A NEW SPECIES, *TRICHOSPORON DOMESTICUM*,
ISOLATED FROM THE HOUSE OF A SUMMER-TYPE
HYPERSENSITIVITY PNEUMONITIS
PATIENT IN JAPAN

TAKASHI SUGITA, AKEMI NISHIKAWA, TAKAKO SHINODA,*
KAZUKO YOSHIDA,¹ AND MASAYUKI ANDO¹

Department of Microbiology, Meiji College of Pharmacy, Tanashi 188, Japan

¹First Department of Internal Medicine, Kumamoto University Medical School,
Kumamoto 860, Japan

(Received June 12, 1995; Accepted August 16, 1995)

A new species, *Trichosporon domesticum* Sugita, Nishikawa et Shinoda, is proposed for a strain isolated from the house of a summer-type hypersensitivity pneumonitis patient in Kumamoto, Japan. The nuclear DNA of *T. domesticum* showed intermediate relatedness to that of *T. montevidense*; however, their genome sizes were significantly different. These findings suggest that they represent separate species. *T. domesticum* is distinguished from *T. montevidense* by its inability to assimilate D-arabinose, ribitol, and dulcitol. The type strain of *T. domesticum* is strain M 9401, which has been deposited in the Japan Collection of Microorganisms as JCM 9580.

Trichosporon Behrend 1890 is a genus of basidiomycetous yeasts, having septated mycelia, pseudomycelia and arthroconidia (8). Recently, Guého et al. (6) revised the genus *Trichosporon* on the basis of morphological and biochemical properties, nuclear DNA relatedness and 26S ribosomal RNA partial sequence analyses. Later, Sugita et al. (18) reclassified *T. cutaneum* and its synonyms by nuclear DNA relatedness and proposed a new classification. *Trichosporon* is a medically important genus which contains the causative agents of white piedra and trichosporonosis (5). In addition, Ando and his coworkers (2,3,14,16,21,22) suggested that *T. cutaneum* is the causative agent of summer-type hypersensitivity pneumonitis (SHP) in Japan. During isolation of yeasts from an SHP patient's house, we recovered a strain that is taxonomically closely related to *T. montevidense* (Aciole de Queiroz) Guého & M. Th. Smith. A new species, *T. domesticum* Sugita, Nishikawa et Shinoda, is proposed for the yeast.

* Address reprint requests to: Dr. Takako Shinoda, Department of Microbiology, Meiji College of Pharmacy, 1-22-1 Yato-cho, Tanashi 188, Japan.

MATERIALS AND METHODS

Yeast strain. Strain M 9401 (type strain) was isolated from a damp and rotten wooden sideboard in the kitchen of an SHP patient's house in September 1985 in Kumamoto, Japan. The organism was obtained by swabbing with a cotton applicator (Transwab Medical Wire and Equipment, Wilshire, U.K.) and then was recovered from three kinds of Sabouraud agar plates: agar plates containing 20 U of penicillin per milliliter, or 40 μ g of streptomycin per milliliter and an antibiotic-free plate. *Trichosporon montevidense* and *T. brassicae* were used for taxonomic comparisons (Table 1).

Physiological, biochemical and morphological characteristics. Physiological and biochemical characteristics were investigated by the method of van der Walt and Yarrow (19). Colonies grown on YM agar plates (Difco Laboratories, Detroit, MI, U.S.A.) at 27°C for 14 days were examined morphologically. Microscopic morphology was determined from slide cultures on cornmeal agar and from YM broth cultures, both incubated at 27°C for 3 days. Growth at 20, 27 and 37°C was determined on YM agar.

Ubiquinone system. Ubiquinone isoprenologues were extracted and purified as described by Yamada and Kondo (20). The ubiquinone system was identified by high-performance liquid chromatography (HPLC) by comparing the retention time with those of standard ubiquinones. HPLC was performed with an LC-9A Liquid Chromatograph and an SPD-2A spectrometric detector (Shimadzu, Kyoto, Japan) with a column (4.6 mm I.D. by 25 cm) of Zorbax ODS (Du Pont Co., Wilmington, DE, U.S.A). The ubiquinone was eluted with an ethanol-H₂O (97:3, v/v) mobile phase at a flow rate of 1.0 ml/min and was detected at 285 nm.

DNA isolation and purification. Nuclear DNA was isolated by the method of Restrepo and Barbour (15) with some modification as described by Sugita et al. (18). Nuclear DNA was purified with hydroxyapatite (Bio-Rad Laboratories,

Table 1. Strains used and their coenzyme Q systems and sources.

Species	Strain ^a	Ubiquinone system	Source
<i>Trichosporon domesticum</i> Sugita, Nishikawa et Shinoda	M 9401 ^T	Q9	Damp and rotten wooden sideboard in a kitchen
<i>Trichosporon montevidense</i> (Aciole de Queiroz) Guého et Smith	CBS 6721 ^T	Q9 ^b	Water purification tank
<i>Trichosporon montevidense</i> (Aciole de Queiroz) Guého et Smith	CBS 8261	Q9	Human faeces
<i>Trichosporon brassicae</i> Nakase	CBS 6382 ^T	Q9 ^b	Cabbage

^a M, Meiji College of Pharmacy, Tokyo, Japan; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands.

^b Guého et al. (6).

^T, type strain.

Richmond, CA, U.S.A.) by a batch method (11). The quality of the purified nuclear DNA was determined from thermal profiles.

DNA base composition. Nuclear DNA base composition was determined by nucleotide separation by HPLC after nuclear DNA digestion with Nuclease P1 (Yamasa Shoyu, Chiba, Japan) as described by Sugita et al. (18).

DNA reassociation by chemiluminescence. Nuclear DNA-DNA hybridization was determined by the method of Adnan et al. (1) in the manner we described previously (18). The nuclear DNA-DNA hybridization reaction involved annealing of biotin-labeled nuclear DNA to nuclear DNA that had been covalently bound to wells in a microplate. The plate was examined for chemiluminescence with a Luminous CT-9000 (DIA-IATRON, Tokyo, Japan) after incubation with Lumi-Phos 530 (Wako Pure Chemical Industries Ltd., Osaka, Japan). *Escherichia coli* JH nuclear DNA was used as a negative control.

DNA reassociation by spectroscopy. Reassociation was performed with a model 2600 Spectrophotometer equipped with a model 2527 Thermoprogrammer (Gilford Instruments Inc., Oberlin, OH, U.S.A.). Sheared nuclear DNA was dialyzed against $5 \times \text{SSC}$ (SSC : 0.15 M NaCl, 0.015 M citric acid, pH 7.0) containing 20% dimethylsulfoxide. The nuclear DNA concentration of the sample was adjusted to 75 $\mu\text{g/ml}$ ($A_{260} = 1.5$). After the nuclear DNA sample was denatured to single strand at $T_m + 10^\circ\text{C}$, the temperature was reduced to $T_m - 25^\circ\text{C}$, where it was maintained. Estimates of relative genome size were determined by comparisons of $C_0t_{0.5}$ values (C_0 = initial nucleotide concentration in moles per liter $\times$ time in seconds) obtained from reassociation kinetics.

RESULTS AND DISCUSSION

Trichosporon domesticum Sugita, Nishikawa et Shinoda, sp. nov.

In liquido "YM" post dies 3 ad 27°C , cellulae ellipsoideae aut ovoideae, $3.7\text{--}4.8 \times 5.2\text{--}12.8 \mu\text{m}$. In agar "YM" post dies 14 ad 27°C , cultura in striis flavida, rugosa, madida, margine undulata. Arthroconidia formantur. Fermentatio nulla. Glucosum, galactosum, L-sorbose, sucrose, maltose, cellobiosum, trehalose, lactose, melezitose, amylose, inulinum, D-xylose, L-arabinose, D-ribose, glycerolum, D-mannitolum, inositolum, D-sorbitolum (exiguum), ethanolum, DL-lacticum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum glucuronicum, N-acetyl-glucosaminum, (–)-acidum malicum, acidum succinicum (exiguum), acidum citricum, salicinum (exiguum), α -methyl-D-glucosidum, glucono- δ -lactonum et acidum D-glucosaminum assimilantur at non melibiosum, raffinose, D-arabinose, L-rhamnose, erythritolum, ribitolum nec dulcitolum. Kalium nitricum, ethylaminum, cadaverinum et natrium nitrosum at non L-lysinum assimilantur. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 56.8 (per HPLC). Ubiquinonum majus: Q-9. Teleomorphosis ignota.

Holotypus: Isolata ex mensa putrida in culina, Kumamoto, Japan, 1985, JCM

9580 (originaliter M 9401) conservatur in collectionibus culturarum quas "Japan Collection of Microorganisms," Wako, Saitama sustentat.

Growth in YM broth: After 3 days at 27°C, the cells are ellipsoidal or long ovoidal, $3.7\text{--}4.8 \times 5.2\text{--}12.8\ \mu\text{m}$ (Fig. 1A).

Growth on YM agar: After 14 days at 27°C, the streak culture become yellowish, wrinkled all over the surface, moist and has an undulate margin.

Slide culture on corn meal agar: Arthroconidia are formed (Fig. 1B).

Fermentation:

Glucose	—	Lactose	—
Galactose	—	Trehalose	—
Sucrose	—	Melibiose	—
Maltose	—	Raffinose	—

Assimilation of carbon compounds:

Glucose	+	Erythritol	—
Galactose	+	D-Mannitol	+
L-Sorbose	+	Inositol	+
Sucrose	+	D-Sorbitol	+
Maltose	+	Ribitol	—
Cellobiose	+	Ethanol	+
Trehalose	+	Dulcitol	—
Lactose	+	DL-Lactic acid	+
Melibiose	—	2-Keto-gluconic acid	+
Raffinose	—	5-Keto-gluconic acid	+
Melezitose	+	Glucuronic acid	+

Fig. 1. Cell morphology of *Trichosporon domesticum* sp. nov.

(A) Vegetative cells of M 9401 grown in YM broth for 3 days at 27°C.

(B) Arthroconidia of M 9401 that developed on cornmeal agar after 3 days incubation at 27°C. Scale bar indicates 10 μm.

Soluble starch	+	N-Acetyl-glucosamine	+
Inulin	+	(-)-Malic acid	+
D-Xylose	+	Succinic acid	+ (weak)
L-Arabinose	+	Citric acid	+
D-Arabinose	-	Salicin	+ (weak)
D-Ribose	+	α -Methyl-D-glucoside	+
L-Rhamnose	-	Glucono- δ -lactone	+
Glycerol	+	D-Glucosamine	+
Assimilation of nitrogen compounds:			
Potassium nitrate	+	L-Lysine hydrochloride	+
Sodium nitrite	-	Cadaverine dihydrochloride	+
Ethylamine hydrochloride	+		

Growth temperature: 20°C, positive; 27°C, positive; 37°C, positive.

Cycloheximide resistance: 100 ppm, positive; 1,000 ppm, negative.

Diazonium blue B color reaction: Positive.

Splitting of arbutin: Positive.

Growth on 50% (w/w) glucose-yeast extract agar: Negative.

Growth in vitamin-free medium: Negative.

G + C content of nuclear DNA: 56.8 mol% (by HPLC).

Major ubiquinone system: Q-9.

Holotype strain: JCM 9580 (originally M 9401), isolated from a damp and rotten wooden sideboard in the kitchen of an SHP patient's house in Kumamoto, Japan in 1985 by Kazuko Yoshida. This strain has been deposited in The Japan Collection of Microorganisms, Wako, Saitama.

It has been suggested that *T. cutaneum* is one of the causative agents of SHP by Ando and his colleagues (2,3,14,16,21,22). They have isolated *Trichosporon* species from an SHP patient's house in Japan (2,3,16,22). We have already reported that *Trichosporon* species have several serotypes (3,10,21). Our serological survey found that the serotype of *T. domesticum* was identical to those of *T. montevidense* and *T. brassicae* (unpublished data). On the other hand, Guého et al. (6) divided the genus *Trichosporon* into five major taxonomic groups based on 26S rRNA partial sequence analyses. Of the five groups, group 5 contains only two species, *T. montevidense* and *T. brassicae*. Nuclear DNA relatedness between the five groups was less than 34% (6,18). As stated above, we thought that our new species, *T. domesticum* might be taxonomically positioned in group 5 and compared it with the two species *T. montevidense* and *T. brassicae*. Three species, *T. domesticum*, *T. montevidense* and *T. brassicae*, have similar G + C content of their nuclear DNA (Table 2). *T. domesticum* showed 56.8 mol% G + C by the HPLC method. *T. montevidense* and *T. brassicae* ranged from 56.0 to 57.2 mol%. In nuclear DNA-DNA hybridization, *T. domesticum* exhibited very low nuclear DNA relatedness values, 0.3–17.2% to *T. brassicae*. However, the nuclear DNA of *T. domesticum* was shown to be intermediately related to that of the two strains of *T.*

montevideense (46.9 and 61.2%) (Table 2). Intermediate values such as our data have been reported infrequently. These values are generally regarded as representing a more differentiated strain within a species such as a variety or sibling species (7). In fact, we proposed that they should be reclassified as varieties in the same species but not as different species based on the limited nuclear DNA relatedness levels (40 to 56%), although *T. asahii*, *T. coremiforme* and *T. faecale*, which belong to group 3, were described as different species by Guého et al. (6). Similar cases have been reported for the three varieties of *Cryptococcus albidus* (17) and the two varieties of *Filobasidiella neoformans* (4). On the other hand, Martini and Kurtzman (12) have reported that *Saccharomyces pastorianus* exhibits intermediate nuclear DNA relatedness to both *S. cerevisiae* and *S. bayanus* (58 and 70%, respectively) even though these latter two species demonstrated 10% relatedness to each other. These observations, together with the genome sizes, led the authors to suggest that *S. pastorianus* is a partial amphidiploid which arose as a natural hybrid of *S. cerevisiae* and *S. bayanus*. Another example of apparent ploidy differences influencing speciation is to be found in comparisons of *S. exiguus* and its anamorph *Candida holmii* (9). The type strain *S. exiguus* and *C. holmii* show 76% nuclear DNA relatedness and have the same genome size. Other strains of each of the two species have a somewhat smaller genome despite relatively high relatedness with their respective type strains. These results were confirmed by electrophoretic karyotyping (13). From the above observations, it is important to demonstrate whether the genome is significantly larger or not. In the case of our new species, the observed $C_{0.5}$ values of *T. domesticum* and *T. montevideense* were 1.76 and 1.07 mol·s/l, respectively (Table 3). Therefore, the genome size of *T. domesticum* is estimated to be approximately 1.6 times as large as that of *T. montevideense*. We speculate that the relatively high nuclear DNA relatedness between the two species is due to the difference in their genome sizes. Therefore, we propose *T. domesticum* as a new species, not a variety of *T. montevideense*, though their nuclear DNAs exhibit intermediate relatedness. Practically, *T. domesticum* is distinguished from *T. montevideense* by its inability to assimilate D-arabinose, ribitol and dulcitol, and

Table 2. Mol% G+C of DNA and % DNA relatedness among *Trichosporon domesticum* and related species.

Species	Strain	Mol% G+C ^a	% DNA relatedness with		
			<i>T. domesticum</i> M 9401 ^T	<i>T. montevideense</i> CBS 6721 ^T	<i>T. montevideense</i> CBS 8261
<i>T. domesticum</i>	M 9401 ^T	56.8±0.5	100.0 ^b		
<i>T. montevideense</i>	CBS 6721 ^T	57.2±0.5	61.2	100.0 ^b	
<i>T. montevideense</i>	CBS 8261	57.0±0.4	46.9	82.3	100.0 ^b
<i>T. brassicae</i>	CBS 6382 ^T	56.0±0.4	17.2	0.3	5.7

^a Mean ± SD (n=6).

^b 100% related by definition.

^T, type strain.

Table 3. C_{50} values of *Trichosporon domesticum* and *T. montevidense*.

Strain		Observed C_{50} ^a (mol·s/l)
<i>T. domesticum</i>	M 9401 ^T	1.76±0.18
<i>T. montevidense</i>	CBS 6721 ^T	1.07±0.13

^a Mean±SD (n=5).^T, type strain.Table 4. Biochemical characteristics of *Trichosporon domesticum* and related species.

Assimilation of carbon compounds	<i>T. domesticum</i> M 9401 ^T	<i>T. montevidense</i> CBS 6721 ^T	<i>T. montevidense</i> CBS 8261	<i>T. brassicae</i> CBS 6382 ^T
L-Sorbose	+	—	—	+
Cellobiose	+	+	+	—
Lactose	+	+	+	—
Melezitose	+	+	+	—
D-Arabinose	—	+	+	—
Ribitol	—	+	+	—
Dulcitol	—	+	+	—
Salicin	+	+	+	—
α -Methyl-D-glucoside	+	+	+	—

+, positive; —, negative; w, weakly positive. ^T, type strain.

from *T. brassicae* by its ability to assimilate cellobiose, lactose, melezitose, salicin and α -methyl-D-glucoside (Table 4). *T. domesticum* was isolated from the SHP patient's house, but it is not known whether this species is the causative agent of the disease or not, because we do not yet have any evidence of inhalation challenge with this species. Our new species, *T. domesticum*, has been deposited as JCM 9580 in The Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN) in Wako, Saitama, Japan.

We thank Prof. J. Sugiyama, Institute of Molecular and Cellular Biosciences, The University of Tokyo, for his help in the preparation of the Latin diagnosis, and Dr. T. Nakase, The Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN) for depositing the culture.

REFERENCES

- 1) Adnan, S., Li, N., Miura, H., Hashimoto, Y., Yamamoto, H., and Ezaki, T., Covalently immobilized DNA plate for luminometric DNA-DNA hybridization to identify viridans streptococci in under 2 hours. *FEMS Microbiol. Lett.*, **106**, 139–142 (1993).
- 2) Ando, M., Arima, K., Yoneda, R., and Tamura, M., Japanese summer-type hypersensitivity pneumonitis. *Am. Rev. Respir. Dis.*, **144**, 765–769 (1991).
- 3) Ando, M., Sakata, T., Yoshida, K., Yamasaki, H., Araki, S., Onoue, K., and Shinoda, T., Serotype-related antigen of *Trichosporon cutaneum* in the induction of summer-type hypersensitivity pneumonitis: Correlation between serotype of inhalation challenge-positive antigen and that of

- the isolates from patients' homes. *J. Allergy Clin. Immunol.*, **85**, 36–44 (1990).
- 4) Aulakh, H. S., Straus, S. E., and Kwon-Chung, K. J., Genetic relatedness of *Filobasidiella neoformans* (*Cryptococcus neoformans*) and *Filobasidiella bacillispora* (*Cryptococcus bacillisporus*) as determined by deoxyribonucleic acid base composition and sequence homology studies. *Int. J. Syst. Bacteriol.*, **31**, 97–103 (1981).
 - 5) Guého, E., Improvisi, R., de Hoog, G. S., and Dupont, B., *Trichosporon* on humans: A practical account. *Mycoses*, **37**, 3–10 (1994).
 - 6) Guého, E., Smith, M. Th., de Hoog, G. S., Billon-Grand, G., Christen, R., and Batenburg-van der Vegte, W. H., Contributions to a revision of the genus *Trichosporon*. *Antonie van Leeuwenhoek*, **61**, 289–316 (1992).
 - 7) Kreger-van Rij, N. J. W., The species. In *The Yeasts: A Taxonomic Study*, 3rd ed., ed. by Kreger-van Rij, N. J. W., Elsevier Science Publishers B. V., Amsterdam (1984), p. 16.
 - 8) Kreger-van Rij, N. J. W., Genus 16. *Trichosporon* Behrend. In *The Yeasts: A Taxonomic Study*, 3rd ed., ed. by Kreger-van Rij, N. J. W., Elsevier Science Publishers B. V., Amsterdam (1984), p. 933–962.
 - 9) Kurtzman, C. P., Prediction of biological relatedness among yeasts from comparisons of nuclear DNA complementarity. *Stud. Mycol.*, **30**, 459–468 (1987).
 - 10) Kwon-Chung, K. J., Kozel, T. R., Edman, J. C., Polacheck, I., Ellis, D., Shinoda, T., and Dromer, F., Recent advances in biology and immunology of *Cryptococcus neoformans*. *J. Med. Vet. Mycol.*, **30** (Suppl. 1), 133–142 (1992).
 - 11) Lachance, M. A., Simple method for determination of deoxyribonucleic acid relatedness by thermal elution in hydroxyapatite microcolumns. *Int. J. Syst. Bacteriol.*, **30**, 433–436 (1980).
 - 12) Martini, A. V. and Kurtzman, C. P., Deoxyribonucleic acid relatedness among species of the genus *Saccharomyces sensu stricto*. *Int. J. Syst. Bacteriol.*, **35**, 508–511 (1985).
 - 13) Martini, A. V., Martini, A., and Cardinali, G., Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces*. *Antonie van Leeuwenhoek*, **63**, 145–156 (1993).
 - 14) Mizobe, T., Yamasaki, H., Doi, K., Ando, M., and Onoue, K., Analysis of serotype-specific antibodies to *Trichosporon cutaneum* type I and II in patients with summer-type hypersensitivity pneumonitis with monoclonal antibodies to serotype-related polysaccharide antigens. *J. Clin. Microbiol.*, **31**, 1949–1951 (1993).
 - 15) Restrepo, B. I. and Barbour, A. G., Cloning of 18S and 25S rDNAs from the pathogenic fungus *Cryptococcus neoformans*. *J. Bacteriol.*, **171**, 5596–5600 (1989).
 - 16) Shimazu, K., Ando, M., Sakata, T., Yoshida, K., and Araki, S., Hypersensitivity pneumonitis induced by *Trichosporon cutaneum*. *Am. Rev. Respir. Dis.*, **130**, 407–411 (1984).
 - 17) Sugita, T., Nishikawa, A., and Shinoda, T., DNA relatedness among the three varieties of *Cryptococcus albidus*. *J. Gen. Appl. Microbiol.*, **38**, 83–86 (1992).
 - 18) Sugita, T., Nishikawa, A., and Shinoda, T., Reclassification of *Trichosporon cutaneum* by DNA relatedness by using the spectrophotometric method and the chemiluminometric method. *J. Gen. Appl. Microbiol.*, **40**, 397–408 (1994).
 - 19) Van der Walt, J. P. and Yarrow, D., Methods for the isolation, maintenance, classification and identification of yeasts. In *The Yeasts, A Taxonomic Study*, 3rd ed., ed. by Kreger-van Rij, N. J. W., Elsevier Science Publishers B. V., Amsterdam (1984), p. 45–105.
 - 20) Yamada, Y. and Kondo, K., Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast like genera *Sporobolomyces* and *Rhodospiridium*. *J. Gen. Appl. Microbiol.*, **19**, 59–77 (1973).
 - 21) Yamasaki, H., Ando, M., Sakata, T., Araki, S., and Onoue, K., Importance of serotype-related antigen in the induction of experimental hypersensitivity pneumonitis by *Trichosporon cutaneum* in rabbits. *Am. Rev. Respir. Dis.*, **141**, 734–742 (1990).
 - 22) Yoshida, K., Ando, M., Sakata, T., and Araki, S., Environmental mycological studies on the causative agent of summer-type hypersensitivity pneumonitis. *J. Allergy Clin. Immunol.*, **81**, 475–483 (1988).