Two new yeasts, *Trichosporon debeurmannianum* sp. nov. and *Trichosporon dermatitis* sp. nov., transferred from the *Cryptococcus humicola* complex

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***Cryptococcus humicola*, as currently defined, shows intraspecific rRNA gene sequence differences. Three strains of this species produced arthroconidia on cornmeal agar and belonged to the genus *Trichosporon* in a molecular phylogeny. They clustered with the species possessing Q10 as the major ubiquinone and were serotype I. Sequence analyses clearly revealed that they were two new *Trichosporon* species. The names *Trichosporon dermatitis* sp. nov. (= CBS 2043^T) and *Trichosporon debeurmannianum* sp. nov. (= CBS 1896^T) are proposed for these strains.**

Keywords: *Trichosporon debeurmannianum* sp. nov., *Trichosporon dermatitis* sp. nov., rDNA

INTRODUCTION

Basidiomycetous anamorphic yeasts of the genus *Trichosporon* Behrend are characterized by the production of arthroconidia, the possession of ubiquinone Q9 or Q10 and the presence of xylose in the cell wall. Approximately 20 species have been described in the genus (Guého *et al.*, 1992; Sugita *et al.*, 1995a; Sugita & Nakase, 1998a; Middelhoven *et al.*, 1999, 2000). Sugita & Nakase (1998b) and Fell *et al.* (2000) reported that the genus *Trichosporon* was monophyletic on the basis of 18S and 28S (D1/D2 regions) rDNA sequences, and each cluster correlated well with the molecular type of ubiquinone. Specifically, of three main clusters, one consists of the species possessing Q10 and the remaining two possess Q9. Serologically, there are four serotypes, I, II, III and I-III, in the genus *Trichosporon* (Ikeda *et al.*, 1996; Nishiura *et al.*, 1997). Serotype I-III species react to both antisera I and III. These serotypes also correlate well with the clusters on the tree.

While investigating the intraspecies diversity of *Cryptococcus humicola*, we found three strains that belong to the genus *Trichosporon*. This paper describes two new yeasts, *Trichosporon dermatitis* sp. nov. and *Trichosporon debeurmannianum* sp. nov., proposed for these strains.

METHODS

Strains examined. Strains CBS 1896^T, 1897 and 2043^T were purchased from Centraalbureau voor Schimmelcultures (CBS) as *Cryptococcus humicola*. The first two were isolated from the bronchial secretions of a man and the latter from an infected human skin lesion (http://www.cbs.knaw.nl/search_ydb.html).

Morphological, biochemical and physiological characteristics. Most of the morphological, biochemical and physiological characteristics were examined using the methods described by Yarrow (1998). Biochemical characteristics were also investigated using ID32C (bioMérieux) in accordance with the manufacturer's instructions.

Examination of chemotaxonomic characteristics. Extraction, purification and identification of ubiquinones were carried out according to the method of Sugita *et al.* (1999). The DNA base composition (mol% G + C) was determined by nucleotide analysis, using HPLC after digesting the DNA with Nuclease P1 (Yamasa Shouyu) as described by Sugita *et al.* (1994). Xylose in cell wall polysaccharide was detected by GC. Cells were collected in 0.02 M sodium citrate buffer (pH 7.0) and autoclaved at 121 °C for 90 min. Cell wall polysaccharide was precipitated from the supernatant by

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The GenBank accession numbers for the 28S rDNA D1/D2 sequences of *Trichosporon debeurmannianum* sp. nov. and *Trichosporon dermatitis* sp. nov. are AB044568 and AB044569, respectively.

Fig. 1. Neighbour-joining tree of *Trichosporon debeurmannianum* sp. nov., *T. dermatitis* sp. nov. and related species based on the D1/D2 regions of 28S rDNA sequences. The numerals represent percentages from 100 replicate bootstrap samplings (values less than 50% are not shown). The serotype and major ubiquinone type of the *Trichosporon* species are also indicated. K_{nuc} , Kimura's parameter (Kimura, 1980).

adding 2 vols ethanol. The precipitate was dissolved, dialysed against water and lyophilized. The polysaccharide was hydrolysed in 2 M trifluoroacetic acid at 120 °C for 3 h. The hydrolysate was analysed by TLC on silica gel plates with an n-butanol/ethanol/water (40:11:19, by vol.) solvent

system. The hydrolysate was reduced with NaBH_4 and then acetylated with acetic anhydride/pyridine (1:1, v/v). Alditol acetate was analysed with a Shimadzu gas chromatograph GC-7 in a Gas Chrom Q glass column containing 3% ECNSS-S (Shinwa) at 190 °C.

Table 1. Biochemical characteristics of *T. debeurmannianum* sp. nov., *T. dermatis* sp. nov. and other Q10-serotype I species by ID32C

+, Positive; v, variable; —, negative.

Compound	<i>T. debeurmannianum</i> sp. nov.	<i>T. dermatis</i> sp. nov.	<i>T. cutaneum</i>	<i>T. mucoides</i>	<i>T. jirovecii</i>	<i>T. moniliiforme</i>
D-Galactose	—	+	+	+	+	+
Cycloheximide (0.01 %)	+	+	—	+	+	+
Sucrose	+	+	+	+	+	+
N-Acetylglucosamine	+	+	+	+	+	+
DL-Lactate	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+
Cellobiose	+	+	v	+	v	+
Raffinose	+	+	v	+	+	+
Maltose	+	+	+	+	v	+
Trehalose	+	+	v	+	+	+
2-Keto-D-gluconate	+	+	+	+	+	+
α-Methyl-D-glucoside	+	+	+	+	v	+
Mannitol	+	+	+	+	v	+
Lactose	+	+	+	+	+	+
Inositol	+	+	+	+	+	+
Sorbitol	+	+	+	+	—	+
D-Xylose	+	+	+	+	+	+
D-Ribose	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
L-Rhamnose	+	+	v	v	v	+
Palatinose	+	+	+	+	v	+
Erythritol	+	+	+	+	+	+
Melibiose	+	+	v	v	+	+
Glucuronate	+	+	+	+	+	+
Melezitose	—	+	+	+	+	+
Glucurionate	+	+	+	+	+	+
Laevulinate	—	—	—	—	—	—
Glucose	+	+	+	+	+	+
Sorbose	—	+	—	v	+	—
Glucosamine	+	+	v	+	+	+
Aesculin	+	+	+	+	—	+

Direct DNA sequencing. Nuclear DNA was extracted by the method of Makimura *et al.* (1994). The D1/D2 regions of 28S rDNA were directly determined using PCR products with the primers NL-1 (5'-GCATATCAATAAGCGGAG-GAAAAG) and NL-4 (5'-GGTCCGTGTTTCAAGACG-G) (Kurtzman & Robnett, 1997). The PCR products were sequenced with an ABI PRISM Cycle Sequencing kit (Perkin-Elmer Applied Biosystems).

Molecular phylogenetic analysis. The sequences were aligned with the computer program CLUSTAL W, version 1.8 (Thompson *et al.*, 1994). The evolutionary distance for the neighbour-joining method (Saitou & Nei, 1987) was calculated according to Kimura (1980). Sites where gaps existed in any sequences were excluded. A bootstrap analysis was conducted with 100 replications (Felsenstein, 1985).

In vitro susceptibility tests. The MICs of amphotericin B, 5-fluorocytosine, fluconazole, itraconazole and miconazole were determined using an Eiken kit (Eiken Chemical) in accordance with the manufacturer's instructions.

RESULTS AND DISCUSSION

We previously reported that strains CBS 1896^T and 1897 (*T. debeurmannianum*) and 2043^T (*T. dermatis*) used in this study were positioned in the lineage *Trichosporon cutaneum*–*Trichosporon mucoides* in a phylogenetic tree based on the sequences of 18S rDNA (Sugita *et al.*, 2000). They possess Q10 as the major ubiquinone and are serotype I. *T. cutaneum*, *Trichosporon jirovecii*, *Trichosporon moniliiforme* and *T. mucoides* also have the same ubiquinone type and serotype as *T. debeurmannianum* and *T. dermatis*. These six species formed a cluster with high bootstrap value (99%) on a tree constructed using 18S rDNA sequences (Sugita *et al.*, 2000). In this study, the D1/2 regions of 28S rDNA were also sequenced. These six species again formed a single cluster on the tree with a 100% bootstrap value (Fig. 1). Sugita *et al.* (1999) demonstrated a relationship between internal trans-

Table 2. *In vitro* susceptibility of *T. debeurmannianum* and *T. dermatitis* to antifungal agents

AMPH, amphotericin B; 5-FC, 5-fluorocytosine; FLCZ, fluconazole; ITZ, itraconazole; MCZ, miconazole.

Species	Strain	MIC ($\mu\text{g ml}^{-1}$)				
		AMPH	5-FC	FLCZ	ITZ	MCZ
<i>T. debeurmannianum</i>	CBS 1896 ^T	0.125	> 64	2	0.06	< 0.06
	CBS 1897	0.25	> 64	4	0.125	< 0.06
<i>T. dermatitis</i>	CBS 2043 ^T	0.25	> 64	4	0.5	0.25

Fig. 2. Arthroconidia of *Trichosporon debeurmannianum* sp. nov. (a) and *T. dermatitis* sp. nov. (b) produced on cornmeal agar after growth for 3 d at 25 °C. Bars, 10 μm .

cribed spacer (ITS) sequence similarity and nuclear DNA relatedness and found that conspecific strains differed by fewer than 1% of nucleotides in the ITS1 and ITS2 regions overall. Compared to serotype I species, the two new species showed 89.4–99.1% similarity to ITS1 and 88.6–99.4% to ITS2. Overall, the ITS regions show 89.4–97.1% similarity among Q10-serotype I species. From these overall ITS similarities, it was concluded that these strains are two new species.

For rapid differentiation of Q10-serotype I species, the commercially available ID32C kit was used. The new species can be differentiated from known Q10-serotype I species on the basis of ability to assimilate galactose, sorbitol, melezitose, sorbose and aesculin, and resistance to 0.01% cycloheximide (Table 1).

Trichosporon is a medically relevant genus that includes the causative agents of deep-seated and superficial infections, including white piedra. *Trichosporon* species are also involved in summer-type hypersensitivity pneumonitis (SHP). Six species are responsible for these infections and allergies, and the causative agent of trichosporonosis differs in each type of infection (Herbrecht *et al.*, 1993; Guého *et al.*, 1994; Sugita *et al.*, 1995b). *Trichosporon asahii* and *T. mucoides* are involved in deep-seated infections and are the major causative antigens of SHP (Nishiura *et al.*, 1997). *Trichosporon asteroides* and *T. cutaneum* are associated with superficial infections. The appressoria-forming yeasts *Trichosporon ovoides* and *Trichosporon inkin* are responsible for white piedra of the head and genital area, respectively. These six medically relevant species are serotype I or II. Serotypes III and I-III do not seem to be responsible for infection or allergy. The three strains examined in this study are serotype I. Both new species proved to be resistant to the antifungal agent 5-fluorocytosine (Table 2). The *in vitro* susceptibilities of the two new species to the five agents in Table 2 are similar to those of the other six pathogenic *Trichosporon* species (Guého *et al.*, 1994). Strains CBS 1896^T and 1897 were isolated from human bronchial secretions and strain CBS 2043^T from an infected human skin lesion. However, it is unknown whether these two species are pathogenic to humans.

Latin diagnosis of *Trichosporon debeurmannianum* Sugita, Takashima, Nakase *et* Shinoda sp. nov.

In liquido 'YM', post dies 3 ad 25 °C, cellulae vegetativae ovoideae, ellipsoideae vel elongatae (2–8 × 2–15 μm), singulae, binae, in catenis aut in fasciculis, propagantes gemmarum blastocarum. Sedimentum formatur. Post unum mensem ad 17 °C, pellícula fragilis et incompleta aut completa et sedimentum formantur. In agar 'YM', post unum mensem ad 17 °C, cultura pallida aut xanthoalba, glabra aut rugosa, nitida,

Table 3. Physiological and chemotaxonomic characterization of *T. debeurmannianum* sp. nov. and *T. dermatitis* sp. nov.

+, Positive; D, delayed positive; DW, delayed and weakly positive; w, weakly positive; s, slow positive; —, negative.

Character	<i>T. debeurmannianum</i> sp. nov.	<i>T. dermatitis</i> sp. nov.
Fermentation of carbon compounds		
D-Glucose	—	—
Assimilation of carbon compounds		
D-Glucose	+	+
D-Galactose	—	+
L-Sorbose	DW	+
Sucrose	+	+
Maltose	+	+
Cellobiose	+	+
α,α -Trehalose	+ /s	+
Lactose	+ /w	+
Melibiose	+	+
Raffinose	+	+
Melezitose	w/DW	+
Inulin	— /DW	—
Soluble starch	+	+
D-Xylose	+	+
L-Arabinose	+ /DW	+
D-Arabinose	+ /DW	+
D-Ribose	+	+
L-Rhamnose	+	+
D-Glucosamine	DW	+
N-Acetyl-D-glucosamine	+	+
Methanol	—	—
Ethanol	+ /DW	+
Glycerol	+	+
Erythritol	+ /D	+
Ribitol	+ /D	+
Galactitol	—	DW
D-Mannitol	+ /D	+
D-Glucitol	+ /D	+
α -Methyl-D-glucoside	+	+
Salicin	+	+
Glucono- δ -lactone	—	+
D-Gluconic acid	+	+
2-Ketogluconic acid	+	+
5-Ketogluconic acid	+	+
DL-Lactic acid	+	+
Succinic acid	+	+
Citric acid	+	+
Inositol	+	+
n-Hexadecane	—	—
Saccharic acid	—	w
Xylitol	s/DW	+
L-Arabinitol	—	+
1,2-Propanediol	+	+
2,3-Butanediol	s/—	DW
D-Glucuronic acid	+	+
D-Galacturonic acid	+ /DW	+
Assimilation of nitrogen compounds		
Ammonium sulfate	+	+
Nitrate	—	—

Table 3 (cont.)

Character	<i>T. debeurmannianum</i> sp. nov.	<i>T. dermatis</i> sp. nov.
Nitrite	w	+
Ethylamine hydrochloride	+	+
L-Lysine	+	+
Cadaverine	+	+
Growth at:		
30 °C	+	+
35 °C	+	+
37 °C	—	+
40 °C	—	—
Cycloheximide resistance		
0.01 %	+	+
0.1 %	—	+
Production of starch-like substances	—	—
Growth on 50 % (w/w) glucose-yeast extract agar	—	—
Urease reaction	+	+
Diazonium Blue B reaction	+	+
Liquefaction of gelatin	—	—
G + C content of nuclear DNA (mol %)	59.8	60.3
Major ubiquinone	Q10	Q10
Xylose in the cell wall	Present	Present

butyracea et margine glabra. Arthroconidiorum et mycelium formantur. Fermentatio nulla. D-Glucosum, L-sorbosum (lente et infirme), saccharosum, maltosum, cellobiosum, trehalosum (lente), lactosum (infirmum), melibiosum, raffinose, melezitosum (infirmum vel lente et infirmum), inulinum (lente et infirmum), amyllum solubile, D-xylosum, L-arabinosum (lente et infirmum), D-arabinosum (lente et infirmum), D-ribosum, L-rhamnosum, D-glucosaminum (lente et infirmum), N-acetylglucosaminum, ethanolum (lente et infirmum), glycerolum, erythritolum (lente), ribitolum (lente), D-mannitolum (lente), D-glucitolum (lente), α -methyl-D-glucosidum, salicinum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum, xyli-torum (lente et infirmum), 1,2-propanediolum, 2,3-butanediolum (lente), acidum D-glucuronicum, et acidum D-galacturonicum (lente et infirmum) assimilantur, autem D-galactosum, methanolum, galactitolum, glucono- δ -lactonum, n-hexadecanum, acidum D-saccharicum et L-arabinitolum non assimilantur. Ammonium sulfatum, kalium nitricum (infirmum), ethylaminum, L-lysinum et cadaverinum assimilantur, natrium nitrosum non assimilatur. Typus: CBS 1896^T, Centraalbureau voor Schimmelcultures, Delphi, Batavorum.

Description of *Trichosporon debeurmannianum* Sugita, Takashima, Nakase et Shinoda sp. nov.

Trichosporon debeurmannianum (de.beur.man.ni. a.num. M.L. n. *debeurmannianum* in honour of L. De Beurmann, who described *Oidium cutanum*

(= *Trichosporon cutaneum*) and reported it to be a causative agent of superficial fungal infection).

After 3 d growth in YM broth at 25 °C, the vegetative cells are ovoid, ellipsoid and elongate (2–8 × 2–15 µm), and single, paired or in clusters. Cells reproduce by budding. Sediment is formed. After 1 month growth in YM broth at 17 °C, an incomplete or complete fragile pellicle and sediment are present. After 1 month growth on YM agar at 17 °C, streak cultures are pale to yellowish white, semi-shiny, smooth and wrinkled near the bottom, butyrous, and have an entire and sporadically fringed margin. After 3 d in Dalmau plate culture on cornmeal agar at 25 °C, arthroconidia and mycelia are produced (Fig. 2a). For physiological and chemotaxonomic characteristics, see Table 3. The type strain is CBS 1896^T; it was isolated from the bronchial secretions of a man in Utrecht, The Netherlands, and deposited in the Centraalbureau voor Schimmelcultures (CBS) by Swierenga in June 1954. The other strain, CBS 1897, has also been maintained in the CBS.

Latin diagnosis of *Trichosporon dermatis* Sugita, Takashima, Nakase et Shinoda sp. nov.

In liquido 'YM', post dies 3 ad 25 °C, cellulæ vegetativæ ovoideæ, ellipsoideæ vel elongatæ (2–8 × 2–15 µm), singulæ, binæ, in catenis aut in fasciculis, propagantes gemmarum multilateraliter. Sedimentum formatur. Post unum mensem ad 17 °C, pellicula fragilis et tenuis, et sedimentum formantur. In agaro 'YM', post unum mensem ad 17 °C, cultura xanthoalba, rugosa, nitida, butyracea et margine mycelica. Arthroconidiorum et mycelium formantur. Ferm-

entatio nulla. *D*-Glucosum, *D*-galactosum, *L*-sorbosum, saccharosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, amyllum solubile, *D*-xylosum, *L*-arabiosum, *D*-arabiosum, *D*-ribosum, *L*-rhamnosum, *D*-glucosaminum, *N*-acetylglucosaminum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum (*lente et infirme*), *D*-mannitolum, *D*-glucitolum, α -methyl-*D*-glucosidum, salicinum, glucono- δ -lactonum, acidum *D*-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum *DL*-lacticum, acidum succinicum, acidum citricum, inositolum, acidum *D*-saccharicum (*infirme*), xylitolum, *L*-arabinitolum, 1,2-propanediolum, 2,3-butanediolum (*lente et infirme*), acidum *D*-glucuronicum et acidum *D*-galacturonicum assimilantur, autem inulinum, methanolum et *n*-hexadecanum non assimilantur. Ammonium sulfatum, kalium nitricum, ethylaminum, *L*-lysinum et cadaverinum assimilantur, natrium nitrosum non assimilatur. Typus: CBS 2043^T, Centraalbureau voor Schimmelcultures, Delphi, Batavorum.

Description of *Trichosporon dermatis* Sugita, Takashima, Nakase et Shinoda sp. nov.

Trichosporon dermatis (der.ma'tis. Gr. n. *dermatis* pertaining to skin, from which the organism was isolated).

After 3 d growth in YM broth at 25 °C, the vegetative cells are ovoid, ellipsoid and elongate (2–8 × 2–15 µm), and single, paired or in clusters. They reproduce by multilateral budding. Sediment is formed. After 1 month growth in YM broth at 17 °C, a thin fragile pellicle and thick sediment are present. After 1 month growth on YM agar at 17 °C, streak cultures are yellowish white, semi-shiny, wrinkled, butyrous and have a fringed margin. After 3 d in Dalmau plate culture on cornmeal agar at 25 °C, arthroconidia and mycelia are produced (Fig. 2b). For physiological and chemotaxonomic characteristics, see Table 3. The type strain is CBS 2043^T; it was isolated from infected human skin in Tübingen, Germany, and deposited in the Centraalbureau voor Schimmelcultures (CBS) by Schmitz in December 1954.

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