Taxonomic Position of Deep-Seated, Mucosa-Associated, and Superficial Isolates of *Trichosporon cutaneum* from Trichosporonosis Patients

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Clinical isolates of *Trichosporon cutaneum*, the causative agent of trichosporonosis, were identified on the basis of DNA relatedness. Of the 10 strains from deep-seated and mucosa-associated infections, 9 were identified as *T. asahii* and 1 was identified as *T. ovoides*. The two superficial strains were identified as *T. cutaneum* and a variety of *T. montevidense*. These findings suggest that *T. cutaneum* is a heterogeneous species in clinical samples and that the causative agents of trichosporonosis exist in four or more species.

Trichosporon cutaneum (de Beurmann, Gougerot et Vaucher) Ota 1926 has been known as the causative agent of white piedra, an innocuous hair shaft infection encountered mostly in tropical and temperate countries. The species is widely distributed in the environment and has been recovered from normal skin and oral microflorae (31, 32). Since disseminated infection caused by *T. cutaneum* was first reported by Watson and Kallichurum in 1970 (33), reports of this rare disease in immunocompromised patients have been increasing (4, 11, 12, 18, 29, 32). The clinical picture of disseminated trichosporonosis consists of pneumonia, hepatitis, encephalitis, and septicemia (32). *T. cutaneum* is isolated mainly from blood, sputum, urine, skin, and feces. Factors that may predispose a patient to disseminated infection include cytotoxic chemotherapy-induced granulocytopenia, corticosteroids, prosthetic valve surgery, hemochromatosis, and AIDS (4, 11, 18, 24). Although most patients already have a severe or life-threatening underlying disease, trichosporonosis is associated with a high mortality rate (11). In addition to its role in disseminated trichosporonosis, serological studies point to *T. cutaneum* as a causative agent of summer-type hypersensitivity pneumonitis (2, 3, 35).

It has been pointed out that the taxon *T. cutaneum* represents a heterogeneous species on the basis of various criteria (8, 9, 19, 26, 34). Recently, Guého et al. (7) revised the genus *Trichosporon* on the basis of morphological and biochemical properties, coenzyme Q systems, DNA relatedness, and partial 26S rRNA sequences. Later, Sugita et al. (28) proposed a new classification of *T. cutaneum* based on DNA relatedness. For the clinical isolates, however, taxonomic analyses have rarely been reported. Kemker et al. (13) examined *T. cutaneum* clinical and environmental isolates for similarities based on morphological features, biochemical profiles, isoenzyme patterns, and restriction fragment length polymorphisms of ribosomal DNA. They found that strains that caused invasive disease were distinct from the superficial and the nonclinical isolates and that the skin and mucosa yielded a number of different organisms. However, they did not identify the clinical isolates taxonomically. In more recent papers, Guého et al. (6) and Herbrecht et al. (10) reported that six different species are

involved in *Trichosporon* infection. In this study, we identified the *T. cutaneum* clinical isolates to clarify their taxonomic position in the genus *Trichosporon* by DNA-DNA reassociation studies. We use the name *T. cutaneum* instead of *T. beigeli* on the basis of the statement of Guého et al. (5) that the name *T. beigeli* is of doubtful validity. Indeed, *T. beigeli* was not retained as a taxon in their taxonomic study of *Trichosporon* spp.

The 12 clinical isolates used in this study are listed in Table 1. The new isolates recovered from urine specimens on CT agar (peptone, 10 g; yeast extract, 5 g; glucose, 40 g; copper sulfate, 0.28 g; copper nitrate, 0.15 g; methylene blue, 0.01 g; Tween 80, 3 g; agar, 15 g/1,000 ml; Serotec, Hokkaido, Japan) and Sabouraud agar plates (Difco, Detroit, Mich.) were identified by production of arthroconidia and from physiological profiles obtained by using the ID 32C kit (Bio-Mérieux, Marcy l'Etoile, France). The other six strains from sources other than urine have been described previously by Kemker et al. (13) and Lee et al. (19). The isolates studied included eight strains from deep-seated infections, two mucosa-associated strains, and two superficial strains. DNA was isolated by the method of Restrepo and Barbour (25), with some modification. Yeast cells were incubated with NovoZym 234 (Novo Industry Laboratories, Bagsvaerd, Denmark) at 30°C and then lysed with a lysing solution (0.45 M EDTA, 10 mM Tris-HCl [pH 7.5], 1% Sarkosyl [ICN Biomedicals Inc., Plainview, Calif.] containing 100 µg of proteinase K per ml [Boehringer GmbH, Mannheim, Germany]). Unbroken cells and cell wall debris were removed by centrifugation at 9,000 × g for 5 min. The supernatant was extracted with phenol-chloroform-isoamyl alcohol (25:24:1 [vol/vol/vol]). DNA was precipitated with 2 volumes of cold ethanol. The DNA was dissolved in SSC (0.15 M NaCl, 0.015 M sodium citrate [pH 7.0]) and incubated with 50 µg of RNase (Boehringer) per ml and 100 µg of proteinase K per ml. It was then reextracted with phenol-chloroform-isoamyl alcohol. DNA was purified with hydroxyapatite (Bio-Rad Laboratories, Richmond, Calif.) by a batch method (17). The quality of the purified DNA was assessed from thermal profiles.

DNA-DNA hybridization was determined by the method of Adnan et al. (1), in the manner that we described previously (28). Briefly, the DNA-DNA hybridization reaction involved the annealing of biotin-labeled DNA to DNA that had been bound to wells in a microtiter plate. *Escherichia coli* JH DNA

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TABLE 1. *T. cutaneum* clinical isolates examined

Type of infection and strain ^a	Source or site of isolate	Source ^b
Deep-seated infections		
M 9402	Blood	UMSMT2
M 9403	Blood	UMSMT3
M 9404	Urine	KULM 92-2166
M 9405	Urine	KULM 92-3527
M 9406	Urine	KULM 92-5130
M 9407	Urine	KULM 93-0157
M 9408	Urine	KULM 93-1170
M 9409	Urine	KULM 93-2779
Mucosa-associated infections		
M 9410	Feces	NYSDH 297-87
M 9411	Sputum	NYSDH 958-85
Superficial infections		
M 9412	Toenail	NYSDH 342-85
M 9413	Skin (leg)	ATCC 38300

^a M, Meiji College of Pharmacy, Tokyo, Japan.^b ATCC, American Type Culture Collection, Rockville, Md.; KULM, Kitasato University Laboratory of Mycology, Kanagawa, Japan; NYSDH, New York State Department of Health, Albany, N.Y.; UMSMT, University of Maryland School of Medical Technology, Baltimore.

was used as a negative control. Strains with DNA relatedness of 80% or greater are defined as conspecific.

Table 2 shows the identification of the *T. cutaneum* clinical isolates. The nomenclature was used according to the proposal

of Sugita et al. (28). Of the eight isolates from deep-seated infections, seven were identified as *T. asahii*, including the three varieties *asahii*, *faecalis*, and *coremiformis*. The remaining strain was *T. ovoides*, which is the type species of the genus *Trichosporon*. The two mucosa-associated strains were also identified as *T. asahii* var. *asahii*. The isolates from blood, urine, and mucosal sites were mostly *T. asahii* (9 of 10), and a large number of them were variety *asahii* (7 of 9). Of the superficial strains, M 9413 was *T. cutaneum*. However, M 9412 did not show 80% or more DNA relatedness to any type strain. M 9412 appears to be defined most accurately as a variety of *T. montevidense* because the strain had intermediate DNA relatedness (58%) to the type strain, CBS 6721. Phylogenetically, *T. montevidense* is very closely related to *T. brassicae* (7), but DNA of M 9412 exhibited only 14% relatedness to that of *T. brassicae*. Only 1 strain was *T. cutaneum*, even though 12 strains were named *T. cutaneum* by the current method of diagnosis (15). These results suggest that *T. cutaneum* is heterogeneous in clinical samples and that there can be more than one species at most sites of infection.

Recently, Guého et al. (6) and Herbrecht et al. (10) described the genus *Trichosporon* as containing six human pathogenic species: *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides*, and *T. ovoides*. Of the six species, *T. ovoides* and *T. inkin* are the main causative agents of white piedra. The latter one has rarely been isolated from systemic mycosis. However, there are no reports that *T. ovoides* has been isolated from cases other than white piedra. In our investigation, it is interesting that *T. ovoides* was isolated from a urine sample. The six species were isolated from superficial sites (6, 10); in addition,

TABLE 2. Identification of *T. cutaneum* clinical isolates by DNA relatedness

Type of infection and strain	% DNA relatedness with ^a :						Identification
	CBS 2479 ^b	CBS 2482 ^c	CBS 4828 ^d	CBS 6721 ^e	CBS 7556 ^f	IFO 1198 ^g	
Deep-seated infections							
M 9402	86	42	40				<i>T. asahii</i> var. <i>asahii</i>
M 9403	81	49	52				<i>T. asahii</i> var. <i>asahii</i>
M 9404	68	97	61				<i>T. asahii</i> var. <i>coremiformis</i>
M 9405	100	58	51				<i>T. asahii</i> var. <i>asahii</i>
M 9406	84	58	42				<i>T. asahii</i> var. <i>asahii</i>
M 9407	10	9	14		99		<i>T. ovoides</i>
M 9408	103	46	43				<i>T. asahii</i> var. <i>asahii</i>
M 9409	62	53	97				<i>T. asahii</i> var. <i>faecalis</i>
Mucosa-associated infections							
M 9410	86	40	38				<i>T. asahii</i> var. <i>asahii</i>
M 9411	92	43	59				<i>T. asahii</i> var. <i>asahii</i>
Superficial infections							
M 9412				58		9	Variety of <i>T. montevidense</i>
M 9413						92	<i>T. cutaneum</i>
Reference strains ^h							
<i>T. asahii</i> var. <i>coremiformis</i> CBS 2482	55						
<i>T. asahii</i> var. <i>faecalis</i> CBS 4828	53	56					
<i>T. montevidense</i> CBS 6721	6	14	12				
<i>T. ovoides</i> CBS 7556	27	26	11	10			
<i>T. cutaneum</i> IFO 1198	7	11	5	15	4		

^a CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IFO, Institute for Fermentation, Osaka, Japan.^b Type strain of *T. asahii* var. *asahii*.^c Type strain of *T. asahii* var. *coremiformis*.^d Type strain of *T. asahii* var. *faecalis*.^e Type strain of *T. montevidense*.^f Type strain of *T. ovoides*.^g Type strain of *T. cutaneum*.^h From the study by Sugita et al. (28).

we identified the strain which can be defined as a variety of *T. montevidense* from the patient's toenail. In deep-seated infections, a large number of *T. asahii* strains were also isolated, as reported by Guého et al. (6).

Of course, rapid identification of the causative agents of infectious diseases is very important in the diagnosis and treatment of patients. However, some fungi, such as *T. cutaneum*, are often misidentified. Currently, *T. cutaneum* isolates are identified on the basis of morphological and biochemical properties, and they are characterized by the presence of hyphae, pseudohyphae, arthroconidia, and blastoconidia (15). *T. cutaneum* does not ferment carbohydrates; it assimilates glucose, galactose, sucrose, maltose, and lactose and hydrolyzes urea. DNA-DNA reassociation studies, as reported herein, would be applicable to isolates that are not identifiable from their biochemical properties. We have not performed physiological and biochemical studies using the clinical isolates. Guého et al. (6) mentioned that *T. asahii*, *T. asteroides*, *T. cutaneum*, *T. inkin*, *T. mucoides*, and *T. ovoides* were clearly differentiated by several key characteristics, a combination of assimilation of carbon compounds, cycloheximide resistance, and growth ability at 37°C. For three varieties of *T. asahii*, it is difficult to make clear distinctions between them to the variety level by physiological and biochemical properties. However, their DNA relatedness is clear (28).

On the other hand, a serological technique for identification of *T. cutaneum* has been reported previously (3, 14, 23, 30). However, the cell wall antigen of *T. cutaneum* cross-reacts with the capsular polysaccharide of *Cryptococcus neoformans* (16, 20, 21, 27). In addition, it is reported that *T. cutaneum* has several serotypes (3, 16, 22, 35). The relationship of these serotypes to this species should be explored.

In conclusion, we have shown the taxonomic position of several clinical isolates of *Trichosporon* by use of a DNA-DNA reassociation technique. We expect that a rapid identification method can be developed to allow routine identifications of this group of organisms by the clinical microbiology laboratory.

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