

Trichosporon asahii, a new record for Taiwan

Chih-Jen Tien¹, Pi-Han Wang², and Fwu-Ling Lee^{1*}

1. Food Industry Research and Development Institute, P. O. Box 246, Hsinchu 30099, Taiwan, R.O.C.

2. Department of Life Science, Tunghai University, P. O. Box 851, Taichung 407, Taiwan, R.O.C.

(Accepted: ?? ??, 2006)

ABSTRACT

Yeast *Trichosporon asahii* is a new record from Taiwan. Morphological and physiological characteristics of the species are described and illustrated.

Keywords: Taiwan, taxonomy, *Trichosporon asahii*, yeast.

Introduction

Trichosporon with 19 species under the genus represents a natural group of arthrosporous anamorphs that belong to Basidiomycota, was assigned to the Cryptococcales (Kurtzman and Fell, 2000), and phylogenetically relatively close to *Cryptococcus* (Guého *et al.*, 1994). The genus is a clinically important one that not only causes infections (trichosporonosis) but also induces summer-type hypersensitivity pneumonitis, and the cases increase recently (Leblond *et al.*, 1986; Young *et al.*, 1993; Sugita *et al.*, 1995; Itoh *et al.*, 1996; Ebright *et al.*, 2001; Meyer *et al.*, 2002; Abliz *et al.*, 2002). According to the references listed above, invasive *Trichosporon* infections are due to *T. asahii* in most cases and is widely distributed throughout the environment, such as soil, plant, fruit juice, cheese, seafood, polluted sea and fresh water (Woollett *et al.*, 1970; Buck *et al.*, 1977; Hagler and Mendonça-Hagler, 1981; Viljoen and

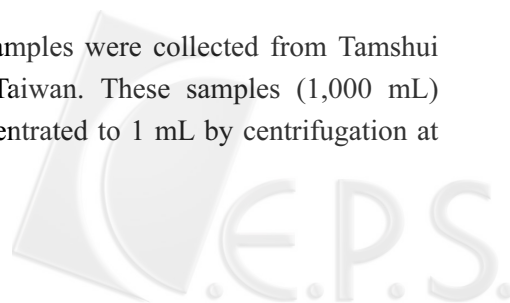
Greyling, 1995; Sláviková and Vadkertiová *et al.*, 1997; Sugita *et al.*, 2000; Heras-Vazquez *et al.*, 2003).

In Taiwan, isolates of *Trichosporon* including *T. ovoides* (synonyms: *T. beigeli*) have been isolated from human (Young *et al.*, 1993), however no study described species found from environment (Wang *et al.*, 1999; Tzean *et al.*, 2005). In this study, *T. asahii* was collected from Tamshui River, Taipei County, and is recorded for the first time in Taiwan. Morphological and physiological characteristics were described. The culture was preserved in Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, R.O.C.

Materials and Methods

Water samples were collected from Tamshui River in Taiwan. These samples (1,000 mL) were concentrated to 1 mL by centrifugation at

* Corresponding author, E-mail: fl@firdi.org.tw



1,000 ×g for 5 min. The concentrated samples were plated on acidified (pH 4.4) YM agar (yeast extract-malt extract agar, yeast extract 3 g, malt extract 3 g, peptone 5 g, glucose 10 g, agar 18 g, distilled water 1,000 mL) plates that contain 40 mg/L chloramphenicol (Kurtzman and Fell, 1998) and incubated 3 to 5 days at 25°C. Yeast isolates were purified by four-way streak-plate method.

Isolated yeasts were grown on YM agar and incubated at 25°C. Morphological characteristics were observed by using light microscope and scanning electron microscope (Instrumentation Center of National Taiwan University). Physiological characteristics tests were performed with Biolog yeast identification kits (Biolog, California, U.S.A.).

Genomic DNA was extracted by the CTAB method as previously described by Wang *et al.* (2003) which was adapted from Doyle and Doyle (1990). Internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) was amplified by primer ITS1 (5' -TCC GTA GGT GAA CCT GCG G- 3') and ITS4 (5' -TCC TCC GCT TCA TTG ATA TGC- 3'), and D1/D2 domain of large sub-unit (LSU) rDNA was amplified by primer F63 (5' -GCA TAT CAA TAA GCG GAG GAA AAG- 3') and LR3 (5' -GGT CCG TGT TTC AAG AAC G- 3'). Polymerase chain reaction (PCR) was performed as described by Wang *et al.* (2003). PCR product was sequenced (Mission Biotech, Taipei, Taiwan) and checked against the GeneBank databases using BLAST.

Taxonomy

Trichosporon asahii Akagi ex Sugita, Nishikawa & Shinoda, 1994.

Growth on YM agar. After 7 days at 25°C: Colonies are white, farinose at the center, with wide, dry, often finely zonate margin with deep transverse fissures. Growth is odorless (Fig. 1A). After 3 days at 25°C the cells are cylindrical/rectangular, 5.0 × 5.0–13.0 μm, usually cluster in pairs (Fig. 1B, Fig. 1C).

Dalmau plate culture on Corn Meal Agar: True hyphae are abundant and easily disarticulate into regularly arthroconidia that measure 1.5 × 1.5–3.0 μm (Fig. 1D–1F).

Physiological characteristics. see Table 1.

Specimen examined. Tamshui River in Taipei Country. 17 Aug, 2000. Strain isolated from river water. Isolation number ST8H-6a.

Discussion

Sequence analysis of D1/D2 domain of 26S rDNA for strain ST8H-6a (AY359869) showed the highest similarity (100%) with type strain *T. asahii* CBS 2479^T. Sequence of Internal transcribed spacer (ITS) region of 18S rDNA for strain ST8H-6a (AY055381), also showed the similar result that have highest similarity (100%) with *T. asahii*. The physiological and biochemical characteristics of the strains were tested by Biolog yeast identification system. The result showed that *T. inkin* was the most closely related species. To compare characteristics obtained by Biolog of strain ST8H-6a and description of *T. asahii* by Kurtzman (1998), the characteristics of strain ST8H-6a, *T. asahii* and *T. inkin* were very similar to each other. To compare morphology characteristics, strain ST8H-6a has hyphae with liberated, cubic arthroconidia as *T. asahii*, the character can be distinguished from *T. inkin* that has hyphae with appressoria, and cylindrical arthroconidia.

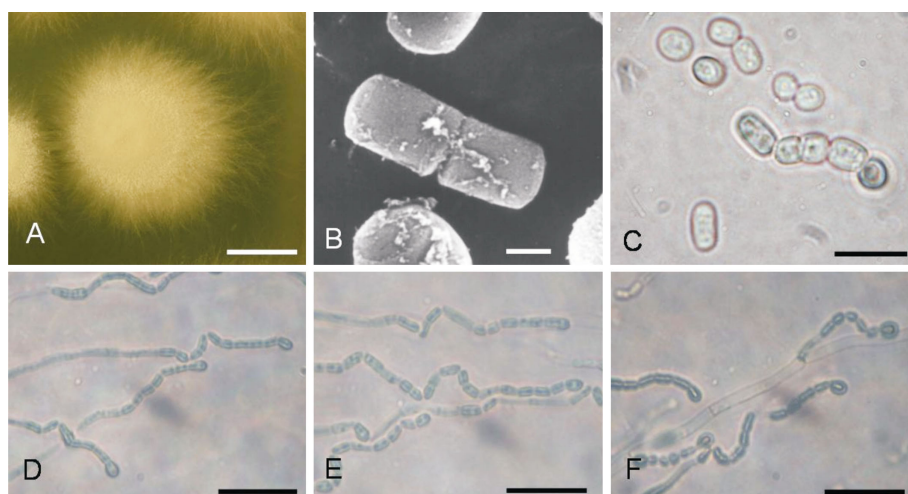


Fig. 1. *Trichosporon asahii* ST8H-6a. (A) Colony morphology, 7 days at 25°C in YM agar. Bar = 5 mm. (B) Vegetative cells after 3 days at 25°C in YM agar, Bar = 1 µm. (C) Vegetative cells after 3 days at 25°C in YM agar, Bar = 10 µm (D, E, & F) Hyphae disarticulating into arthroconidia after 3 days at 25°C in CMA agar. Bar = 10 µm. A, DM (Nikon SMZ-10A), B, SEM, C, D, E & F, LM (Nikon ECLIPSE E800).

Table 1. Physiological characteristics of *Trichosporon asahii* ST8H-6a isolated from river water in Taiwan.

Oxidation test	-D-glucose	+ Maltose	+ Adonitol	-
Acetic acid	v D-galactose	+ Maltotriose	+ D-arabitol	-
Formic acid	v D-psiocese	v D-melezitose	- Xylitol	-
Propionic acid	- L-sorbose	+ D-melibiose	- i-erythritol	+
Succinic acid	+ Salicin	v Palatinose	+ Glycerol	-
Methyl succinate	+ D-mannitol	- D-raffinose	- Tween 80	+
L-aspartic acid	+ D-sorbitol	v Stachyose	- L-arabinose	+
L-glutamic acid	+ D-arabitol	- Sucrose	+ D- arabinose	v
L-proline	+ Xylitol	- D-trehalose	+ D-ribose	+
D-gluconic acid	+ Glycerol	- Turanose	+ D-xylose	+
Dextrin	+ Tween 80	+ N-acetyl-D-glucosamine	+ Methyl succinate+D-xylose	+
Inulin	v Assimilation test	D-glucosamine	+ N-acetyl-L- glutamic acid + D-xylose	+
Cellobiose	+ Fumaric acid	+ -D-glucose	+ Quinic acid+D-xylose	+
Gentiobiose	+ L-malic acid	- D-galactose	+ D-glucuronic acid + D-xylose	+
Maltose	+ Methyl succinate	+ D- psiocese	v Dextrin + D-xylose	+
Maltotriose	+ Bromo succinic acid	v L- rhamnose	+ -D-lactose + D-xylose	+
D-melezitose	- L-glutamic acid	+ L-sorbose	+ D-melibiose + D-xylose	+
D-melibiose	- -amino butyric acid	+ -methyl-D-glucoside	+ D-galactose + D-xylose	+
Palatinose	+ -keto- glutamic acid	- -methyl-D-glucosidase	+ m-inositol+ D-xylose	+
D-raffinose	- 2-keto-D-guconic acid	+ Amygdalin	+ 1,2-propanediol + D-xylose	+
Stachyose	- D-gluconic acid	+ Arbutin	+ acetoin + D-xylose	v
Sucrose	+ Dextrin	+ Salicin	+	
D-trehalose	+ Inulin	- Maltitol	+ Growth at 37°C	+
Turanose	+ Cellobiose	+ D- mannitol	+	
N-acetyl-D-glucosamine	+ Gentiobiose	+ D- sorbitol	-	

Based on Biolog YT MicroPlate instructions. All reactions optically resembling the negative control wells are scored as “negative” (-) and all wells with a noticeable increase in absorbance at 590 nm are scored as “positive” (+). Well with an extremely slight increase in absorbance at 590 nm are scored as “borderline” (∅).

Based on these characteristics, strain ST8H-6a was identified as *T. asahii*.

As strain ST8H-6a exhibited a physiological profile identical to *T. inkin*, and *T. asahii* was not included in the database of the Biolog yeast identification system, it was misidentified as *T. inkin*.

Acknowledgements

This study was supported by the National Science Council, Taiwan, R.O.C., grant number NSC89-2320-B-031-002 and the Ministry of Economic Affairs, Taiwan, R.O.C (project no. 05G270-03).

Reference

- Abliz, P., K. Fukushima, K. Takizawa, R. Yang, R. Li, and K. Nishimur. 2002. Identification of the first isolates of *Trichosporon asahii* var. *asahii* from disseminated trichosporosis in China. *Diagn. microbiol. infect. dis.* 44: 17–22.
- Buck, J.D., P.M. Bubucis, and T.J. Combs. 1977. Occurrence of human-associated yeasts in bivalve shellfish from Long Island Sound. *Appl. Environ. Microbiol.* 33: 370–378.
- Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15.
- Ebright, J.R., M.R. Fairfax, and J.A. Vazquez. 2001. *Trichosporon asahii*, a non-*Candida* Yeast that caused fatal septic shock in a patient without cancer or neutropenia. *Clin. infect. dis.* 33: 28–30.
- Guého, E., L. Improvisi, G.S. de Hoog, and B. Dupont. 1994. *Trichosporon* on humans: a practical account. *Mycoses* 37: 3–10.
- Kurtzman, C.P. and J.W. Fell. Elsevier, Amsterdam, Netherlands.
- Hagler, A.N. and L.C. Mendonça-Hagler. 1981. Yeasts from Marine and estuarine waters with different levels of pollution in the state of Rio de Janeiro, Brazil. *Appl. Environ. Microbiol.* 41: 173–178.
- Heras-Vazquez, F.J.L., L. Mingorance-Cazorla, J.M. Clemente-jimenez, and F. Rodriguez-Vico. 2003. Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of 5.8S rRNA gene and the two internal transcribed spacers. *FEMS Yeast Research* 3: 3–9.
- Itoh, T., H. Hosokawa, U. Kohdera, N. Toyazaki, and Y. Asada. 1996. Disseminated infection with *Trichosporon asahii*. *Mycoses* 39: 195–199.
- Kurtzman, C.P. and J.W. Fell. 1998. *The Yeasts: A Taxonomic Study*, 4th ed. Elsevier Science, B.V. Amsterdam, Netherlands.
- Leblond, V., O. Saint-Jean, A. Detry, G. Lecso, C. Frances, S. Bellefiqu, M. Gentilini, and J.L. Binet. 1986. Systemic infection with *Trichosporon beigellii* (*cutaneum*). *Cancer* 58: 2399–2405.
- Meyer, M.H., V. Letscher-Bru, J. Waller, P. Lutz, L. Marcellin, and R. Herbrecht. 2002. Chronic disseminated *Trichosporon asahii* infection in a leukemic child. *Clin. infect. dis.* 35: e22–e25.
- Sláviková, E. and R. Vadkertiová. 1997. Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. *Antonie van Leeuwenhoek* 72: 77–80.
- Sugita, T., A. Nishikawa, T. Ichikawa, R. Ikeda, and T. Shinoda. 2000. Isolation of *Trichosporon asahii* from environmental ma-

- terials. *Medical Mycology* 38: 27–30.
- Sugita, T., A. Nishikawa, T. Shinoda, K. Yoshida, and M. Ando. 1995. A new species, *Trichosporon domesticum*, isolated from the house of a summer-type hypersensitivity pneumonitis patient in Japan. *J. Gen. Appl. Microbiol.* 41: 429–436.
- Tzean, S.S., W.H. Hsieh, T.T. Chang, and S.H. Wu. 2005. Fungal flora of Taiwan. 1st ed. Pp. 1–2422. National Science Council, Taiwan, R.O.C.
- Viljoen, B.C. and T. Greyling. 1995. Yeast associated with Cheddar and Gouda making. *Int. F. Food Microbiol.* 28: 79–88.
- Wang, P.H., C.W. Chang, and Y.T. Wang. 2003. Phylogenetic analysis of five *Rhodotorula* spp. *Taiwanese Journal of Agricultural Chemistry and Food Science* 41: 101–112.
- Wang, Y.Z., S.H. Wu, Y.N. Chou, T.T. Chang, K.Y. Chen, S.F. Chen, J.L. Chen, S.S. Tzean, C.H. Liu, W.H. Hsieh, H.J. Hsieh, C.H. Chung, and C.Y. Chien. 1999. List of the fungi in Taiwan. 1st ed. Pp. 1–289. The Council of Agriculture of the Executive Yuan of the R.O.C.
- Woollett, L.L. and L.R. Hedrick. 1970. Ecology of yeasts in polluted water. *Antonie van Leeuwenhoek* 36: 427–435.
- Young, C., M.H. Chang, and J.M. Chen. 1993. Fungal Esophagitis in children. *Acta. Paed. Sin.* 34: 436–442.

臺灣新紀錄的絲孢酵母菌

田志仁¹、汪碧涵²、李福臨¹

1. 食品工業發展研究所，郵政信箱第 246 號，新竹市，臺灣，中華民國
2. 東海大學生命科學系，郵政信箱第 581 號，臺中市，臺灣，中華民國

摘 要

本文描述由淡水河河水中分離的到之 *Trichosporon asahii* (阿氏絲孢酵母)，為臺灣之新紀錄種。

關鍵詞： *Trichosporon asahii*、分類學、酵母菌、臺灣。



