materials and Methods

Strains. Two groups of C. albicans strains were selected for MLST analysis. The first group included 26 epidemiologically unrelated clinical isolates recovered between 1993 and 2000 from 26 patients in 10 different hospitals located in four French regions and also the two reference strains C. albicans ATCC 36322 and SC5314. The genome sequence of strain SC5314 is currently being determined at the Stanford Genome Technology Center (27). The second group of strains studied comprised 14 epidemiologically related clinical isolates, including 11 which came from 11 patients hospitalized in the same intensive care unit and were isolated during the same 3-month period and 3 isolated from a pregnant woman and her fetus during an episode of systemic candidiasis which ended in abortion. Clinical isolates of Candida glabrata, Candida tropicalis, Candida dubliniensis, Candida krusei, Candida parapsilosis, Candida lusitaniae, and Saccharomyces cerevisiae were also tested. Strains were stored at −80°C in Cryo-bille tubes (Laboratoire AES, Combourg, France).

DNA extraction. After subculture on Sabouraud agar, two colonies of each isolate were inoculated into 10 ml of YPD broth (2% glucose, 2% Bacto Peptone, and 1% yeast extract) in a sterile tube and grown overnight at 37°C with shaking. Two milliliters of the culture was centrifuged, the pellet was resuspended in 200 μl of buffer (50 mM Tris-HCl [pH 8], 25 mM EDTA [pH 8], and 1% [vol/vol] β-mercaptoethanol) containing 0.5 μg of Zymolyase (25,000 U; ICN Pharmaceuticals, Costa Mesa, Calif.), and the mixture was incubated for 1 h at 37°C. After centrifugation, the pellet was resuspended in 200 μl of lysis buffer (200 mM diethanolamine, 80 mM EDTA [pH 9], and 1% [wt/vol] sodium deoxycholate) and incubated for 30 min at 65°C. Following addition of 100 μl of 5 M potassium acetate, incubation was continued for 45 min on ice. DNA was precipitated with 3.5 volumes of ethanol–7.5 M ammonium acetate (6:1), rinsed with 70% ethanol, dried, and dissolved in 50 μl of sterile water.

Choice of loci. Murat et al. (18) reported the identification of 3,313 putative open reading frames (ORFs) from assembly 3 of the C. albicans genome sequence carried at the Stanford DNA Sequencing and Genome Technology Center (27). Comparison of these ORFs to the S. cerevisiae proteome revealed a set of C. albicans ORFs with very high similarity to S. cerevisiae ORFs, all of them encoding housekeeping functions. The amino acid sequences corresponding to these C. albicans ORFs and their homologues in S. cerevisiae sequences were aligned, and the most variable regions were identified. Among these regions, those of roughly 150 to 200 amino acids in length and flanked by highly conserved regions were selected. Primers were designed using the nucleotide sequences of the conserved regions in the C. albicans ORF. Each pair of primers was designed to amplify a 500- to 700-bp fragment.

Amplification and nucleotide sequence determination. PCRs were carried out in 100-μl reaction volumes containing about 0.1 μg of extracted DNA, 100 pmol of each primer, 2.5 μl of Taq DNA polymerase (Amersham Pharmacia Biotech, Orsay, France), 10 μl of 10× buffer (supplied with the Taq polymerase), and a 200 μM concentration of each deoxynucleoside triphosphate (Boehringer Mannheim, Meylan, France). PCRs were performed with an initial 5-min denaturation step at 93°C, followed by 30 cycles of 93°C for 30 s, 55°C for 1 min, and 72°C for 1 min, with a final extension step of 4 min at 72°C. The amplified fragments were purified using a PCR purification kit (Qiagen, Courtaboeuf, France). Purified fragments were sequenced on both strands by using the same primers as those used in the initial amplification. Sequencing reactions were prepared using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems) according to the manufacturer’s recommendations for cycle sequencing on the GeneAmp PCR 9700 system. The reactions were analyzed on an ABI PRISM 3700 Genetic Analyzer (PE Applied Biosystems).

Results

Sequence diversity in C. albicans MLST. Using the genome sequence of the C. albicans reference strain SC5314 available at the Stanford DNA Sequencing and Genome Technology Center (27), we identified regions in six C. albicans housekeeping genes showing relatively high divergence from the corresponding regions in the S. cerevisiae ortholog and flanked by regions that are highly conserved between C. albicans and S. cerevisiae (Table 1). Table 2 lists the oligonucleotides used to amplify these regions (loci) from all 42 C. albicans isolates tested. Analysis of the sequences obtained from all strains and loci showed that all of the sequences obtained from a single locus could be aligned without gaps or insertions. In all, 2,354 nucleotides from six loci were studied for each isolate. Sixty-eight polymorphic nucleotide sites (2.9%) were detected among the 42 C. albicans isolates. The number of polymorphic sites for each locus ranged from 6 in locus CaRPN2 to 16 in locus CaVPS13 (Table 3). The number of nucleotide substitutions from the C. albicans SC5314 genome sequence that were not conservative at the amino acid level ranged from one at locus CaRPN2 to nine at locus CaVPS13 (Table 3).

C. albicans is almost always diploid as isolated, and evidence has been obtained for heterozygosity of complementary chromosomes (23). Therefore, amplification of genomic DNA is expected to result in PCR products that arise from both chromosomes and that upon direct sequencing yield profiles that correspond to the superimposition of the two heterozygous alleles. In the six loci investigated here, polymorphic nucleotide sites were indeed either homozygous or heterozygous, confirming that the oligonucleotides used in this study could amplify both chromosomal alleles. None of the isolates was 100% homozygous. The presently available genome sequence

Candida Albicans Multilocus Sequence Typing

Menininating, Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, and Campylobacter jejuni (4, 6–8).

Although MLST was originally developed for haploid organisms, a similar sequence typing methodology has been described for C. albicans, which is an assexual diploid organism. It is based on the sequencing of 12 anonymous loci and has been successfully used for genetic population analysis (9). However, this method has not been evaluated for analysis of isolates from the standpoint of understanding nosocomial infections.

In the present investigation, we developed an MLST scheme that appeared to be highly resolutive, in order to clarify the relationships between C. albicans isolates with well-defined clinical and epidemiological links.
for *C. albicans* strain SC5314 is composed mostly of homoyzygous nucleotide sequences derived from the two complementary chromosomes. However, in the present study, we found one heterozygosity at position 35 in locus *CaADP1*. Thus, altogether, and including reference strain SC5314, at least one heterozygosity was observed among the 68 polymorphic sites in the 42 isolates investigated. These results underline the fact that local heterozygosity is frequent in the sequences of the six housekeeping loci studied.

Each of the different sequences in a locus defined a distinct genotype, even if it differed from the others by only a single nucleotide. The different genotypes were numbered in the order of their identification. The number of genotypes identified for each of the six loci investigated ranged from 10 for *CaACC1* to 24 for *CaVPS13* (Table 3).

### TABLE 1. Housekeeping loci used

<table>
<thead>
<tr>
<th>Locus</th>
<th><em>C. albicans</em> ORF no.</th>
<th>Size of <em>C. albicans</em> ORF (bp)</th>
<th>Locus position</th>
<th><em>S. cerevisiae</em> ORF ortholog no.</th>
<th>Putative function of gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CaACC1</em></td>
<td>6–8718</td>
<td>6,816</td>
<td>3251–3769</td>
<td>YNR016c</td>
<td>Acetyl-coenzyme A carboxylase</td>
</tr>
<tr>
<td><em>CaVPS13</em></td>
<td>6–4223</td>
<td>9,252</td>
<td>5151–5891</td>
<td>YLL040c</td>
<td>Vacuolar protein sorting protein</td>
</tr>
<tr>
<td><em>CaGLN4</em></td>
<td>6–4849</td>
<td>2,400</td>
<td>109–591</td>
<td>YOR168w</td>
<td>Glutaminyl-tRNA synthetase</td>
</tr>
<tr>
<td><em>CaADP1</em></td>
<td>6–2855</td>
<td>3,117</td>
<td>918–454</td>
<td>YCR011c</td>
<td>ATG-dependent permease</td>
</tr>
<tr>
<td><em>CaRPN2</em></td>
<td>6–7682</td>
<td>2,859</td>
<td>1083–1529</td>
<td>YIL075c</td>
<td>26S proteasome regulatory subunit</td>
</tr>
<tr>
<td><em>CaSYA1</em></td>
<td>6–2925</td>
<td>2,910</td>
<td>2400–2942</td>
<td>YOR335c</td>
<td>Alanine-RNA synthetase</td>
</tr>
</tbody>
</table>

* ORF numbers refer to the genome sequence of *C. albicans* SC5314 published by Stanford University (http://sequence-www.stanford.edu/group/candida).

* ORF numbers refer to the genome sequence of *S. cerevisiae* (http://genome-www.stanford.edu/Saccharomyces).

* *CaSYA1* is the ortholog of the *ScALAI* gene encoding alanyl-tRNA synthetase; however, the designation *CaALAI* could not be used because it corresponds to a gene encoding an agglutinin-like adhesin (11).

### TABLE 2. Oligonucleotide primers used for *C. albicans* MLST

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CaACC1</em></td>
<td>466 forward</td>
<td>GCAAGAGAAATTTTAATTCAATG</td>
<td>519</td>
</tr>
<tr>
<td></td>
<td>466 reverse</td>
<td>TCTATCAACATCATCACAATG</td>
<td></td>
</tr>
<tr>
<td><em>CaVPS13</em></td>
<td>552 forward</td>
<td>TCGTTGAGGATATTCGACCT</td>
<td>741</td>
</tr>
<tr>
<td></td>
<td>552 reverse</td>
<td>ACGGATGATGTCCTCCAGCC</td>
<td></td>
</tr>
<tr>
<td><em>CaGLN4</em></td>
<td>598 forward</td>
<td>GAGATAGTCAAGAAATATAAALAAAAGT</td>
<td>483</td>
</tr>
<tr>
<td></td>
<td>598 reverse</td>
<td>ATCTCCTCCTCCTTGGAC</td>
<td></td>
</tr>
<tr>
<td><em>CaADP1</em></td>
<td>904 forward</td>
<td>GAGGCCAAGTATGAATGATTTG</td>
<td>537</td>
</tr>
<tr>
<td></td>
<td>904 reverse</td>
<td>TTGATCAACAACACCGATAAT</td>
<td></td>
</tr>
<tr>
<td><em>CaRPN2</em></td>
<td>1041 forward</td>
<td>TTCACTGACATGCTGACTAC</td>
<td>447</td>
</tr>
<tr>
<td></td>
<td>1041 reverse</td>
<td>TAATCCCATACCAAACCCGAC</td>
<td></td>
</tr>
<tr>
<td><em>CaSYA1</em></td>
<td>1369 forward</td>
<td>AGAAGAATGTGTTGTTGTTG</td>
<td>543</td>
</tr>
<tr>
<td></td>
<td>1369 reverse</td>
<td>GTTACCTTACCAACACGCTT</td>
<td></td>
</tr>
</tbody>
</table>

* ORF numbers refer to the genome sequence of *C. albicans* SC5314 published by Stanford University (http://sequence-www.stanford.edu/group/candida).

### TABLE 3. Characteristics of the six housekeeping loci studied

<table>
<thead>
<tr>
<th>Locus</th>
<th>Size of fragment (bp)</th>
<th>No. of genotypes identified</th>
<th>No (%) of polymorphic nucleotide sites</th>
<th>No. of polymorphic amino acid sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CaACC1</em></td>
<td>407</td>
<td>10</td>
<td>6 (1.5)</td>
<td>2</td>
</tr>
<tr>
<td><em>CaVPS13</em></td>
<td>403</td>
<td>24</td>
<td>16 (4)</td>
<td>9</td>
</tr>
<tr>
<td><em>CaGLN4</em></td>
<td>404</td>
<td>15</td>
<td>11 (2.7)</td>
<td>7</td>
</tr>
<tr>
<td><em>CaADP1</em></td>
<td>443</td>
<td>16</td>
<td>13 (2.9)</td>
<td>6</td>
</tr>
<tr>
<td><em>CaRPN2</em></td>
<td>306</td>
<td>16</td>
<td>11 (3.6)</td>
<td>1</td>
</tr>
<tr>
<td><em>CaSYA1</em></td>
<td>391</td>
<td>13</td>
<td>11 (2.8)</td>
<td>4</td>
</tr>
</tbody>
</table>

* ORF numbers refer to the genome sequence of *C. albicans* SC5314 published by Stanford University (http://sequence-www.stanford.edu/group/candida).

These 37 DSTs, 7 pairs differed at only one locus (DSTs 18 and 20, 31 and 33, 32 and 33, 31 and 38, 33 and 34, 14 and 36, and 16 and 30). The other DSTs differed at two to six loci. When MLST analysis of our 42 *C. albicans* isolates was performed using the data from only the three loci *CaVPS13*, *CaGLN4*, and *CaADP1*, 38 unique combinations of genotypes could be identified instead of the 39 DSTs identified using the data from the six loci.

### Species specificity of primers of loci

No amplification was obtained with the *CaACC1* and *CaGLN4* oligonucleotides when the DNAs of the six *Candida* non-*C. albicans* species, or of *S. cerevisiae*, were used as templates. When oligonucleotides for loci *CaVPS13*, *CaADP1*, and *CaRPN2* were used, an amplicon was generated from *C. dubliniensis* DNA but not from the DNA of any of the six other species tested. When the oligonucleotides for *CaSYA1* were used, an amplicon was generated from all of the species tested except *C. krusei*.

These results demonstrated that the oligonucleotides used for *CaACC1* and *CaGLN4* were specific for *C. albicans*; that those used for *CaVPS13*, *CaADP1*, and *CaRPN2* were specific for both *C. albicans* and *C. dubliniensis*; but that those used for *CaSYA1* were not species specific. The sequences of the PCR products obtained from the *C. dubliniensis* CdVPS13, CdADP1, and CdSYA1 loci were compared to those of the corresponding loci in *C. albicans*. CdSYA1 displayed polymorphic nucleotide sites similar to those observed in *C. albicans*. In contrast, comparison of the CdVPS13 and CdADP1 sequences with the corresponding ones in *C. albicans* showed 41 and 35 divergent nucleotides, respectively. These divergent nucleotides were not at sites associated with polymorphism in *C. albicans* (data not shown). These results are in agreement with the close relationship between *C. albicans* and *C. dubliniensis*, which were nevertheless recently demonstrated to be separate species (5).

### Stability and reproducibility of *C. albicans* MLST

The sequence stability of the six loci was evaluated by sequencing the
FIG. 1. Positions of the polymorphic nucleotide sites and genotypes identified at loci CaACC1 (A), CaVPS13 (B), CaGLN4 (C), CaADP1 (D), CaRPN2 (E), and CaSYA1 (F). The nucleotides present at each variable site among the 42 C. albicans strains tested are shown for genotype 1. For the other genotypes, only sites that differ from those in genotype 1 are shown; sites that are the same as those in genotype 1 are shown by dots. The numbers of isolates with the same genotype are indicated in parentheses. The position of each polymorphic site relative to the fragment sequenced is shown at the top in vertical format. Y, C or T; R, A or G; K, G or T; M, A or C; S, G or C; W, A or T.
PCR products obtained using DNAs from six subclones of *C. albicans* strain ATCC 36232 that were obtained after 30 successive subcultures (about 400 generations). No difference was observed between the subclones and the original isolate. Reproducibility was tested with nine separate isolates by using DNAs from two independent extractions. Again, the sequences obtained from the two extractions were strictly identical, thus confirming the high reproducibility of the method.

**MLST analysis of related and unrelated *C. albicans* isolates.**

In all, 27 DSTs were found among the 28 epidemiologically unrelated isolates investigated (26 clinical isolates and 2 reference strains). Only two isolates (isolates 17 and 21) had identical DSTs. All of the other 26 had a unique DST and could be separated from each other (Table 4). The DSTs of 16 of these 26 isolates (61%) differed from each other at 3 loci or more. For the present sample of 28 epidemiologically unrelated isolates, the discriminatory power of the MLST scheme for typing *C. albicans*, calculated using a numerical index as previously described (13), was 99.7. The results of MDS and MST analyses showed that six groups of genetically related isolates were identifiable on the basis of bootstrap values of greater than 900 (Fig. 2). Isolates 8 and 13 were separated, respectively, from groups III and V on the basis of low bootstrap values, which ranged from 554 to 896. Within groups, the lengths of the branches linking the strains were variable, suggesting that some isolates were genetically very close whereas others were more distant. Contrasting patterns were observed. In groups I, II, and V, the isolates were connected by short branches, whereas in group VI, the branches were longer. These results provided a measure of the genetic diversity at multiple loci within a set of epidemiologically unrelated clinical isolates.

We observed that among the 14 epidemiologically related clinical isolates, isolates CP7, CP8, and CP9, which were recovered from a single episode of infection (mother and fetus)
shared the same DST (Table 4). The 11 other isolates, which were isolated during the same 3-month period from patients hospitalized in the same intensive care unit, had different DSTs. In 8 of these 11 isolates, the DSTs differed by only one or two of the six genotypes.

A dendrogram depicting the relationships between the 42 isolates studied showed that 8 of the 11 isolates from the patients hospitalized in the same unit (CP1, CP2, CP3, CP5, CP10, CP12, CP13, and CP14) were grouped in a cluster, whereas the 28 unrelated isolates were not distributed in a specific cluster (Fig. 3).

DISCUSSION

In this study we showed that from an epidemiological standpoint, MLST provides valuable information for the characterization of C. albicans isolates. MLST is a highly resolutive method based on analysis of the nucleotide polymorphism of internal fragments from several housekeeping genes present in all isolates within a given species (16). It has been used to characterize several bacterial pathogens which are strictly haploid microorganisms (4, 6–8). By contrast, the possible occurrence of heterozygosity in the sequences of diploid organisms makes MLST analysis more difficult. However, sequence typing of randomly amplified DNA fragments from C. albicans anonymous DNA regions has been used to investigate the genetic structures of typical and atypical populations of C. albicans from different geographic origins (9). This analysis has demonstrated that the structure of these populations was predominantly clonal but that there was also evidence for recombination and very low levels of gene flow between them (9, 12).

In the present study, we characterized six loci, each corresponding to an internal fragment of a distinct C. albicans housekeeping gene. At each locus, we observed a wide variety of genotypes among the isolates studied, although the number of polymorphic nucleotide sites was relatively limited. This was the consequence, at least in part, of a high frequency of heterozygosity, which increased sequence diversity at each locus,
and thus allowed the identification of a greater number of genotypes. Indeed, heterozygosity was detected at all polymorphic nucleotide sites except three (positions 139, 180, and 327 at locus CaGLN4), and each *C. albicans* isolate had at least one heterozygous site within the 68 polymorphic sites studied. The presence of heterozygosity at the DNA level has been previously reported for *C. albicans* (2, 3, 12). When using 13 anonymous DNA regions to analyze the genetic structure of populations of typical *C. albicans* isolates, Forche et al. found that the frequency of polymorphic nucleotide sites in these regions was 1.1% and that there was evidence for heterozygosity at 35 of the 56 polymorphic nucleotide sites that they identified (9). The presence of heterozygosity has also been reported in the sequence of the *CaERG11* gene, which encodes lanosterol 14-α-demethylase (10). However, the overall sequence diversity and levels of heterozygosity previously reported for these DNA regions were lower than those we observed at the six loci which we investigated and which included 68 polymorphic sites (2.9%). Of these, 65 were heterozygous.

In this study, we confirmed the reproducibility of the MLST method and the genomic stability of the loci studied. The fact that neither a loss nor a gain in the heterozygosity was detected at any polymorphic nucleotide site when the subclones obtained after successive subcultures were compared also confirmed that heterozygosity was stable in our isolates. These results agree with previous observations, based on an experimental *C. albicans* population, that over 330 generations, no change occurred in five DNA regions known to be heterozygous in the progenitor genotype (2).

The MLST method proved to be highly resolutive for *C. albicans* strain differentiation. The number of theoretically different DSTs resulting from the combination of the 10 to 24 genotypes present at each of the six loci is in the range of 10^7, and this should increase as new genotypes are described after the use of MLST with additional *C. albicans* isolates. The discriminatory power of the method, measured with a sample of 28 epidemiologically unrelated strains, was 99.7. Two isolates (isolates 17 and 21) had the same DST, which was unexpected because they were epidemiologically unrelated. However, due to the diploid nature of *C. albicans*, strains with the same DST may be different if they are heterozygous at several sites, and these two isolates were indeed heterozygous at 21 of the 68 polymorphic sites. Sequencing did not allow us to determine which allele is associated with which nucleotide at heterozygous positions, a limit common to all methods using nucleotide polymorphism analysis at several heterozygous loci in diploid organisms. Despite this limitation, the discriminatory power of our MLST method was greater than that of the other methods previously proposed for typing of *C. albicans* by using DNA sequence data. The discriminatory power of a method using analysis of the polymorphism of one microsatellite region was only 88 (1). A multilocus genotyping system using oligonucleotide probes for identification of the nucleotide state at only one of the polymorphic nucleotide sites of each of 16 distinct loci distinguished only 64 genotypes in a sample of 84 *C. albicans* isolates (3). Recently, it was shown by this method that the ability to invade the bloodstream is widespread among *C. albicans* isolates (15). The discriminatory power of our MLST method was similar to that of fingerprinting with the moderately repetitive sequence Ca3, which provided the highest resolution reported so far for typing of *C. albicans* isolates (25). However, MLST has several advantages over fingerprinting. First, the technology used, based on PCR amplification followed by the sequencing of six well-characterized loci, is easy to perform and can be done quickly as automatic sequencing becomes more widely available. The PCR conditions used in this study were very robust, as judged by the 100% success rate obtained in more than 700 assays. Second, the results of MLST are unambiguous, and sequence data can be shared and compared between different laboratories.

The *C. albicans* isolates that we studied in this work were specifically chosen to constitute a sampling of strains, with some having specific epidemiological links and some being unrelated. We have described the genetic relationships within a set of epidemiologically unrelated isolates of *C. albicans* by using a two-dimensional representation (Fig. 2). This representation allowed to identify groups of strains which are genetically close. We have observed that within the 28 isolates investigated, 26 were associated within six different groups. This method appears to be suitable for identification of the presence or absence of specific groups of strains among *C. albicans* isolates and could be useful for understanding the transmission and population dynamics in hospitalized patients or in healthy carriers. We showed that MLST can distinguish not only between epidemiologically unrelated strains but also between epidemiologically related strains and can identify a unique strain represented by different isolates. The dendrogram depicting the relationships between all of the isolates studied (Fig. 3) showed that as high a proportion as 73% of the related isolates were grouped in a cluster, whereas the unrelated isolates were not. This suggests that the epidemiologically related isolates, which had been recovered within a short period of time from patients hospitalized in the same intensive care unit, were genetically very close, although they were not strictly identical. These results agreed with those reported by others, who found that the isolates from patients in the same ward were genetically closer than other isolates (17, 24). By contrast, the three isolates recovered from a mother and her fetus were strictly identical. Thus, together our results emphasize that MLST can detect minute variations in the genomes of related isolates of *C. albicans* and can be used by epidemiologists to trace strain transmission. During epidemiological investigations, a large number of isolates often have to be typed. The fact that MLST was highly discriminatory even when only three loci were studied should make the method highly suitable for such settings.

Taken together, our results showed that MLST is highly reproducible and discriminatory with *C. albicans*. The sequence data obtained with this method can be used to construct an online global database which should allow laborato ries to compare their local isolates and should prove useful both for global epidemiology and for investigating the transmission of *C. albicans* infections.

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