JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2008, p. 3237–3242 Vol. 46, No. 10
0095-1137/08/$08.00+0 doi:10.1128/JCM.01451-08
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Debaryomyces hansenii (*Candida famata*), a Rare Human Fungal Pathogen Often Misidentified as *Pichia guilliermondii* (*Candida guilliermondii*)▽†

Marie Desnos-Ollivier,[1] Marie Ragon,[1]‡ Vincent Robert,[2] Dorothée Raoux,[1] Jean-Charles Gantier,[1] and Françoise Dromer[1]*

Institut Pasteur, Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire; CNRS URA 3012, Paris, France,[1] and Centraalbureau voor Schimmelcultures, Comparative Genomics and Bioinformatics, Utrecht, The Netherlands[2]

Received 29 July 2008/Accepted 1 August 2008

Debaryomyces hansenii **is a hemiascomycetous yeast commonly found in natural substrates and in various types of cheese.** ***Pichia guilliermondii*** **is widely distributed in nature and is a common constituent of the normal human microflora. Both species have been described in human infections but are extremely difficult to differentiate phenotypically. Thus, frequent errors in identification occur. The 62 clinical and environmental isolates sent between 2000 and 2007 to the French National Reference Center for Mycoses and Antifungals as** ***D. hansenii*** **or** ***P. guilliermondii*** **were analyzed by using the carbon assimilation pattern, the presence of pseudohyphae, and sequencing of the ITS and D1/D2 regions of the rRNA gene. The objective of this study was to assess using nucleotide sequences whether phenotypic identification was accurate and whether phenotypic characteristics could be used to differentiate the two species when sequencing was not available. We found that 58% of the isolates were misidentified and belong to seven different species:** ***P. guilliermondii*****,** ***P. caribbica*****,** ***P. jadinii*****,** ***D. hansenii*****,** ***Candida palmioleophila*****,** ***C. haemulonii*** **type II, and** ***Clavispora lusitaniae*****. In conclusion,** ***D. hansenii*** **may not be as common a human pathogen as previously thought. Sequencing of either ITS or D1/D2 regions is a good tool for differentiating the species more frequently confused with** ***D. hansenii*****, keeping in mind that reliable databases should be used.**

Debaryomyces hansenii is a hemiascomycetous yeast commonly found in natural substrates and in various types of cheese (3, 26). It has been described in human infections (11, 31, 35). However, its incidence during candidemia is low based on data surveillance implemented by the Centers for Disease Control and Prevention (9), whereas one review on worldwide-collected isolates states that *D. hansenii* accounts for 0.08 to 0.5% of isolates recovered during invasive candidiasis (22). *Pichia guilliermondii* is widely distributed in nature (routinely isolated from insects, soil, plants, atmosphere, seawater, the exudates of various trees, and processed foods) and is a common constituent of the normal human microflora (19). Globally, this species and its anamorphic state *Candida guilliermondii* accounts for 1 to 2% of all candidemia (14, 23). However, the species *D. hansenii* (*Candida famata*) and *P. guilliermondii* are extremely difficult to differentiate phenotypically (17, 18). Thus, frequent errors in identification occur. We undertook a retrospective analysis of all isolates, mostly recovered from clinical specimens sent to the French National Reference Center for Mycoses and Antifungals (NRCMA) as *D. hansenii* or *P. guilliermondii*. The objective of the present study was to assess using nucleotide sequences whether phenotypic identification was correct and whether phenotypic characteristics could be used to differentiate the two species when sequencing was not available.

MATERIALS AND METHODS

Strains. All of the epidemiologically unrelated clinical or environmental isolates ($n = 41$) sent between September 2000 and April 2007 as *D. hansenii* or *P. guilliermondii* to the NRCMA for confirmation of the identification were selected. In addition, 21 epidemiologically unrelated clinical or environmental isolates were collected for this specific study (Table 1). Type strains for the anamorph of each species were obtained from the Centraalbureau voor Schimmelcultures (CBS; Utrecht, The Netherlands) (see Table S1 in the supplemental material). All isolates were stored frozen in 40% glycerol at −80°C.

Phenotypic characterization of isolates. Carbon assimilation patterns were obtained with the commercialized strips (ID32C; bioMérieux, Marcy l'Etoile, France). The presence of pseudohyphae was determined after growth for 24 h and 5 days of incubation at 30°C in potato-carrot-ox bile medium (used routinely in France instead of cornmeal agar or rice agar) (5). The MICs of amphotericin B, flucytosine, fluconazole, voriconazole, and caspofungin were determined by using the EUCAST microdilution method (7). The concentrations corresponding to the MICs that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were determined.

Molecular characterization. After 24 h of incubation at 27°C on Sabouraud dextrose agar plates, single colonies were transferred to 1 ml of distilled water in a microcentrifuge tube, and DNA extraction was performed by using a High-Pure PCR template preparation kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's instructions. Universal fungal primers were used for the amplification of the ITS1-5.8S-ITS2 (primers V9D [8] and LS266 [15]) and 26S (primers NL1 and NL4) (20) ribosomal DNA regions. Reaction volumes of 50 μl contained 3 μl of genomic DNA, 2.5 U of AmpliTaq Gold, 5 μl of PCR buffer 10×, 5 μl of $MgCl_2$ 25 mM, 5 μl of 2.5 mM deoxynucleoside triphosphate (Roche), and 1.25 μl of 20 μM primers. The PCR products were amplified by using an iCycler thermocycler (Bio-Rad, Marnes-La-Coquette, France) set up with a first cycle of denaturation for 10 min at 95°C, followed by 30 cycles of denaturation at 94°C for 30 s and 30 s at 58°C and elongation at 72°C for 30 s, with a final extension step of 10 min at 72°C. Both strands of purified amplified fragments were sequenced at the Genopole of the Pasteur Institute, on an ABI Prism 3700 DNA analyzer (Applied Biosystems,

* Corresponding author. Mailing address: Centre National de Référence Mycologie et Antifongiques, Unité de Mycologie Moléculaire, CNRS URA 3012, Institut Pasteur, 25 Rue du Docteur Roux, 75724 Paris, France. Phone: 33 1 40 61 33 89. Fax: 33 1 45 68 84 20. E-mail: dromer@pasteur.fr.

† Supplemental material for this article may be found at http://jcm.asm.org/.

‡ Present address: Département Génétique et Ecologie Evolutives, Laboratoire Ecologie Systématique et Evolution, CNRS UMR8079, Université de Paris-Sud, Orsay, France.

▽ Published ahead of print on 13 August 2008.

TABLE 1. Molecular identification, ID32C code, and production of pseudohyphae for the 62 clinical and environmental isolates sent to the NRCMA between September 2000 and April 2007 as *D. hansenii* or *P. guilliermondii*

Strain	Site of isolation	Yr of isolation	City, country	First identification	Identification sequence	ID32C code	Presence (+) or absence (–) of pseudohyphae
200500815	Blood	2001	Hambourg, Germany	*D. hansenii*	*D. hansenii*	5777375317 E+	–
200600362	Sputum	2006	Paris, France	*D. hansenii*	*D. hansenii*	5777174137 E+	–
200600935	Table surface	2006	Fort de France, Martinique	*D. hansenii*	*D. hansenii*	5777751137 E-	–
200501145	Sputum	2005	Paris, France	*D. hansenii*	*P. caribbica*	7577752117 E+	+
200501146	Mouth	2005	Paris, France	*D. hansenii*	*P. caribbica*	7577352117 E+	+
200600033	Blood	2006	Reims, France	*D. hansenii*	*P. caribbica*	5577370117 E+	+
200700035	BAL[a]	2006	Paris, France	*D. hansenii*	*P. caribbica*	7577770117 E+	+
200700175	Blood	2006	Paris, France	*D. hansenii*	*P. caribbica*	7577350117 E+	+
200700593	Blood	2007	Paris, France	*D. hansenii*	*P. caribbica*	7577350117 E+	+
200500086	Blood	2005	Paris, France	*D. hansenii*	*P. guilliermondii*	7577350117 E+	+
200500816	Environment	2001	Saint Malo, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	–
200500821	Environment	2002	Dreux, France	*D. hansenii*	*P. guilliermondii*	7577350117 E+	–
200501141	Stomach	2001	Paris, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	+
200501142	Urine	2001	Paris, France	*D. hansenii*	*P. guilliermondii*	7577350117 E+	+
200501144	BAL	2005	Paris, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	+
200501305	Blood	2005	Pontoise, France	*D. hansenii*	*P. guilliermondii*	7577350117 E+	+
200700040	Blood	2000	Reims, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	+
200700041	Blood	2000	Reims, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	+
200700261	Blood	1999	Reims, France	*D. hansenii*	*P. guilliermondii*	7577352117 E+	+
200400824	Sputum	2004	Fort de France, Martinique	*D. hansenii*	*C. haemulonii* type II	7167370317 E+	–
200600079	Catheter	2001	Paris, France	*D. hansenii*	*C. haemulonii* type II	7167370317 E-	+
200500823	Blood	2002	Paris, France	*D. hansenii*	*C. lusitaniae*	5357350117 E+	–
200501223	Tongue	2002	Paris, France	*D. hansenii*	*C. lusitaniae*	7357370117 E+	+
200500813	Catheter	2001	Bicêtre, France	*D. hansenii*	*C. palmioleophila*	5367352315 E-	–
200500825	Mouth	2002	Reims, France	*D. hansenii*	*C. palmioleophila*	5367352315 E-	–
200500840	Kidney	2003	Paris, France	*D. hansenii*	*C. palmioleophila*	5367352315 E+	–
200500808	Nose	2000	Paris, France	*P. guilliermondii*	*P. caribbica*	7577352117 E-	+
200500812	Blood	2003	Paris, France	*P. guilliermondii*	*P. caribbica*	7577750117 E-	+
200500862	Blood	2003	Paris, France	*P. guilliermondii*	*P. caribbica*	7577352117 E+	+
200500949	Blood	2005	Paris, France	*P. guilliermondii*	*P. caribbica*	7577350117 E+	+
200501000	Blood	2005	Paris, France	*P. guilliermondii*	*P. caribbica*	7577370117 E+	+
200501201	Blood	2005	Paris, France	*P. guilliermondii*	*P. caribbica*	7577750117 E+	+
200501316	BAL	2005	Paris, France	*P. guilliermondii*	*P. caribbica*	7577350117 E+	+
200501317	Sputum	2002	Paris, France	*P. guilliermondii*	*P. caribbica*	7577350117 E+	+
200600196	Sputum	2006	Paris, France	*P. guilliermondii*	*P. caribbica*	7577350117 E+	+
200600368	BAL	2006	Paris, France	*P. guilliermondii*	*P. caribbica*	7577370117 E+	–
200601082	Sputum	2006	Paris, France	*P. guilliermondii*	*P. caribbica*	7577750117 E+	+
200601194	Blood	2006	Paris, France	*P. guilliermondii*	*P. caribbica*	7577750117 E+	+
200500030	Blood	2004	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352115 E+	+
200500719	Blood	2005	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200500809	Selles	2003	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200500861	Blood	2003	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577372137 E+	+
200500863	Blood	2004	Paris, France	*P. guilliermondii*	*P. guilliermondii*	5577352117 E+	–
200500864	Spleen	2004	Marseille, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200500950	Blood	2005	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352115 E+	–
200501202	Blood	2005	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577350117 E+	–
200501314	Blood	2002	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200501319	Throat	2002	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577350117 E+	+
200501320	Sputum	2002	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200501343	Urine	2002	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200600032	Blood	2005	Angers, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200600130	Blood	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577350117 E+	+
200600335	Blood	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577350117 E+	+
200600561	BAL	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200600803	Blood	2006	Bondy, France	*P. guilliermondii*	*P. guilliermondii*	7577772117 E+	+
200600938	Blood	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E-	–
200601010	BAL	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200601023	Blood	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200601042	Blood	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E+	+
200601191	BAL	2006	Paris, France	*P. guilliermondii*	*P. guilliermondii*	7577352117 E-	+
200700329	Blood	2007	Chambéry, France	*P. guilliermondii*	*P. guilliermondii*	7577372117 E+	+
200500811	Blood	2003	Paris, France	*P. guilliermondii*	*P. jadinii*	4374350117 E+	+

[a] BAL, bronchoalveolar lavage.

Courtaboeuf, France), with the same primers that were used in the PCR step. Sequences were edited with Chromas Pro version 1.33 (Technelysium Pty., Ltd., Australia).

The sequences of the ITS1-5.8S-ITS2 regions were delimited by the sequences of the primers ITS1 and ITS4 (TCCGTAGGTGAACCTGCGG/GCATATCA ATAAGCGGAGGA) (36). In the same way, the sequences of the D1/D2 region of the 26S subunit were delimited by the sequence TTGRAATC (R = A or G) and by the sequence of the primer U1 (GTGAAATTGTTGAAAGGGAA) (29). The sequences were compared to those of the type strains to confirm species identification. The D1/D2 and ITS bounded sequences, including the sequences of the type strains, were aligned by using CLUSTAL W software (32). Phylogenetic analyses were performed by using the Bayesian Markov chain Monte Carlo method based on MrBayes software (28) and were based on a concatenated alignment of the ITS and D1/D2 datasets.

RESULTS

Among the 36 isolates first identified as *P. guilliermondii*, species identification was confirmed for 23 isolates, 12 were identified as *P. caribbica* based on the comparison of their ITS and 26S sequences with the type strain sequences (CBS 2022), and one was identified as *P. jadinii* based on both carbon assimilation profile and nucleotide sequences. Of the 26 isolates initially identified as *D. hansenii*, only 3 were confirmed as such, 10 were identified as *P. guilliermondii*, 6 were identified as *P. caribbica*, 3 were identified as *Candida palmioleophila*, and 2 belong to the currently recognized species *Candida haemulonii* type II and 2 to *Clavispora lusitaniae* (Table 1).

A Bayesian tree was constructed by using the bounded sequences of the ITS and 26S regions of all isolates, including the type strains (Fig. 1). The isolates of *P. guilliermondii* are divided into two closely related groups: one group included the type strain (CBS 566) and 20 of 33 isolates, and the second group contained 13 isolates. *P. caribbica* isolates were clustered into three groups: one group included the type strain (CBS 2022) and 15 of 18 isolates, and the second and the third groups included, respectively, 2 isolates and 1 isolate. Both species were closely related. The isolates of *D. hansenii*, including the type strain CBS 1795, were closely related to those of *C. palmioleophila.* Isolates of *P. jadinii*, *C. lusitaniae*, and *C. haemulonii* type II are well separated from these species and would have been using only one of the nucleotide sequences.

Analysis of carbon assimilation patterns by each species showed that all three isolates of *D. hansenii* assimilated DL-lactate, whereas none of the *P. guilliermondii* and *P. caribbica* isolates did so (Table 2). None of the *D. hansenii* isolates was resistant to 0.01% cycloheximide, whereas >90% of *P. guilliermondii* and *P. caribbica* isolates were. For *C. palmioleophila*, the carbon assimilation patterns were ambiguous with *D. hansenii* profiles. For *C. haemulonii* type II, *C. lusitaniae*, and *P. jadinii* the majority of the codes were lacking in the ID32C database or show a low discrimination with *D. hansenii*. None of the *D. hansenii* isolates was able to produce pseudohyphae, whereas 27 of 33 and 17 of 18 isolates of *P. guilliermondii* and *P. caribbica*, respectively, produce them. For isolates eventually identified as *P. guilliermondii* ($n = 33$) or *P. caribbica* ($n = 18$), different carbon assimilation profiles were obtained (eight and nine, respectively), some of which were shared by both species, one being a frequent code for both (see Table S2 in the supplemental material). Each isolate of *D. hansenii* had a unique profile that was not found for any of the *P. guilliermondii* and *P. caribbica* isolates. An unweighted pair-group method for arithmetic averages tree was constructed by using the significant carbon sources of the ID32C assimilation pattern for the 62 clinical and environmental isolates (see Fig. S1 in the supplemental material).

MICs of amphotericin B and flucytosine were similar for all *P. guilliermondii*, *P. caribbica*, and *D. hansenii* isolates (data not shown). The MICs of fluconazole (range, 0.124 to 2 μg/ml), and to a lesser degree those of voriconazole (<0.015 μg/ml) and caspofungin (<0.015 to 0.03 μg/ml), were lower for *D. hansenii* than those determined for *P. guilliermondii* (MIC_{50}/MIC_{90} [range] = 8/64 μg/ml [2 to ≥64] for fluconazole, 0.06/0.5 μg/ml [0.03 to 2] for voriconazole, and 0.125/1 μg/ml [0.014 to 2] for caspofungin) and for *P. caribbica* (MIC_{50}/MIC_{90} [range] = 4/64 μg/ml [1 to ≥64] for fluconazole, 0.125/0.25 μg/ml [0.06 to ≥8] for voriconazole, and 0.125/0.25 μg/ml [0.03 to 0.5] for caspofungin) isolates.

DISCUSSION

D. hansenii (teleomorph of *C. famata*) has been repeatedly associated with catheter-related bloodstream infection and rarely with other infections (4, 12, 24, 25, 27, 31). However, in most of the reported cases, the species was identified by using phenotypic parameters. Based on our results and on previous reports, one may wonder whether some of these cases could not have been misidentified. In our study, 36 of 62 (58%) of the isolates classified as *D. hansenii* or *P. guilliermondii* were misidentified. Isolates initially identified as *D. hansenii* belong to six different species (*D. hansenii*, *P. guilliermondii*, *P. caribbica*, *C. palmioleophila*, *C. haemulonii* type II, and *C. lusitaniae*). Among the 36 isolates initially identified as *P. guilliermondii*, 12 belong to the very closely related species *P. caribbica* and one was *P. jadinii* (Table 1). *D. hansenii* may thus be a frequent species of the gut microflora in individuals who like cheese. It does not seem to represent a frequent cause of fungemia. Whether nucleotide sequencing was used for the identification of the isolates to the species level in the ARTEMIS study reporting up to 0.5 and 1% of *C. famata* and *C. guilliermondii* among isolates responsible for invasive candidiasis is unknown. In the current active surveillance program on yeast fungemia in the Paris area, no cases were due to *D. hansenii*, whereas *P. guilliermondii* and *P. caribbica* were recovered in, respectively, 1 and 0.5% of the 1975 cases recorded thus far over the 5-year survey period (YEASTS program, unpublished data).

D. hansenii is an uncommon yeast characterized by its cryo- and halotolerance (6), but these criteria, as well as the production of ascospores, are not used in routine yeast identification. Analysis of carbon assimilation patterns showed that, in contrast to what is found for the majority of *P. guilliermondii* and *P. caribbica* isolates, all isolates of *D. hansenii* assimilated DL-lactate but were unable to produce pseudohyphae and were susceptible to 0.01% cycloheximide (Table 2) (3). No single *D. hansenii* carbon assimilation profile was shared with the other species studied here. Furthermore, there was a trend toward lower azole and caspofungin MICs than for *P. guilliermondii*, a finding not reported for echinocandins by Pfaller et al. (21).

P. guilliermondii is a genetically heterogeneous complex comprising several phenotypically indistinguishable taxa that have been brought into synonymy (3, 13, 34) or assigned new names (30). One of these synonymous species is *Candida fer-*

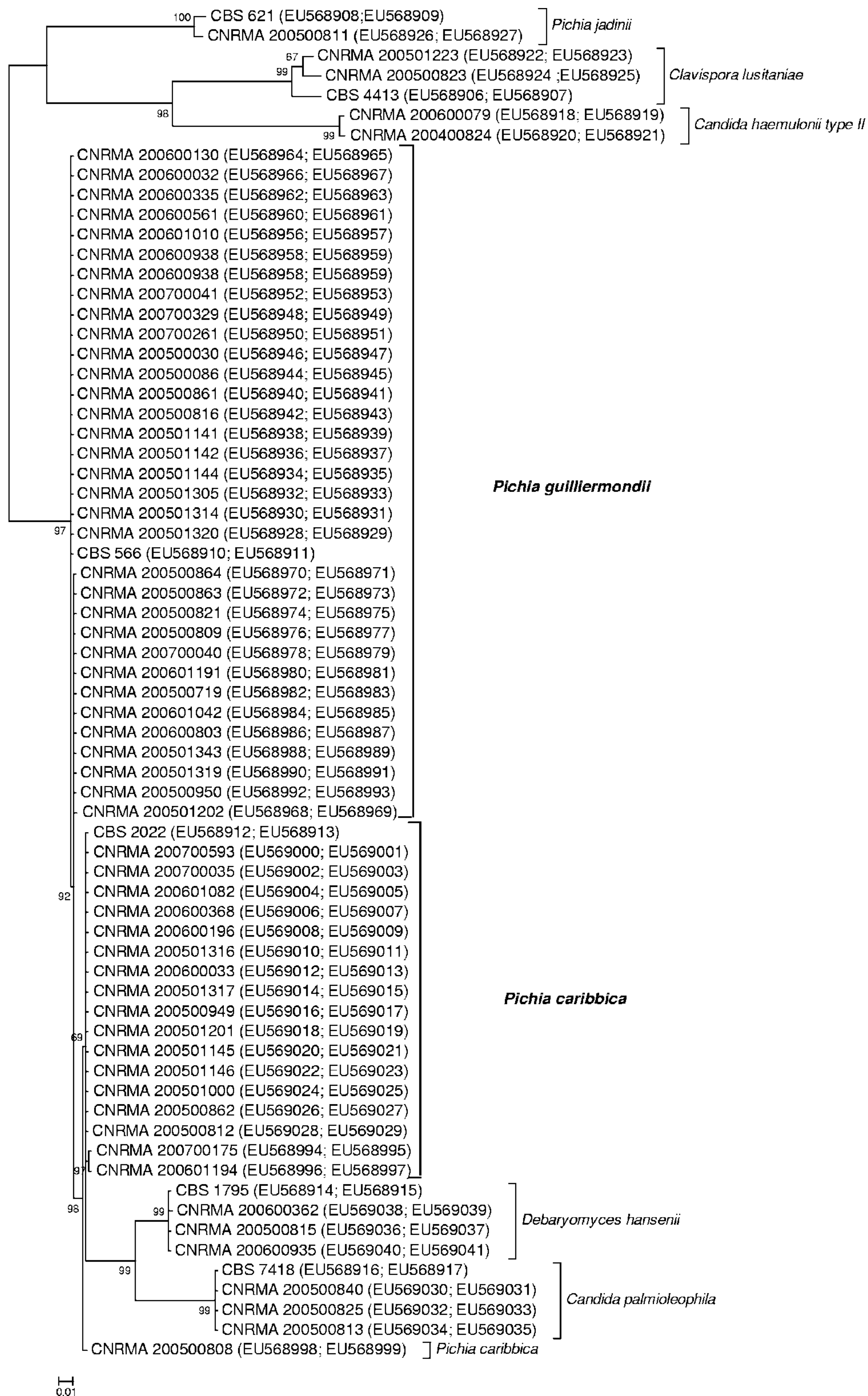

FIG. 1. Bayesian phylogenetic majority rule consensus tree of the combined D1/D2 and ITS datasets for the 62 clinical and environmental isolates and the type strains of *D. hansenii*, *P. guilliermondii*, *P. caribbica*, *C. palmioleophila*, *C. lusitaniae,* and *P. jadinii.* CBS numbers correspond to type strains. GenBank accession numbers D1/D2 and ITS datasets are indicated in parentheses. The scale bar represents the distance between strains (10%).

TABLE 2. Percentage of the clinical and environmental isolates of *P. guilliermondii*, *P. caribbica*, and *D. hansenii* forming pseudohyphae and exhibiting growth in the presence of discriminatory carbon sources[a]

Carbon source	% Species		
	P. guilliermondii (*n* = 33)	*P. caribbica* (*n* = 18)	*D. hansenii* (*n* = 3)
Cycloheximide (0.01%)	97	94	0
DL-Lactate	0	0	100
D-Xylose	100	100	66
Ribose	6	33	33
Ramnose	9	22	66
Erythritol	0	0	66
Melibiose	74	22	0
Glucuronate	0	0	66
Gluconate	0	0	33
Lactose	3	0	66
Sorbose	94	100	100
Esculine	91	89	66
Pseudohyphae	83	94	0

[a] *n*, Number of isolates.

mentati, described by Bai in 1996 (1). Changes in nomenclature could also explain additional mistakes such as the *P. jadinii* synonym of *C. guilliermondii* var. *nitratophila*. The teleomorphic state of *C. fermentati* has been recently described by Vaughan-Martini in 2005 based on molecular data and is named *Pichia caribbica* (34). Since *P. caribbica* has been described, many analyses using molecular tools (RAPD [random(ly) amplified polymorphic DNA], electrophoretic karyotyping, DNA composition, chromosomal DNA banding profiles, and D1/D2 sequences) reclassified clinical and environmental isolates phenotypically identified as *P. guilliermondii* into several species, including *P. guilliermondii* but also *P. caribbica* (2, 14, 30, 34). In our analyses, some carbon assimilation profiles were shared by the two species, one of the codes being a frequent code for both (see Table S2 in the supplemental material), and the similarity of the ITS and 26S sequences was greater than 98%.

In 1997, Nishikawa et al. designed specific primers in the large subunit region to differentiate *D. hansenii* and *P. guilliermondii* (17). Lan and Xu in 2006 proposed the sequencing of the *RIBO* gene to differentiate *P. caribbica* and *P. guilliermondii*, but the sequences seem too divergent within the same species (14). Recently, Tsui et al. (33) demonstrated that a polygenic sequencing method is best able to differentiate closely related species such as *P. guilliermondii* and *P. caribbica*. Here, sequencing of the ITS and 26S regions was sufficient, but using both enhances the chances of differentiation.

We also want to underline another pitfall related to these species' identification. Sequences recorded before 2004 and the complete sequencing of *D. hansenii* genome by Dujon et al. (10) in the GenBank database are sometimes erroneous. For example, the ITS sequence of strain CBS8417 (accession no. AF209874) deposited in 2001 as *D. hansenii* var. *fabryii* was reidentified in the CBS database (http://www.cbs.knaw.nl/yeast/BioloMICS.aspx) as *P. guilliermondii* in 2006. In the same way, the ITS sequence of strain BPY-01 recorded in GenBank as *D. hansenii* var. *fabryii* in 2002 (accession no. AY125962) is homologous with the ITS sequence of *P. guilliermondii* type strain CBS566. This underlines the importance of using databases such as that of the CBS, where taxonomy is more reliable than in public repositories as pointed out by Nilsson et al. (16).

In conclusion, *D. hansenii* may not be as common a human pathogen as previously thought. Sequencing of either the ITS or the D1/D2 region is a good tool for differentiating the species more frequently confused with *D. hansenii*, keeping in mind that reliable databases should be used.

ACKNOWLEDGMENTS

We thank the Institut de Veille Sanitaire for its financial support. The technical help of the sequencing facilities and specifically that of Isabelle Iteman, Anne-Sophie Delannoy, Rachel Lavenir, and Laure Diancourt (PF-8) is gratefully acknowledged.

REFERENCES

1. **Bai, F. Y.** 1996. Separation of *Candida fermentati* comb. nov. from *Candida guilliermondii* by DNA base composition and electrophoretic karyotyping. Syst. Appl. Microbiol. **19:**178–181.
2. **Bai, F. Y., H. Y. Liang, and J. H. Jia.** 2000. Taxonomic relationships among the taxa in the *Candida guilliermondii* complex, as revealed by comparative electrophoretic karyotyping. Int. J. Syst. Evol. Microbiol. **50**(Pt. 1):417–422.
3. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 2000. Yeasts: characteristics and identification, 3rd ed. Cambridge, University Press, Cambridge.
4. **Carrega, G., G. Riccio, L. Santoriello, M. Pasqualini, and R. Pellicci.** 1997. *Candida famata* fungemia in a surgical patient successfully treated with fluconazole. Eur. J. Clin. Microbiol. Infect. Dis. **16:**698–699.
5. **Casal, M., and M. J. Linares.** 1981. The comparison of six media for chlamydospore production by *Candida albicans*. Mycopathologia **76:**125–128.
6. **Corredor, M., A. M. Davila, S. Casaregola, and C. Gaillardin.** 2003. Chromosomal polymorphism in the yeast species *Debaryomyces hansenii*. Antonie van Leeuwenhoek **84:**81–88.
7. **Cuenca-Estrella, M., C. B. Moore, F. Barchiesi, J. Bille, E. Chryssanthou, D. W. Denning, J. P. Donnelly, F. Dromer, B. Dupont, J. H. Rex, M. D. Richardson, B. Sancak, P. E. Verweij, J. Rodríguez-Tudela, et al.** 2003. Multicenter evaluation of the reproducibility of the proposed antifungal susceptibility testing method for fermentative yeasts of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST). Clin. Microbiol. Infect. **9:**467–474.
8. **de Hoog, G. S., and G. A. H. van den Ende.** 1998. Molecular diagnostics of clinical strains of filamentous basidiomycetes. Mycoses **41:**183–189.
9. **Diekema, D. J., S. A. Messer, A. B. Brueggemann, S. L. Coffman, G. V. Doern, L. A. Herwaldt, and M. A. Pfaller.** 2002. Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa organisms study. J. Clin. Microbiol. **40:**1298–1302.
10. **Dujon, B., D. Sherman, G. Fischer, P. Durrens, S. Casaregola, I. Lafontaine, J. De Montigny, C. Marck, C. Neuveglise, E. Talla, N. Goffard, L. Frangeul, M. Aigle, V. Anthouard, A. Babour, V. Barbe, S. Barnay, S. Blanchin, J. M. Beckerich, E. Beyne, C. Bleykasten, A. Boisrame, J. Boyer, L. Cattolico, F. Confanioleri, A. De Daruvar, L. Despons, E. Fabre, C. Fairhead, H. Ferry-Dumazet, A. Groppi, F. Hantraye, C. Hennequin, N. Jauniaux, P. Joyet, R. Kachouri, A. Kerrest, R. Koszul, M. Lemaire, I. Lesur, L. Ma, H. Muller, J. M. Nicaud, M. Nikolski, S. Oztas, O. Ozier-Kalogeropoulos, S. Pellenz, S. Potier, G. F. Richard, M. L. Straub, A. Suleau, D. Swennen, F. Tekaia, M. Wesolowski-Louvel, E. Westhof, B. Wirth, M. Zeniou-Meyer, I. Zivanovic, M. Bolotin-Fukuhara, A. Thierry, C. Bouchier, B. Caudron, C. Scarpelli, C. Gaillardin, J. Weissenbach, P. Wincker, and J. L. Souciet.** 2004. Genome evolution in yeasts. Nature **430:**35–44.
11. **Gupta, A., H. Mi, C. Wroe, B. Jaques, and D. Talbot.** 2006. Fatal *Candida famata* peritonitis complicating sclerosing peritonitis in a peritoneal dialysis patient. Nephrol. Dial. Transplant. **21:**2036–2037.
12. **Krcmery, V., and A. Kunova.** 2000. *Candida famata* fungemia in a cancer patient: case report. J. Chemother. **12:**189–190.
13. **Kurtzman, C. P., and J. W. Fell.** 1998. The yeasts: a taxonomic study. Elsevier Science B.V., Amsterdam, The Netherlands.
14. **Lan, L., and J. Xu.** 2006. Multiple gene genealogical analyses suggest divergence and recent clonal dispersal in the opportunistic human pathogen *Candida guilliermondii*. Microbiology **152:**1539–1549.
15. **Masclaux, F., E. Gueho, G. S. de Hoog, and R. Christen.** 1995. Phylogenetic relationships of human-pathogenic *Cladosporium* (*Xylohypha*) species inferred from partial LS rRNA sequences. J. Med. Vet. Mycol. **33:**327–338.
16. **Nilsson, R. H., M. Ryberg, E. Kristiansson, K. Abarenkov, K. H. Larsson, and U. Köljalg.** 2006. Taxonomic reliability of DNA sequences in public sequence databases: a fungal perspective. PLos ONE **1:**e59.
17. **Nishikawa, A., T. Sugita, and T. Shinoda.** 1997. Differentiation between *Debaryomyces hansenii*/*Candida famata* complex and *Candida guilliermondii* by polymerase chain reaction. FEMS Immunol. Med. Microbiol. **19:**125–129.

18. **Nishikawa, A., H. Tomomatsu, T. Sugita, R. Ikeda, and T. Shinoda.** 1996. Taxonomic position of clinical isolates of *Candida famata*. J. Med. Vet. Mycol. **34:**411–419.
19. **Odds, F. C.** 1988. Ecology of *Candida* and epidemiology of candidosis, p. 68–82. *In Candida* and candidosis: a review and bibliography, 2nd ed. Baillière Tindall, London, England.
20. **O'Donnell, K.** 1993. Fusarium and its near relatives, p. 225–233. *In* D. R. Reynolds and J. W. Taylor (ed.), The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics. CAB International, Wallingford, United Kingdom.
21. **Pfaller, M. A., L. Boyken, R. J. Hollis, J. Kroeger, S. A. Messer, S. Tendolkar, and D. J. Diekema.** 2008. In vitro susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. J. Clin. Microbiol. **46:**150–156.
22. **Pfaller, M. A., and D. J. Diekema.** 2007. Epidemiology of invasive candidiasis: a persistent public health problem. Clin. Microbiol. Rev. **20:**133–163.
23. **Pfaller, M. A., D. J. Diekema, M. Mendez, C. Kibbler, P. Erzsebet, S. C. Chang, D. L. Gibbs, and V. A. Newell.** 2006. *Candida guilliermondii*, an opportunistic fungal pathogen with decreased susceptibility to fluconazole: geographic and temporal trends from the ARTEMIS DISK antifungal surveillance program. J. Clin. Microbiol. **44:**3551–3556.
24. **Prinsloo, B., G. F. Weldhagen, and R. W. Blaine.** 2003. *Candida famata* central nervous system infection. S. Afr. Med. J. **93:**601–602.
25. **Quindos, G., F. Cabrera, M. C. Arilla, A. Burgos, R. Ortiz-Vigon, J. L. Canon, and J. Ponton.** 1994. Fatal *Candida famata* peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis who was treated with fluconazole. Clin. Infect. Dis. **18:**658–660.
26. **Quiros, M., P. Martorell, M. J. Valderrama, A. Querol, J. M. Peinado, and M. I. de Siloniz.** 2006. PCR-RFLP analysis of the IGS region of rDNA: a useful tool for the practical discrimination between species of the genus *Debaryomyces*. Antonie van Leeuwenhoek **90:**211–219.
27. **Rao, N. A., A. V. Nerenberg, and D. J. Forster.** 1991. *Torulopsis candida* (*Candida famata*) endophthalmitis simulating *Propionibacterium acnes* syndrome. Arch. Ophthalmol. **109:**1718–1721.
28. **Ronquist, F., and J. P. Huelsenbeck.** 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19:**1572–1574.
29. **Sandhu, G. S., B. C. Kline, L. Stockman, and G. D. Roberts.** 1995. Molecular probes for diagnosis of fungal infections. J. Clin. Microbiol. **33:**2913–2919.
30. **San Millan, R. M., L. C. Wu, I. F. Salkin, and P. F. Lehmann.** 1997. Clinical isolates of *Candida guilliermondii* include *Candida fermentati*. Int. J. Syst. Bacteriol. **47:**385–393.
31. **St-Germain, G., and M. Laverdiere.** 1986. *Torulopsis candida*, a new opportunistic pathogen. J. Clin. Microbiol. **24:**884–885.
32. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:**4876–4882.
33. **Tsui, C. K., H. M. Daniel, V. Robert, and W. Meyer.** 2008. Re-examining the phylogeny of clinically relevant *Candida* species and allied genera based on multigene analyses. FEMS Yeast Res. **8:**651–659.
34. **Vaughan-Martini, A., C. P. Kurtzman, S. A. Meyer, and E. B. O'Neill.** 2005. Two new species in the *Pichia guilliermondii* clade: *Pichia caribbica* sp. nov., the ascosporic state of *Candida fermentati*, and *Candida carpophila* comb. nov. FEMS Yeast Res. **5:**463–469.
35. **Wagner, D., A. Sander, H. Bertz, J. Finke, and W. V. Kern.** 2005. Breakthrough invasive infection due to *Debaryomyces hansenii* (teleomorph *Candida famata*) and *Scopulariopsis brevicaulis* in a stem cell transplant patient receiving liposomal amphotericin B and caspofungin for suspected aspergillosis. Infection **33:**397–400.
36. **White, T. J., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct sequencing of fungal rRNA genes for phylogenetics, p. 315–322. *In* M.A Innis et al. (ed.), PCR protocols. Academic Press, Inc., San Diego, CA.