Short Communication

A basidiomycetous anamorphic yeast, *Trichosporon terricola* sp. nov., isolated from soil

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(Received July 3, 2002; Accepted September 17, 2002)

Key Words—new species; soil; *Trichosporon terricola*

Trichosporon species are basidiomycetous anamorphic yeasts characterized by the production of arthroconidia. Twenty-six species have been listed in the genus *Trichosporon*, which includes medically relevant species that cause opportunistic infections and induce summer-type hypersensitivity pneumonitis (SHP) (Ando et al., 1995; Guého et al., 1994, 1998; Middelhoven et al., 1999, 2000a, b; Sugita et al., 2001). Besides pathogens, *Trichosporon* species are widely distributed in the environment, and are also used as a biological sensor in industry to determine biochemical oxygen demand (Yang et al., 1996). While analyzing the diversity of *Trichosporon* in the soil, we found a new *Trichosporon* species. In this paper, we propose a new species, *Trichosporon terricola* sp. nov., for the isolates.

Strains M9900, M9952, and M9953 used in this study were isolated in July 1997 from soil in Niiza, Saitama, Japan. Most of their morphological, biochem-

ical, and physiological characteristics were examined using the methods described by Yarrow (1998). Their biochemical characteristics were also investigated using ID32C (bioMérieux SA, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. Ubiquinone molecules were identified according to the method of Nakase and Suzuki (1986). The DNA base composition (mol% G+C) was determined by nucleotide analysis, using HPLC after digesting the DNA with Nuclease P1 (Yamasa Shouyu, Chiba) as described by Tamaoka and Komagata (1984). Xylose was detected in cell wall polysaccharides by gas chromatography as previously described by Sugita et al. (2001). The internal transcribed spacer (ITS) regions (Sugita et al., 1999), D1/D2 regions of 26S rDNA (Kurtzman and Robnett, 1997), and intergenic spacer (IGS) 1 region (Sugita et al., 2002) were determined directly using PCR products with primers: pITS-F (5'-GTCGTAACAAGGTTAACCTGCGG) and pITS-R (5'-TCCTCCGCTTATTGATATGC), NL-1 (5'-GCATATCA-ATAAGCGGAGGAAAAG) and NL-4 (5'-GGTCCGT-GTTTCAAGACGG), and 26SF (5'-ATCCTTTGCAGACGACTTGA) and 5SR (5'-AGCTTGACTTCGCAGATCGG), respectively, using an ABI 310 DNA sequencer

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Fig. 1. Phylogenetic tree of *Trichosporon terricola* sp. nov. and other *Trichosporon* species.

The tree was constructed by the Neighbor-Joining method from D1/D2 26S rDNA sequences. The numbers are the percentages of 100 replicate bootstrap samplings (values less than 50% are not shown). K_{nuc}, Kimura's parameter (Kimura, 1980). The accession numbers of sequences retrieved from the DDBJ are indicated in parentheses.

with an ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA) according to the manufacturer's instructions. The sequences were aligned with the computer program CLUSTAL W, Version 1.8 (Thompson et al., 1994). The evolutionary distances for the Neighbor-Joining method (Saitou and Nei, 1987) were calculated according to Kimura (1980). A bootstrap analysis was conducted with 100 replications (Felsenstein, 1985).

The genus *Trichosporon* is divided into three major phylogenetic groups, which correspond well to

ubiquinone molecules (Sugita and Nakase, 1998), i.e., of the three groups, two possess Q-9 and one possesses Q-10. The isolates also possessed Q-10, and were positioned in the *T. mucoides* group in a tree (Fig. 1). D1/D2 26S rDNA, ITS and IGS sequences of three isolates showed complete identity with each other. They showed 5.2 to 9.8% dissimilarities to other Q-10 species on their ITS regions. Sugita et al. (1999) found that conspecific strains have less than 1% dissimilarity in the ITS1 and ITS2 regions, after comparing the nuclear DNA-DNA hybridization values and

similarity in the sequences of the entire ITS and 2 regions using all *Trichosporon* species. Recently, Sugita et al. (2002) demonstrated that the IGS1 sequence more readily distinguishes phylogenetically very closely related species than does the ITS sequence. According to an analysis of all *Trichosporon* species, 99% similarity in the ITS sequences observed between two species corresponds to approximately 55 to 95% IGS1 sequence similarity. The IGS1 sequence of isolate (AB086383) is most similar to that of *T. moniliiforme* (AB066429), but they only share 48.9% similarity. According to these results, the divergence between known *Trichosporon* species and our isolates is sufficient to resolve them as individual species (*T. terricola*). Using physiological characteristics, *T. terricola* is readily distinguished from the other Q-10 species by using the ID32C kit and growth temperature, as shown in Table 1. At 40°C, only *T. terricola* and *T. mucoides* can grow, but *T. terricola* does not assimilate melezitose within 48 h, while *T. mucoides* does assimilate this compound.

Table 1. Rapid identification of Q-10 species by ID32C and growth temperature.

Characteristic	<i>T. terricola</i> sp. nov. ^a	<i>T. debeurmannianum</i> ^b	<i>T. dermatis</i> ^b	<i>T. cutaneum</i> ^c	<i>T. mucoides</i> ^c	<i>T. jirovecii</i> ^c	<i>T. moniliiforme</i> ^c
Assimilation of							
D-Galactose	+	–	+	+	+	+	+
0.01% Cycloheximide	+	+	+	–	+	+	+
Sorbitol	+	+	+	+	+	–	+
Melezitose	–	–	+	+	+	+	+
L-Sorbose	+	–	+	–	V	+	–
Esculine	+	+	+	+	+	–	+
Growth at							
37°C	+	–	+	–	+	–	–
40°C	+	–	–	–	+	–	–

+, positive; V, variable; –, negative.

^aBiocode; 7777775377.

^bSugita et al., 2001.

^cGuého et al., 1994.

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

In liquido 'YM,' post dies 3 ad 25°C, cellulae ovoideae, ellipsoideae aut elongatae, (2–12)×(3–25) µm, singulae et binae. Sedimentum formatur. Post unum mensem ad 17°C, annulus repens, pellicula fragilis et completa, et sedimentum formantur. In agar 'YM,' post unum mensem ad 17°C, cultura luteola, rugosa aut cerebriformis, attolens juxta imum, hebetata, butyracea, margo fimbriata. In cultura Dalmau plate, post dies 3 ad 17°C, arthrospora et mycelia flexuosa formantur.

Fermentatio nulla. Glucosum, galactosum, L-sorbosum, saccharosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitose, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum (lente), galactitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, acidum

(A)

(B)

Fig. 2. (A) Vegetative cells grown in YM broth for 3 days at 25°C and (B) arthroconidia produced on cornmeal agar after growth for 3 days at 25°C. The scale bar indicates 10 µm.

D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum, acidum saccharicum (lente), xylitolum, L-arabinitolum, 1,2-propanediolum, 2,3-butanediolum (lente), acidum D-glucuronicum et acidum D-galacturonicum assimilantur, autem inulinum, methanolum, glucono- δ -lactonum et hexadecanum non assimilantur. Ethylaminum, L-lysinum et cadaverinum assimilantur, autem kalium nitricum et natrium nitrosum non assimilantur. Ad crescentiam biotinum necessarium est. Diazonium caeruleum B positivum. Proportio molaris guanini+cytosini in acido de-

oxyribonucleico: 57.6 mol%. Ubiquinonum majus: Q-10. Xylosum in cellulis presens.

Typus: JCM11688, ex terrae, Niiza, Saitama, Japonia, 1997, T. Sugita (originaliter ut M9900) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms,' Saitama, Japan sustentat.

After 3 days of growth in YM broth at 25°C, the vegetative cells are oval, ellipsoidal, or elongate, (2–12) × (3–25) μ m, single or in pairs (Fig. 2). A sediment is formed. After 1 month at 17°C, a creeping and complete ring, a complete fragile pellicle, and sediment are present. On YM agar, after 1 month at 17°C, the streak

Table 2. Physiological and chemotaxonomic characterization of *Trichosporon terricola* sp. nov.

Fermentation of carbon compounds		Assimilation of carbon compounds (cont'd)	
D-Glucose	–	5-Ketogluconic acid	+
Assimilation of carbon compounds		DL-Lactic acid	+
D-Glucose	+	Succinic acid	+
D-Galactose	+	Citric acid	+
L-Sorbose	+	Inositol	+
Sucrose	+	n-Hexadecane	–
Maltose	+	Saccharic acid	L
Cellobiose	+	Xylitol	+
α,α -Treharose	+	L-Arabinitol	+
Lactose	+	Propane 1, 2 diol	+
Melibiose	+	Butane 2, 3 diol	L
Raffinose	+	D-Glucuronic acid	+
Melezitose	+	D-Galacturonic acid	+
Inulin	–	Assimilation of nitrogen compounds	
Soluble starch	+	Potassium nitrate	–
D-Xylose	+	Sodium nitrite	–
L-Arabinose	+	Ethylamine hydrochloride	+
D-Arabinose	+	L-Lysine	+
D-Ribose	+	Cadaverine	+
L-Rhamnose	+	Growth at	
D-Glucosamine	+	37°C	+
N-Acetyl-D-glucosamine	+	40°C	+
Methanol	–	Vitamin required:	Biotin
Ethanol	+	Cycloheximide resistance	
Glycerol	+	0.01%	+
Erythritol	+	0.1%	+
Ribitol	L	Production of starch-like substances	+
Galactitol	+	Growth on 50% (w/w) glucose-yeast	–
D-Mannitol	+	extract agar	
D-Glucitol	+	Urease reaction	+
α -Methyl-D-glucoside	+	Diazonium Blue B reaction	+
Salicin	+	Liquefaction of gelatin	–
Glucono- δ -lactone	–	G+C content of nuclear DNA (mol%)	57.6
D-Gluconic acid	+	Major ubiquinone	Q-10
2-Ketogluconic acid	+	Xylose in the cell wall	Present

+, positive; L, latent; –, negative.

culture is pale yellow, mat, wrinkled to cerebriform, elevated near the bottom, butyrous, and has a fimbriate margin. In Dalmau plate culture on cornmeal agar, after 3 days at 25°C, arthroconidia and flexuous mycelia are formed (Fig. 2). The physiological and chemotaxonomic characteristics are shown in Table 2. Type strain: JCM11688 (originally M9900), isolated from soil, in Niiza, Saitama, Japan, by T. Sugita in July 1997, is maintained in the Japan Collection of Microorganisms (JCM), Wako, Saitama, Japan. The other strains, M9952 and M9953 have also been deposited in JCM, as JCM11689 and JCM11690, respectively. Etymology: The specific epithet "*terricola*" is derived from the Latin name for the soil where the type strain of this species was obtained.

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