***Trichosporon japonicum* sp. nov. isolated from the air**

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A yeast strain isolated from the air in Japan was found to represent a new species, and was named *Trichosporon japonicum*. This species produced arthroconidia and appressoria. *T. japonicum* formed a cluster with the appressorium-forming species *Trichosporon inkin* and *Trichosporon ovoides* in a phylogenetic tree constructed using small-subunit rDNA sequences. However, they had low relatedness to each other in DNA–DNA hybridization experiments. *T. japonicum* is distinguished from both *T. inkin* and *T. ovoides* by its ability to assimilate inulin, and its inability to assimilate L-rhamnose. JCM 8357^T is the type strain of the species.

Keywords: *Trichosporon japonicum* sp. nov., yeast

INTRODUCTION

Basidiomycetous anamorphic yeasts of the genus *Trichosporon* Behrend are characterized by the production of arthroconidia and the presence of xylose in the cells. In 1992, Guého *et al.* proposed the new taxonomy of the genus *Trichosporon* based on the partial sequences of large-subunit rRNA and DNA relatedness. Presently, 17 species and five varieties are recognized within the genus (Guého *et al.*, 1992; Sugita *et al.*, 1994, 1995). Of these, 13 species possess Q-9 as the major ubiquinone, while the other four possess Q-10. The strain JCM 8357^T used in this study was isolated from the air in 1971. This strain has been maintained in the Central Research Laboratories, Ajinomoto Co. Inc., as *Prototheca* species. However, the isolate seemed to be closely related to *Trichosporon inkin* and *Trichosporon ovoides*, because it produced arthroconidia and appressoria. A comparative taxonomic study among these three yeasts revealed that the strain JCM 8357^T represented a new species. *Trichosporon japonicum* Sugita et Nakase sp. nov. is proposed for this isolate.

METHODS

Strains examined. The strain JCM 8357^T reported in this paper was supplied from Central Research Laboratories, Ajinomoto Co. Inc., Kawasaki, Kanagawa Pref., Japan, as

Abbreviation: SSU, small-subunit.

The GenBank/EMBL/DBJ accession number for the SSU rDNA sequence of JCM 8357^T is AB001749.

Prototheca sp. AJ 14673. It was isolated in 1971 as an air contaminant in a microbiological laboratory.

Morphological, biochemical and physiological characteristics. Most of the morphological, biochemical and physiological characteristics were examined using the methods described by van der Walt (1984). Assimilation of nitrogen compounds was investigated according to the methods of Nakase & Suzuki (1986). Vitamin requirements were examined using the methods of Komagata & Nakase (1967). The maximum growth temperature was determined in YM broth (Difco) using metal heating blocks.

Examination of chemotaxonomic characteristics. Extraction, purification and identification of ubiquinones were carried out according to the methods of Nakase & Suzuki (1988). The presence of xylose in the cells was determined using methods described by Suzuki & Nakase (1988). Isolation and purification of DNA followed the methods of Nakase & Suzuki (1986). The DNA base composition (mol% G + C) was determined by using HPLC after digesting the DNA with nuclease P1 (Yamasa Shouyu) and alkaline phosphatase (Sigma) as described by Nakase *et al.* (1989). DNA–DNA hybridization experiments were performed using the membrane-filter method of Nakase & Suzuki (1985).

Sequencing of small-subunit rDNA (SSU rDNA). The SSU rRNA coding region was amplified by the PCR as described in Suh & Nakase (1995). The PCR product was sequenced by using the Sequi Therm Long-Read Cycle Sequencing Kit (Epicentre Technologies) with the primers described in Suh *et al.* (1996). Analyses of DNA sequence reactions were performed on an ALFexpress DNA sequencer (Pharmacia Biotech).

Phylogenetic analysis. The sequences were aligned using the

CLUSTAL W computer program (Thompson *et al.*, 1994). For phylogenetic analysis, SSU rDNA sequences of related basidiomycetes were obtained from the EMBL, GenBank and DDBJ nucleotide sequence databases. The urediniomycetous yeasts *Rhodosporidium toruloides* (D12806) and *Sporidiobolus johnsonii* (L22261) were used as outgroups. Kimura's two-parameter distances (Kimura, 1980) were used for the neighbour-joining analysis (Saito & Nei, 1987). Sites where gaps existed in any of the sequences were excluded from the analysis. Bootstrap analysis was performed using 1000 random replications (Felsenstein, 1985).

RESULTS AND DISCUSSION

Latin diagnosis of *Trichosporon japonicum* Sugita et Nakase sp. nov.

In liquido 'YM', post dies 5 ad 25 °C, cellulae ovoideae, ellipsoideae, elongatae, (4·5–9·6) × (5·8–9·7) µm, singulae aut binae. Sedimentum formatur. Post unum mensem ad 25 °C, anulus et sedimentum formantur. In agarō 'YM', post unum mensem ad 25 °C, cultura eburnea, margine erosa. Appressorium, arthroconidium aut mycelium formatur. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, saccharosum, maltosum, cellobiosum, trehalosum, lactosum, melezitium, inulinum (exiguum), amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum (exiguum), D-ribosum, ethanolum (exiguum), glycerolum (exiguum), erythritolum, ribitolium (exiguum), galactitolium, D-mannitolium (exiguum), D-glucitolium, methyl α-D-glucosidum, salicinum, glucono-δ-lactonum (exiguum), acidum 2-ketogluconicum, acidum D-glucuronicum (exiguum), acidum D-galacturonicum (exiguum), acidum DL-lacticum, acidum succinicum, acidum citricum (exiguum), et N-acetylglucosaminum assimilantur at non melibiosum, raffinolum, L-rhamnosum, acidum 5-ketogluconicum, inositolium, n-hexadecanum, D-glucosaminum, methanolum nec D-saccharicum. Ammonium sulfatum, ethylaminum, L-lysinum et cadaverinum assimilantur at non kalium nitricum nec natrium nitrosolum. Maxima temperatura crescentiae: 39–40 °C. Ad crescentiam biotinum necessarium est. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 59·8 mol % (per HPLC). Ubiquinonum majus: Q-9. Xylosum in cellulis presens. Holotypus: isolatus ex ventilarum, JCM 8357^T Conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentat.

Description of *Trichosporon japonicum* Sugita et Nakase sp. nov.

Trichosporon japonicum (ja.po'ni.cum. M.L. masc. adj. *japonicum* pertaining to Japan, where the organism was isolated).

Growth in YM broth: After 5 d at 25 °C, cells are ovoidal, ellipsoidal, elongate, (4·5–9·6) × (5·8–9·7) µm, single or in pairs (Fig. 1a). A sediment is formed. After 1 month at 25 °C, a ring and a sediment are present. Growth on YM agar: After 1 month at 25 °C, the

Fig. 1. Morphology of *Trichosporon japonicum* sp. nov. after growth for 5 d at 25 °C. (a) Vegetative cells grown in YM broth. (b) Appressoria produced on corn meal agar. (c) Arthroconidia produced on corn meal agar. Bars, 10 µm.

streak culture is ivory and has an erose margin. Dalmau plate culture on corn meal agar: appressoria, arthroconidia and mycelia are produced (Fig. 1b and 1c). Fermentation: absent. Assimilation of carbon compounds: glucose, +; ribitol, + (weak); galactose, +; galactitol, +; L-sorbose, +; D-mannitol, + (weak); sucrose, +; D-glucitol, +; maltose, +; methyl α-D-glucoside, +; cellobiose, +; salicin, +; trehalose, +; glucono-δ-lactone, + (weak); lactose, +; 2-ketogluconic acid, +; melibiose, –; 5-ketogluconic acid, –; raffinose, –; D-glucuronic acid, + (weak); melezitose, +; D-galacturonic acid, + (weak); inulin, + (weak); DL-lactic acid, +; soluble starch, +; succinic acid, +; D-xylose, +; citric acid, + (weak); L-arabinose, +; inositol, –; D-arabinose, + (weak); n-hexadecane, –; D-ribose, +; D-glucosamine, –; L-rhamnose, –; N-acetylglucosamine, +; ethanol, + (weak); methanol, –; glycerol, + (weak); D-saccharic acid, –; erythritol, +. Assimilation of nitrogen compounds: ammonium sulfate, +; ethylamine hydrochloride, +; potassium nitrate, –; L-lysine hydrochloride, +; sodium nitrite, –; cadaverine dihydrochloride, +. Maximum growth temperature: 39–40 °C. Vitamins required: biotin. Production of starch-like substances: negative. Growth on 50 % (w/w) glucose-yeast extract agar: negative. Urease: positive. Liquefaction of gelatin: negative. Hydrolysis of fat: negative. Diazonium blue B reaction: positive. G+C content of nuclear DNA: 59·8 mol % (by

Fig. 2. Neighbour-joining tree resulting from analysis of SSU rDNA sequences of *Trichosporon japonicum* sp. nov. and related taxa. The numerals represent the confidence level from 1000 replicate bootstrap sampling (the frequency less than 50% was not indicated).

Table 1. DNA relatedness among *Trichosporon japonicum* and phylogenetically closely related species

Species	Strain	G + C content (mol %)	Relative binding (%) of DNA with:		
			JCM 8357 ^T	JCM 9940 ^T	JCM 9195 ^T
<i>Trichosporon japonicum</i>	JCM 8357 ^T	59.8	100	29	29
<i>Trichosporon ovoides</i>	JCM 9940 ^T	58.5*, 60.1†	24	100	23
<i>Trichosporon inkin</i>	JCM 9195 ^T	59.5*	27	27	100

* Guého *et al.* (1992).

† Sugita *et al.* (1994).

HPLC). Major ubiquinone: Q-9. Xylose in the cells: present. Type strain: JCM 8357^T, isolated by M. Hoshino from the air in 1971, Kawasaki, Kanagawa Pref., Japan. This strain has been deposited in the Japan Collection of Microorganisms, Wako, Saitama.

Morphologically, the genus *Trichosporon* is characterized by the production of septate mycelia and arthroconidia. A few species possess additional diagnostic morphological characters, such as the production of appressoria, macroconidia or meristematic

conidiation. Strain JCM 8357^T produced appressoria on a slide culture of corn meal agar. The only species in the genus *Trichosporon* that produce appressoria are *T. inkin* and *T. ovoides*. We have already determined the SSU rDNA sequences for all of the species in the genus *Trichosporon* (Sugita & Nakase, 1997). The sequence data showed that the genus *Trichosporon*, with the exception of *Trichosporon pullulans*, was monophyletic although the members of the genus *Trichosporon* have two different major ubiquinones, Q-9 and Q-10. *T. japonicum* formed a cluster with the

Table 2. Differential characteristics of *Trichosporon japonicum* and phylogenetically closely related *Trichosporon* species

+, Positive; —, negative; w, weak.

Species	Assimilation of:		Appressoria
	Inulin	L-Rhamnose	
<i>T. japonicum</i>	w	—	+
<i>T. inkin</i>	—	—	+
<i>T. ovoides</i>	—	+	+
<i>T. asahii</i> var. <i>asahii</i>	—	+	—
<i>T. asahii</i> var. <i>coremiformis</i>	—	+	—
<i>T. asahii</i> var. <i>faecalis</i>	—	+	—
<i>T. asteroides</i>	—	+	—
<i>T. aquatile</i>	—	—	—

appressorium-producing species, *T. inkin* and *T. ovoides* (Fig. 2). On the basis of this phylogenetic analysis, we also performed a DNA–DNA hybridization experiment. As shown in Table 1, the DNA of *T. japonicum* indicated low relatedness values (23–29%) with either *T. inkin* or *T. ovoides*. This result suggests that *T. japonicum* represents a new species in the genus *Trichosporon*.

In practice, *T. japonicum* is distinguished from the phylogenetically closely related species *Trichosporon asahii* var. *asahii*, *Trichosporon asahii* var. *coremiformis*, *Trichosporon asahii* var. *faecalis*, *Trichosporon asteroides*, *Trichosporon aquatile*, *T. inkin* and *T. ovoides* by its ability to assimilate inulin, its inability to assimilate L-rhamnose, and the production of appressoria (Table 2).

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