JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2002, p. 1283–1289 Vol. 40, No. 4
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.4.1283–1289.2002

Mitochondrial Telomeres as Molecular Markers for Identification of the Opportunistic Yeast Pathogen *Candida parapsilosis*

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Received 25 July 2001/Returned for modification 14 December 2001/Accepted 24 January 2002

Recent studies have demonstrated that a large number of organisms carry linear mitochondrial DNA molecules possessing specialized telomeric structures at their ends. Based on this specific structural feature of linear mitochondrial genomes, we have developed an approach for identification of the opportunistic yeast pathogen *Candida parapsilosis*. The strategy for identification of *C. parapsilosis* strains is based on PCR amplification of specific DNA sequences derived from the mitochondrial telomere region. This assay is complemented by immunodetection of a protein component of mitochondrial telomeres. The results demonstrate that mitochondrial telomeres represent specific molecular markers with potential applications in yeast diagnostics and taxonomy.

Several yeast species are associated with opportunistic infections of humans and other mammals. Among them, candidoses are of the greatest clinical importance. These mycoses manifest themselves as localized, invasive or systemic infections that are frequently associated with immune deficiencies, AIDS, immunosuppressive therapy, anticancer treatments, organ transplantations, and various invasive medical procedures. They are caused mainly by *Candida albicans*, but many recent clinical surveys have illustrated the rising significance of non-*C. albicans* infections. As a result, there has been an increased interest in the biology and taxonomy of *Candida* species originally believed to be nonpathogenic (9, 14, 36).

C. parapsilosis is a widespread pathogen, accounting for up to 30% of nosocomial fungemias. It is also associated with septic arthritis, peritonitis, vaginitis, and nail and skin infections. An increasing prevalence of *C. parapsilosis* has also been observed in cases of endocarditis, either indicating a selective affinity of this yeast for endocardial tissues or reflecting its propensity to colonize damaged skin and gain ingress along intravascular lines (5, 10, 14, 36).

Due to differences in susceptibility of non-*C. albicans* species to antifungal drugs, rapid and accurate species identification is essential for the implementation of appropriate therapy. Methods for identification and classification of clinically important *Candida* species based on phenotypic and/or morphologic characteristics do not always lead to unambiguous results, and identification of a pathogen is sometimes difficult. The recent development of various molecular techniques has brought significant improvements in yeast diagnostics and strain typing (33). Molecular typing approaches for *C. parapsilosis* include restriction fragment length polymorphism analysis, DNA fingerprinting, protein and tRNA profiling, PCR, and electrophoretic karyotype analysis (4, 6, 12, 27, 29, 30, 35).

Although mitochondrial DNA (mtDNA) is typically portrayed as a circular molecule, the mitochondrial genomes of many organisms are linear double-stranded DNA molecules (23). Recent analyses of mtDNA in various yeasts revealed that closely related species differ in the form of the mtDNA. A relatively high occurrence of the linear form of the mitochondrial genome was found in species of the genera *Pichia*, *Williopsis* and *Candida* (8, 21). Among the special molecular features of linear mitochondrial genomes are telomeres, the structures present at the ends of a linear DNA molecule. Inspection of linear mitochondrial genomes revealed several distinct types of mitochondrial telomeres. In the yeast *C. parapsilosis*, mitochondrial telomeres consist of long arrays of tandem repeats of a 738-bp unit. Detailed analysis revealed that the mtDNA molecules terminate with an incomplete repeat unit possessing a 5′ single-stranded extension. The extreme end of the molecule is specifically recognized by the mitochondrial telomere-binding protein (mtTBP) that protects the single-stranded overhang from enzymatic degradation (21, 24, 34).

Molecular diagnostics in clinical microbiology require rapid and highly selective methods for identification of pathogenic microorganisms. In general, species-specific procedures take advantage of the unique traits of a pathogenic microorganism. Since mitochondrial telomeres represent a unique feature of the linear form of mtDNA, we propose that they may represent specific molecular markers suitable for identification of organisms harboring linear mitochondrial genomes. Here we tested the pathogenic yeast *C. parapsilosis*, whose close relatives (e.g., *C. albicans* and *C. tropicalis* [32, 37]) possess circular genomes in their mitochondria. Our results demonstrate that the nucleotide sequence of mitochondrial telomeres and the antigenic properties of the protein specifically binding to this sequence have great potential for facilitating the molecular identification of *C. parapsilosis.*

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MATERIALS AND METHODS

Yeast strains. Yeasts were obtained from the Centraalbureau voor Schimmelcultures (CBS), Delft, The Netherlands. *C. parapsilosis* SR23 (CBS 7157) and *Saccharomyces cerevisiae* W303-1A are laboratory strains from the collection of

the Department of Biochemistry, Comenius University, Bratislava, Slovakia. *C. parapsilosis* strains designated MCO and PL were kindly provided by P. F. Lehmann (Medical College of Ohio, Toledo) and S. A. Meyer (Georgia State University, Atlanta), respectively. Yeast cells were grown on YPD plates (1% [wt/vol] yeast extract [Difco], 1% [wt/vol] Bacto Peptone [Difco], 2% [wt/vol] glucose, 2% [wt/vol] agar) at 28°C.

Amplification by PCR and gel electrophoresis. Yeast cells (approximately 10^4) from a single colony grown overnight on a fresh YPD plate were picked with a yellow tip (Gilson pipette) and resuspended in 20 μl of 50 mM KCl–10 mM Tris-HCl (pH 9.0)–0.1% (wt/vol) Triton X-100. The suspension was heated at 95 to 100°C for 5 to 10 min and then centrifuged briefly (10,000 × *g* for 5 s) to suppress condensation. Alternatively, cells were resuspended in 20 mM NaOH (20 μl) and incubated for 5 to 10 min at room temperature. PCRs (20-μl final volume) were performed with 50 mM KCl–10 mM Tris-HCl (pH 9.0)–0.1% (wt/vol) Triton X-100–1.25 mM $MgCl_2$–0.2 mM each deoxynucleoside triphosphate–0.5 μM each primer–2 μl of cell lysate–*Taq* DNA polymerase (0.5 to 2 U per reaction mixture). PCR primers (5′-CTTGTGCTGGCGATGGTTCA-3′, 5′-GCTCTCAATCTGTCAATCCT-3′, 5′-TAAATTTATGTATATGTTTGCA TATATCTTA-3′, and 5′-TAGGGATTGATTATTTACCTATATATTATCA-3′) were designed with the Vector NTI 4.0 software package (InforMax Inc.) and synthesized by Genset. Reactions were prepared on ice by combining 18 μl of premixed reaction components (master mix) and 2 μl of cell lysate (see above). Amplifications were started in a preheated DNA Thermal Cycler 480 (Perkin-Elmer Cetus) with the following standard three-step program: 3 min at 95°C, followed by 25 cycles of 45 s at 94°C, 1 min at 49°C, and 30 s at 72°C and then 5 min at 72°C. The samples were separated by agarose gel electrophoresis (1.5% [wt/vol] containing 0.5 μg of ethidium bromide per ml) at 5 to 10 V/cm for 45 to 60 min in 90 mM Tris-borate buffer.

Immunoblotting. Yeast cells were grown until the late logarithmic phase in YPD medium (1% [wt/vol] yeast extract, 1% [wt/vol] peptone, 2% [wt/vol] glucose), and cells (0.1 ml of the culture) were washed with double-distilled water and lysed for 5 min at 95°C in 0.1 ml of 1× sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis loading buffer (62.5 mM Tris-HCl [pH 6.8], 2% [wt/vol] SDS, 5% [vol/vol] 2-mercaptoethanol, 10% [vol/vol] glycerol) as described by Horvath and Riezman (13). Proteins were separated by SDS–13% (wt/vol) polyacrylamide gel electrophoresis (16). Resolved proteins were transferred to nitrocellulose filters in transfer buffer (25 mM Tris, 192 mM glycine, 20% [vol/vol] methanol [pH 8.3]) with a semidry electroblotter system (Panther HEP-1; Owl Scientific, Portsmouth, N.H.) for 60 min at 200 mA. Filters were blocked for 2 h at room temperature with blocking solution (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 2% [wt/vol] skim milk [Difco]) and then incubated overnight at 4°C in blocking solution containing anti-mtTBP polyclonal rabbit antibody SE1785 at a 1:200 dilution (24). Membranes were washed four times with rinsing buffer (20 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.05% [vol/vol] Tween 20) and once with rinsing buffer lacking Tween 20 and then incubated with the blocking solution containing a 1:3,000 dilution of a goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Sigma) for 2 h at room temperature. Blots were washed as described above and developed by the addition of 0.3 mg of *p*-nitroblue tetrazolium chloride (Sigma) per ml and 0.15 mg of 5-bromo-4-chloro-3-indolylphosphate toluidine salt (Sigma) per ml in alkaline phosphatase buffer (100 mM $NaHCO_3$, 1 mM $MgCl_2$ [pH 9.8]) for 5 to 20 min at room temperature.

Miscellaneous. mtDNA was prepared as described by Defontaine et al. (7), digested with the restriction endonucleases *Bgl*II, *Hin*dIII, *Pvu*II, and *Eco*RV (New England Biolabs) in accordance with the manufacturer's instructions, and separated by agarose gel electrophoresis. Total cellular DNA was isolated from 5-ml yeast culture samples as described by Phillippsen et al. (26). DNA samples for pulsed-field gel electrophoresis were prepared as described previously (22) and separated on a 0.8% (wt/vol) agarose gel in 45 mM Tris–borate–1 mM EDTA buffer in a Pulsaphor apparatus (LKB) in contour-clamped homogeneous electric field configuration. The pulse switching program for chromosomal DNA separation involved two steps of linear interpolation, i.e., 60 to 65 s for 2.5 h, followed by 65 to 600 s for 69.5 h, at 100 V and 9°C throughout.

Reproducibility of data. All PCR and immunoblot analyses were repeated at least twice with the same results.

RESULTS AND DISCUSSION

Mitochondrial telomeres as molecular markers. The mitochondrial genomes of many organisms were found to be represented by linear DNA molecules possessing telomeres (23). The structure and nucleotide sequence of mitochondrial telomeres appear to be species specific and thus may represent useful molecular markers applicable in molecular diagnostics.

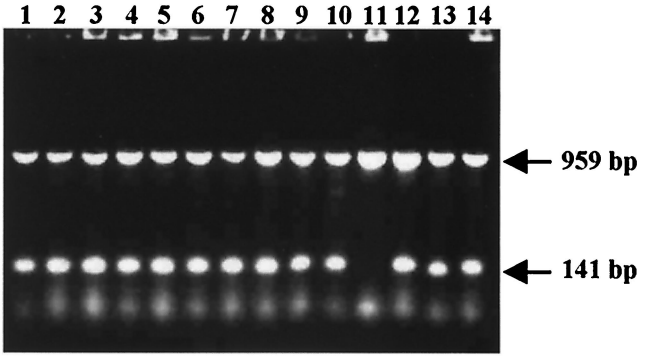

FIG. 1. Identification of *C. parapsilosis* strains with mitochondrial telomere-derived (*C. parapsilosis*-specific) and 18S rRNA gene-derived (yeast-specific) primers. PCR amplification and gel electrophoresis were performed as described in Materials and Methods. Lanes 1 to 14 contained samples of strains CBS 7157, CBS 604^T, CBS 1954, CBS 2152, CBS 2193, CBS 2195, CBS 2197, CBS 2211, CBS 2215, CBS 2916, CBS 5301, CBS 6318, CBS 8050, and CBS 8181. Arrows indicate the positions of the mitochondrial telomere-specific PCR product (141 bp) and the 18S rRNA-specific PCR product (959 bp).

To develop an approach for identification of *C. parapsilosis*, we designed two pairs of oligonucleotide primers for PCR amplification (see Materials and Methods). The oligonucleotides were derived from nucleotide sequences of *C. parapsilosis* mitochondrial telomeres (21) (EMBL data library accession numbers X76196 and X76197) and the conserved region of the nuclear 18S rRNA gene (accession number M60307) to serve as *C. parapsilosis*-specific and yeast-specific primers, respectively. The sensitivity of this system is based on a high redundancy of both molecular markers in *C. parapsilosis* cells. The redundancy of mitochondrial telomeres is due to the repeated nature of telomeric sequences and the presence of multiple copies of the mtDNA. Similarly, the redundancy of the 18S rRNA gene is due to the presence of 100 to 200 copies within the nuclear genome. When cell lysates of *C. parapsilosis* (e.g., type strain CBS 604) were used as the source of template DNA, PCR resulted in amplification of two products of 141 and 959 bp derived from the mitochondrial telomere and the gene encoding the 18S rRNA, respectively (Fig. 1).

Analysis of non-*C. parapsilosis* species. Having an optimized protocol in hand, we were interested in the specificity of this approach. We selected 114 yeast strains belonging to 83 different species (Table 1). This collection contained mainly *Candida* species that are considered to be phylogenetically related to *C. parapsilosis*, including *C. albicans*, *C. dubliniensis*, *C. maltosa*, *C. tropicalis*, and *C. sojae* (1, 18, 19). In addition, yeast species known to harbor a linear mitochondrial genome (such as *Williopsis saturnus*, *Pichia pijperii*, *P. jadinii*, *P. philodendra*, *C. utilis*, *C. salmanticensis*, and *C. vartiovaarae*) were added to the list. Finally, the panel also included 10 strains of *Lodderomyces elongisporus*, as this species was found to be the most closely related to *C. parapsilosis* phylogenetically (15, 19) and previously was even considered to be its teleomorphic form (11, 20). The results of PCR analysis showed that the *C. parapsilosis*-specific product was not amplified in any of these species

TABLE 1. List of yeast species/strains tested for the presence of the *C. parapsilosis* mitochondrial telomere-specific PCR product

Species	Strain[a]	18S rRNA product	Mitochondrial telomere product
Candida akabanensis	CBS 7878^T	+	−
Candida albicans	CBS 562^{NT}	+	−
Candida albicans	CBS 1949	+	−
Candida albicans	CBS 2716	+	−
Candida albicans	CBS 5983	+	−
Candida albicans	CBS 6431	+	−
Candida apicola	CBS 7444	−	−
Candida berthetii	CBS 6113	+	−
Candida boidinii	CBS 7447	+	−
Candida butyri	CBS 6421^T	+	−
Candida cantarellii	CBS 4878^T	+	−
Candida caseinolytica	CBS 7881	+	−
Candida catenulata	CBS 565^T	−	−
Candida catenulata	CBS 2014	−	−
Candida catenulata	CBS 6174	−	−
Candida cellulolytica	CBS 7920^T	+	−
Candida diddensiae	CBS 2214^T	+	−
Candida dubliniensis	CBS 7987^T	+	−
Candida entomaea	CBS 6306^T	+	−
Candida ergatensis	CBS 6248^T	+	−
Candida ernobii	CBS 1737^T	+	−
Candida ethanolica	CBS 8041^T	+	−
Candida fabianii	CBS 5481^T	+	−
Candida fermentati	CBS 8302	+	−
Candida floricola	CBS 7289^T	+	−
Candida fluviatilis	CBS 6776^T	+	−
Candida friedrichii	CBS 4114^T	+	−
Candida fukuyamaensis	CBS 7921^T	+	−
Candida glabrata	CBS 138^T	+	−
Candida homilentoma	CBS 6312^T	+	−
Candida inconspicua	CBS 180^T	−	−
Candida insectamans	CBS 6033^T	+	−
Candida intermedia	CBS 572^T	+	−
Candida ishiwadae	CBS 7401	+	−
Candida krissii	CBS 6519^T	+	−
Candida magnoliae	CBS 166^T	−	−
Candida magnoliae	CBS 2677	−	−
Candida magnoliae	CBS 3086	−	−
Candida magnoliae	CBS 6201	−	−
Candida maltosa	CBS 5611^T	+	−
Candida melibiosica	CBS 5814^T	+	−
Candida membranifaciens	CBS 6060	+	−
Candida nitratophila	CBS 2027^T	+	−
Candida norvegica	CBS 2874	+	−
Candida oleophila	CBS 8269	+	−
Candida ooitensis	CBS 7299^T	+	−
Candida oregonensis	CBS 5036^T	+	−
Candida ovalis	CBS 7298^T	+	−
Candida paludigena	CBS 8005^T	+	−
Candida pararugosa	CBS 1010^T	+	−
Candida pignaliae	CBS 6071^T	+	−
Candida pini	CBS 970^T	+	−
Candida pseudolambica	CBS 2063^T	+	−
Candida pseudotropicalis	CBS 607^T	+	−
Candida quercuum	CBS 6422^T	+	−
Candida rhagii	CBS 4237^T	+	−
Candida santjacobensis	CBS 8183^T	+	−
Candida sake	CBS 159^T	+	−
Candida sake	CBS 5093	+	−
Candida salmanticensis	CBS 5121^T	−	−
Candida savonica	CBS 6563^T	+	−
Candida sequanensis	CBS 8118^T	+	−
Candida shehatae	CBS 5813^T	−	−
Candida schatavii	CBS 6452^T	+	−
Candida silvae	CBS 5498^T	−	−
Candida silvanorum	CBS 6274^T	+	−
Candida sojae	CBS 7871^T	+	−
Candida sonorensis	CBS 6792^T	+	−
Candida sorbophila	CBS 7922	+	−
Candida sp.	CBS 5927	+	−
Candida sp.	CBS 8262	+	−
Candida stellimalicola	CBS 7853^T	+	−
Candida succiphila	CBS 7297	+	−
Candida tenuis	CBS 615^T	+	−
Candida tenuis	CBS 2309	+	−
Candida tropicalis	CBS 94^T	+	−
Candida tropicalis	CBS 643	+	−
Candida tropicalis	CBS 2321	+	−
Candida tropicalis	CBS 2323	+	−
Candida tropicalis	CBS 6719	+	−
Candida tropicalis	CBS 6948	+	−
Candida tropicalis	CBS 5701	+	−
Candida tropicalis	CBS 7923	+	−
Candida utilis	CBS 621^T	+	−
Candida vaccinii	CBS 7318^T	−	−
Candida vartiovaarae	CBS 4289^T	+	−
Candida versatilis	CBS 1752^T	−	−
Candida vini	CBS 639	−	−
Candida vini	CBS 2122	−	−
Candida zeylanoides	CBS 619^{NT}	+	−
Clavispora lusitaniae	CBS 6936^T	+	−
Kluyveromyces lactis	CBS 2359	+	−
Lodderomyces elongisporus	CBS 1946	+	−
Lodderomyces elongisporus	CBS 2605^T	+	−
Lodderomyces elongisporus	CBS 2606	+	−
Lodderomyces elongisporus	CBS 5912	+	−
Lodderomyces elongisporus	CBS 6120	+	−
Lodderomyces elongisporus	CBS 6180	+	−
Lodderomyces elongisporus	CBS 6181	+	−
Lodderomyces elongisporus	CBS 6182	+	−
Lodderomyces elongisporus	CBS 6298	+	−
Lodderomyces elongisporus	CBS 7803	+	−
Pichia canadensis	CBS 1992^T	+	−
Pichia jadinii	CBS 1600^T	+	−
Pichia kluyveri	CBS 7907	+	−
Pichia pastoris	CBS 704^T	−	−
Pichia philodendri	CBS 6075^T	+	−
Pichia pijperi	CBS 2887^T	+	−
Saccharomyces cerevisiae	W303-1A	+	−
Williopsis saturnus var. *mrakii*	CBS 1707^T	+	−
Williopsis saturnus var. *saturnus*	CBS 5761^T	+	−
Williopsis saturnus var. *suaveolens*	CBS 255^T	+	−
Williopsis saturnus var. *suaveolens*	CBS 1670	+	−
Yarrowia lipolytica	CBS 599^T	−	−

[a] A superscript capital T or NT indicates the type or neotype strain of the species, respectively.

TABLE 2. List of *C. parapsilosis* strains tested for the presence of mitochondrial telomere-specific PCR product.

Species	Strain[a]	18S rRNA product	Mitochondrial telomere product
Candida parapsilosis	CBS 604^T (I)	+	+
Candida parapsilosis	CBS 1954	+	+
Candida parapsilosis	CBS 2152	+	+
Candida parapsilosis	CBS 2193	+	+
Candida parapsilosis	CBS 2194	+	+
Candida parapsilosis	CBS 2195	+	+
Candida parapsilosis	CBS 2197	+	+
Candida parapsilosis	CBS 2211	+	+
Candida parapsilosis	CBS 2215 (I)	+	+
Candida parapsilosis	CBS 2916	+	+
Candida parapsilosis	CBS 6318	+	+
Candida parapsilosis	CBS 7157 (SR23)	+	+
Candida parapsilosis	CBS 8050 (I)	+	+
Candida parapsilosis	CBS 8181 (I)	+	+
Candida parapsilosis	CBS 5301	+	−
Candida parapsilosis	MCO 433 (I)	+	+
Candida parapsilosis	MCO 441 (I)	+	+
Candida parapsilosis	MCO 448 (III)	+	+
Candida parapsilosis	MCO 456 (II)	+	−
Candida parapsilosis	MCO 457 (II)	+	−
Candida parapsilosis	MCO 462 (II)	+	−
Candida parapsilosis	MCO 471 (II)	+	+
Candida parapsilosis	MCO 478 (I)	+	+
Candida parapsilosis	PL 429 (III)	+	+
Candida parapsilosis	PL 448 (III)	+	−
Candida parapsilosis	PL 452 (II)	+	−

[a] A superscript capital T indicates the type strain of the species. I, II, or III is the group of *C. parapsilosis* as defined by Lin et al. (17) and Roy and Meyer (28).

(Table 1). In 18 strains belonging to 12 different species, neither the mitochondrial telomere-specific nor the 18S rRNA-derived PCR product was amplified. A closer inspection of these cases revealed that these results were due to the presence of an inhibitor of *Taq* DNA polymerase originating from the cell lysates, since the amplifications performed on purified DNA samples yielded the 18S rRNA PCR product (e.g., *C. apicola*, *C. magnoliae*, *C. salmanticensis*, and *C. shehatae*). Alternatively, a divergence in the nucleotide sequence of the 18S rRNA (e.g., *Yarrowia lipolytica*) may be responsible for the lack of a PCR product.

These results demonstrate the specificity of a mitochondrial telomere-derived marker since the corresponding PCR products could not be generated in samples from non-*C. parapsilosis* species. The high selectivity of this approach is also illustrated by the ability to discriminate between *L. elongisporus* and *C. parapsilosis.*

Survey of *C. parapsilosis* strains. Genetic heterogeneity has been reported in *C. parapsilosis* (2, 4, 5, 18, 25, 30). Recently, it has been demonstrated that *C. parapsilosis* isolates can be divided into three distinct genotype groups (17, 28). The differences between these groups are profound, and it was suggested they may represent distinct species (5, 6, 17, 28). Due to the genetic heterogeneity mentioned above, it was of interest to determine whether a molecular marker based on mitochondrial telomeres could allow discrimination among these groups (Table 2).

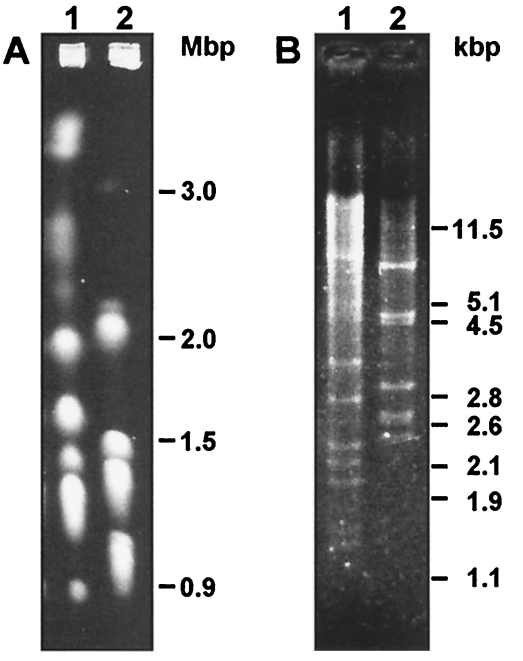

FIG. 2. Comparison of electrophoretic karyotypes (A) and *Bgl*II restriction enzyme digestion patterns of mtDNA (B) of strains CBS 5301 (lane 1) and CBS 604 (lane 2) (see Materials and Methods).

First, we examined 15 different *C. parapsilosis* strains obtained from the CBS yeast collection. All of these strains, except CBS 5301, reproducibly yielded both of the PCR products described above, indicating positive identification as *C. parapsilosis* (Fig. 1). To test the hypothesis that the result obtained with CBS 5301 was caused by its genetic difference from other *C. parapsilosis* strains, we analyzed this case in more detail. To exclude the possibility that the cells used for analysis contained incidental contamination, we analyzed a new sample of this strain from the CBS collection and obtained the same results. Based on physiologic and morphologic criteria, E. Slavikova (Czechoslovak Culture of Yeasts, Chemical Institute of Slovak Academy of Sciences, Bratislava, Slovakia) recognized this strain as non-L-arabinose-utilizing form II of *C. parapsilosis*. However, identification by the API 20C kit (Biomerieux, Marcy l'Etoile, France) revealed that the biotype of CBS 5301 (i.e., 6176171) differs from that typical of *C. parapsilosis* strains (i.e., 6756171) due to the absence of L-arabinose assimilation and weak utilization of D-xylitol. Also, comparison of the electrophoretic karyotypes and mtDNA restriction enzyme digestion patterns of CBS 5301 and CBS 604 revealed remarkable differences in nuclear and mitochondrial genome organization (Fig. 2) corresponding to the absence of mitochondrial telomere-derived PCR products in CBS 5301 cells. Thus, according to the molecular criteria, strain CBS 5301 seems to be substantially different from *C. parapsilosis* type strain CBS 604 and may represent a distinct species.

Next, we analyzed clinical isolates belonging to three different genotype groups of *C. parapsilosis* as defined by Lin et al. (17) and Roy and Meyer (28). PCR amplification on lysates

TABLE 3. Detection of the 15 kDa band in the lysates of various yeast species with anti-mtTBP antibody SE1785

Species	Strain[a]	Presence of 15-kDa protein[b]	Cross-reacting band molecular mass(es) (kDa)
Candida albicans	CBS 562^{NT}	−	
Candida caseinolytica	CBS 7881	−	
Candida catenulata	CBS 565^{T}	−	90
Candida glabrata	CBS 138^{T}	−	45
Candida maltosa	CBS 5611^{T}	−	
Candida parapsilosis	CBS 604^{T}	+	
Candida parapsilosis	CBS 1954	+	
Candida parapsilosis	CBS 2152	+	
Candida parapsilosis	CBS 2193	+	
Candida parapsilosis	CBS 2194	+	
Candida parapsilosis	CBS 2195	+	
Candida parapsilosis	CBS 2197	+	
Candida parapsilosis	CBS 2211	+	
Candida parapsilosis	CBS 2215	+	
Candida parapsilosis	CBS 2916	+	
Candida parapsilosis	CBS 6318	+	
Candida parapsilosis	CBS 7157	+	
Candida parapsilosis	CBS 8050	+	
Candida parapsilosis	CBS 8181	+	
Candida parapsilosis	CBS 5301	−	
Candida parapsilosis	MCO 433 (I)	+	
Candida parapsilosis	MCO 441 (I)	+	
Candida parapsilosis	MCO 448 (III)	+	
Candida parapsilosis	MCO 456 (II)	+	
Candida parapsilosis	MCO 457 (II)	+	
Candida parapsilosis	MCO 462 (II)	+	
Candida parapsilosis	MCO 471 (II)	+	
Candida parapsilosis	MCO 478 (I)	+	
Candida parapsilosis	PL 429 (III)	+	
Candida parapsilosis	PL 448 (III)	+	
Candida parapsilosis	PL 452 (II)	+	
Candida pararugosa	CBS 1010^{T}	−	
Candida pseudotropicalis	CBS 607^{T}	−	
Candida rhagii	CBS 4237^{T}	−	35, 40, 45, 50
Candida sake	CBS 159^{T}	−	
Candida salmanticensis	CBS 5121^{T}	−	25, 30, 33, 43, 50, 60, 70
Candida shehatae	CBS 5813^{T}	−	
Candida tropicalis	CBS 94^{T}	−	
Candida utilis	CBS 621^{T}	−	27, 35, 40, 45
Clavispora lusitaniae	CBS 6936^{T}	−	30, 40, 45, 50
Kluyveromyces lactis	CBS 2359	−	43
Lodderomyces elongisporus	CBS 2605^{T}	−	55
Lodderomyces elongisporus	CBS 2606	−	55
Lodderomyces elongisporus	CBS 5912	−	55
Lodderomyces elongisporus	CBS 6120	−	55
Lodderomyces elongisporus	CBS 6181	−	55
Lodderomyces elongisporus	CBS 6182	−	55
Pichia canadensis	CBS 1992^{T}	−	43
Pichia jadinii	CBS 1600^{T}	−	27, 35, 40, 45
Pichia pastoris	CBS 704^{T}	−	25, 45
Pichia philodendri	CBS 6075^{T}	−	43, 70, 90
Pichia pijperi	CBS 2887^{T}	−	29, 37, 45
Williopsis saturnus var. *mrakii*	CBS 1707^{T}	−	
Williopsis saturnus var. *saturnus*	CBS 5761^{T}	−	43
Williopsis saturnus var. *suaveolens*	CBS 255^{T}	−	43
Williopsis saturnus var. *suaveolens*	CBS 1670	−	43
Yarrowia lipolytica	CBS 599^{T}	−	

[a] A superscript capital T or NT indicates the type or neotype strain of the species. I, II, or III is the group as defined in reference 17 and 28.

[b] Plus and minus signs indicate the presence and absence, respectively, of the 15-kDa protein recognized by the antibody. Molecular masses are those of minor cross-reacting proteins present in lysates of the corresponding yeast strains.

from all group I strains reproducibly yielded both products. However, tests of four strains belonging to group II (MCO456, MCO457, MCO462, and PL452) and one group III strain (PL448) did not display PCR products derived from mitochondrial telomeres. This discrepancy may be attributed to the variability of *C. parapsilosis* mentioned above and requires further examination of the genetic relatedness of strains belonging to different groups by alternative approaches.

The reproducible results of the PCR analysis of the group I strains (type strain CBS 604 group) that predominate among clinical isolates (17) correspond well to the stability of restriction enzyme digestion patterns of mtDNA previously observed by Camougrand et al. (3) and further strengthen the idea that mtDNA-derived markers can be used for appropriate identification of this yeast in clinical samples.

Immunoblotting approach. The use of the mitochondrial telomere sequences for *C. parapsilosis* identification may be complemented by detection of proteins that specifically interact with the terminal structures of mtDNA. We recently purified the first mtTBP from *C. parapsilosis* and subsequently cloned the corresponding gene (24, 34). Rabbit antisera raised against the recombinant form of mtTBP were then examined for suitability for *C. parapsilosis* identifications. Western blot analysis of 57 strains belonging to 24 yeast species demonstrated the presence of a 15-kDa protein corresponding to mtTBP only in *C. parapsilosis* strains (Table 3), illustrating the specificity of this approach. The immunoblot analyses of strains belonging to *C. parapsilosis* groups I, II, and III always demonstrated the presence of the 15-kDa marker, indicating a close relationship among these strains. On the other hand, the differences between the PCR and immunoblot approaches observed in some isolates from groups II and III of *C. parapsilosis* have to be further investigated in terms of genetic polymorphism. Analogous to the results mentioned above (PCR approach, electrophoretic karyotype, mtDNA restriction enzyme pattern, API 20C) CBS 5301 did not display the 15-kDa antigen. Other yeast species (including the most closely related species, *L. elongisporus*) tested by immunoblot analysis yielded either no or only minor cross-reacting bands with higher molecular weights (Table 3).

Taken together, our results illustrate that mitochondrial telomeres are suitable targets for identification of *C. parapsilosis* strains. Moreover, the utilization of mitochondrial telomere-derived molecular markers is not limited to *C. parapsilosis* but, in principle, may be applied to the identification of other microorganisms associated with human or animal infections, provided they possess linear mitochondrial genomes (e.g., *C. utilis* [8] *Theileria annulata*, and *T. parva* [31]). The specificity of mitochondrial telomere-derived molecular markers, together with the sensitivity and versatility of the PCR and immunoblot assays, would enable the adaptation and optimization of this approach for the direct detection of *C. parapsilosis* in clinical samples or mixed fungal populations without prior cultivation and/or purification of yeasts. In addition to PCR and immunoblot analyses, dot blot or DNA microarray methods might become alternative tools for employing mitochondrial telomere-derived molecular markers in molecular diagnostics.

ACKNOWLEDGMENTS

We thank L. Kováč (Comenius University, Bratislava, Slovakia) for continuous support, helpful discussions, and comments; P. F. Lehmann (Medical College of Ohio, Toledo) and S. A. Meyer (Georgia State University, Atlanta) for providing *C. parapsilosis* MCO and PL isolates; A. Vaughan-Martini (University of Perugia, Perugia, Italy) for the gift of the type strain of *L. elongisporus*; and D. Subramanian, R. Stansel, and T. Cesare (University of North Carolina, Chapel Hill) for reading the manuscript and editorial advice.

This work was supported in part by grants from the European Union (BIO4-CT96-0003), the Howard Hughes Medical Institute (55000327), the Slovak grant agency VEGA (1/6168/99 and 1/7248/00), and a Fogarty International Research Collaboration Award (1-R03-TW05654-01). J.N. was supported by a postdoctoral fellowship from the Ministry of Education of the French government.

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