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SHORT COMMUNICATION

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Trichosporon siamense sp. nov. isolated from insect frass in Thailand

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Abstract A strain of yeast isolated from insect frass collected in Thailand was found to represent a hitherto undescribed species of a basidiomycetous anamorphic genus *Trichosporon*. It is described as *Trichosporon siamense*. In the phylogenetic tree based on the D1/D2 region sequences of 26S rDNA, this yeast constitutes a cluster with several Q-9 having species of *Trichosporon* including *T. otae* and *T. brassicae* but is clearly differentiated from these species by 1.8% or more base substitutions. In the internal transcribed spacer region (ITS1 and ITS2), this species differs from *T. scarabaeorum*, the nearest species, by 6.5% base substitution.

Key words New yeast from Thailand · *Trichosporon siamense* sp. nov.

In a course of the survey of yeasts living in the natural environment of Thailand, a strain isolated from insect frass was found to represent a new species of the anamorphic yeast genus *Trichosporon*. It is described here as a new species.

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A yeast strain designated ST-318 was isolated from insect frass collected in Khao-Yaow, Pattani Province, a southern region of Thailand, in March 2001 by S. Jindamorakot. Most morphological, physiological, and biochemical characteristics were examined by the method described by Yarrow (1998). The production of mycelia and arthroconida was examined by slide culture on potato dextrose agar (PDA). The assimilation of nitrogen compounds was examined on solid media. Vitamin requirement was investigated according to Komagata and Nakase (1967). Ubiquinone (coenzyme Q) system and cellular xylose were analyzed by HPLC according to the method of Nakase and Suzuki (1986) and Suzuki and Nakase (1988), respectively.

Isolation and purification of nuclear DNA were performed according to Takashima and Nakase (2000) using freeze-dried cells. The DNA base composition was determined by HPLC after enzymatic digestion of DNA to deoxyribonucleosides as described by Tamaoka and Komagata (1984). A DNA-GC Kit (Yamasa Shoyu, Chiba, Japan) was used as the quantitative standard. The sequencing of ribosomal DNA and phylogenetic analysis were performed as previously described (Fungsin et al. 2002). The sequences of the D1/D2 region of 26S rDNA and internal transcribed spacer (ITS) region including 5.8S rDNA determined in this study were deposited in the DDBJ database under the following accession numbers: ST-318 (BCC 11797 = TISTR 5823 = JCM 12478) ITS region including 5.8S rDNA and D1/D2 domain of 26S rDNA (AB164370), and *T. scarabaeorum* CBS 5601 ITS regions including 5.8S DNA and D1/D2 domain of 26S rDNA (AB164372). Generated sequences were aligned with species of *Trichosporon* and related hymenomycetous yeast taxa using the Clustal X version 1.8 computer program (Thompson et al. 1997). The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbor-joining method (Saitou and Nei 1987). Sites where any gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein 1985) were performed from 1000 random resamplings.

In a phylogenetic tree based on the D1/D2 sequences, strain ST-318 was located in the Trichosporonales lineage

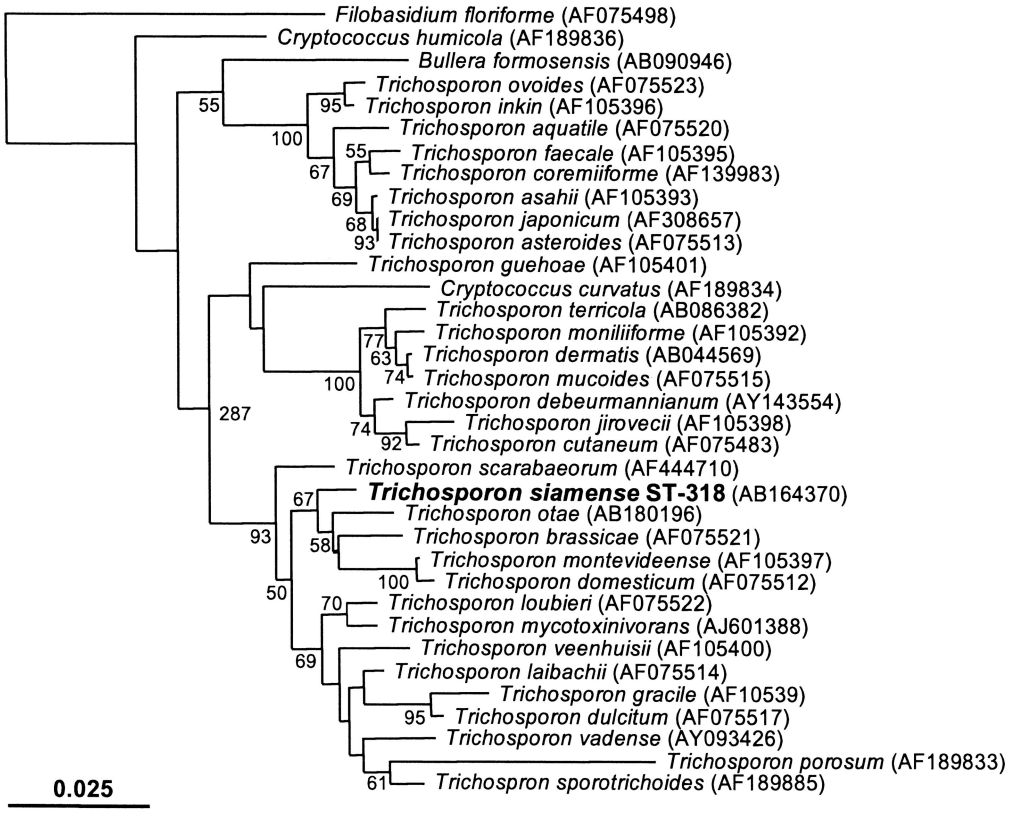

Fig. 1. A phylogenetic tree for *Trichosporon siamense* sp. nov. and related species constructed by the neighbor-joining method based on the D1/D2 region of 26S rDNA sequences. The *numerals* represent the percentages from 1000 random replicate bootstrap resamplings (a frequency of less than 50% is not shown). Sequences were retrieved from the DDBJ database under the accession number indicated (in *parentheses*)

and constituted a cluster with several Q-9 having species of *Trichosporon*, "*T. otae*," *T. brassicae* Nakase (Nakase 1971), *T. montevideense* (L.A. Queiroz) E. Guého & M.T. Smith (Guého et al. 1992), and *T. domesticum* Sugita, A. Nishikawa & Shinoda (Sugita et al. 1995), although the bootstrap confidence level was not high (Fig. 1). In the D1/D2 nucleotide sequences, ST-318 was close to *T. brassicae, T. otae*, and *T. scarabaeorum* Mildhoven, Scorzetti & Fell. *Trichosporon otae* is not yet described, but its D1/D2 sequence is available from DNA data banks (AB180196), and *T. scarabaeorum* was recently described (Middelhoven et al. 2004). However, ST-318 differed from *T. brassicae* and *T. scarabaeorum* by 1.8% base substitutions (12 nucleotides in total 626 and 672 sites compared, respectively) and from *T. otae* by 1.9% base substitution (13 nucleotides in total 674 sites). In the ITS1 and ITS2, ST-318 is most closely related to *T. scarabaeorum* but differed from this species by 6.5% base substitution (18 nucleotides in total 278 sites). These sequence differences clearly indicate the difference of ST-318 from the known species already mentioned at species level. It is described here as *Trichosporon siamense* sp. nov.

Description

Trichosporon siamense Nakase, Jindamorakot, Sugita & H. Kawasaki, sp. nov.

In liquido "YM" post dies 3 ad 25°C, cellulae globosae, rectangulares, cylindratae vel elongatae, singulae, binae vel aliquot catenatae, 2.5–7.5 × 2.5–15 µm. Mycelium formatur. Arthroconidia cylindrata, rectangularia vel subglobosa, 2.5–4.5 × 3–5–10 µm. Pellicula et sedimenta formantur. Ballistoconidium nullum. In agaro "YM" post unum mensem ad 20°C, coloniae in cultura lineali griseo-flavae, pilosae, non nitidae, molles aut butyraceae, margine ciliatae.

Fermentatio nulla. Glucosum, galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melezitosum (fortasse lente), amylum solubile, D-xylosum, D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, ethanolum, glycerolum, ribitolum (lente), D-mannitolum, D-glucitolum, xylitolum, α-methyl-D-glucosidum, glucono-δ-lactonum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, acidum saccharicum, acidum D-glucuronicum, acidum D-galacturonicum, inositolum, propanum 1,2-diolum et butanum 2,3-diolum assimilantur et non melibiosum, raffinosum, inulinum, L-arabinosum, D-arabinosum, methanolum, erythritolum, galactitolum, L-arabinitolum, salicinum nec hexadecanum. Kalium nitricum non assimilatur. Temperatura maxima crescens: 35°–36°C. Ad crescentiam thiaminae necessarium est. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 63.4 mol% (per HPLC). Ubiquinonum majus: Q-9. Xylosum cellulis presaens. Teleomorphosis ignota.

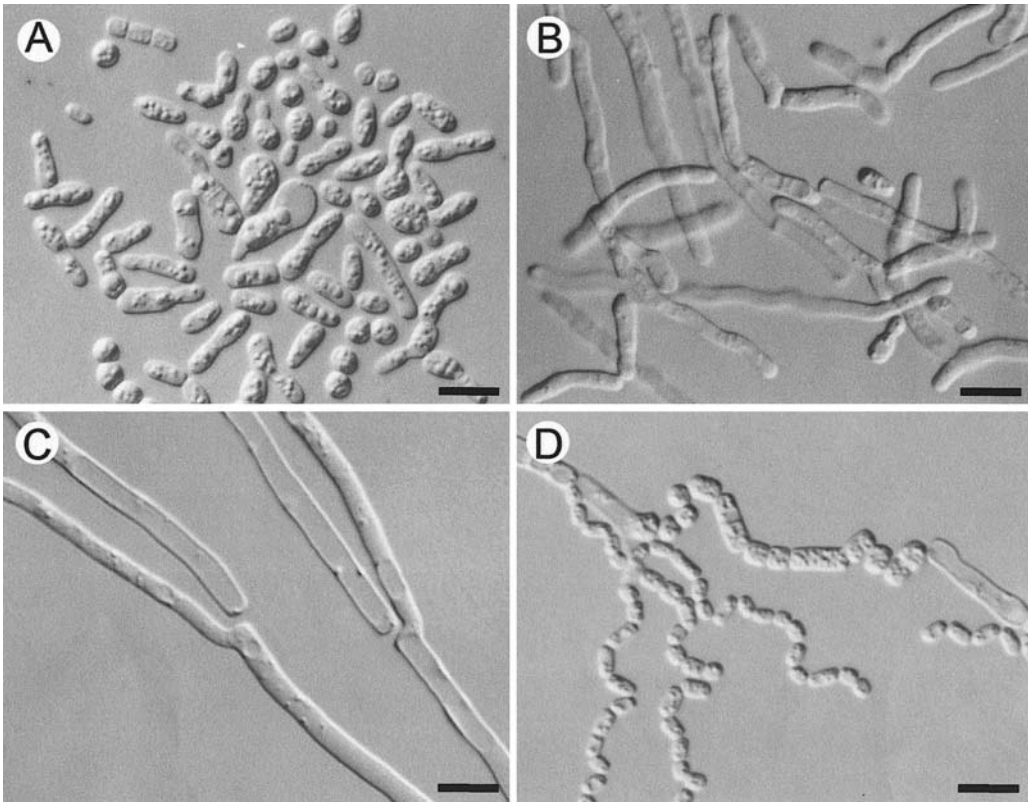

Fig. 2. Morphology of *Trichosporon siamense* sp. nov. **A** Cells grown in YM broth for 3 days at 25°C. **B,C** Mycelia formed on the slide culture with potato dextrose agar (PDA) after 1 week at 25°C. **D** Arthroconidia formed on the slide culture with PDA after 1 week at 25°C. *Bars* 10 μm

Holotypus: BIOTEC-BCC 11797 in statu lyophilo ex stirpe ST-318, cultura viva ex ligno pulvereo ab insecto efferenti, Khao Yaow, Pattani, Thailandia, isolata et in collectione culturarum in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia conservata. Isotypus ut JCM 12478 in statu lyophilo in collectione culturarum in Japan Collection of Microorganisms (JCM), RIKEN, Wako, Saitama, Japonia, et ut TISTR 5823 in statu lyophilo in collectione culturarum in Thailand Institute of Scientific and Technological Research (TISTR), Pathumthani 12120, Thailandia conservata.

Growth in YM broth: After 3 days at 25°C, cell are globose, rectangular, cylindrical, or elongate, single, in pairs or in chains, 2.5–7.5 × 2.5–15 μm (Fig. 2A), often very long, measuring up to 50 μm. Septate mycelia and arthroconidia are also observed. A wrinkled creeping pellicle and a sediment are formed.

Growth on YM agar: After 1 month at 20°C, the streak culture is grayish yellow, dull, soft to butyrous, and has a ciliate margin. The surface of the culture has fine hairs.

Slide culture on potato dextrose agar: Septate and branched mycelia are abundantly produced (Fig. 2B,C). They break up into arthroconidia that are often arranged in a zigzag. Arthroconidia are rectangular, cylindrical, or close to subglobose, 2.5–4.5 × 3–5–10 μm (Fig. 2D).

Fermentation: Absent.

Assmilation of carbon compounds:

Glucose	+
Galactose	+
L-Sorbose	+
Sucrose	+
Maltose	+
Cellobiose	+
Trehalose	+
Lactose	+
Melibiose	–
Raffinose	–
Melezitose	+ (may be latent)
Inulin	–
Soluble starch	+
D-Xylose	+
L-Arabinose	–
D-Arabinose	–
D-Ribose	+
L-Rhamnose	+
D-Glucosamine	+
N-Acetyl-D-glucosamine	+
Methanol	–
Ethanol	+
Glycerol	+
Erythritol	–
Ribitol	+ (latent)
Galactitol	–
D-Mannitol	+
D-Glucitol	+
Xylitol	+
L-Arabinitol	–
α-Methyl-D-glucoside	+
Salicin	–
Glucono-δ-lactone	+

D-Gluconic acid	+
2-Ketogluconic acid	+
5-Ketogluconic acid	+
DL-Lactic acid	+
Succinic acid	+
Citric acid	+
Saccharic acid	+
D-Glucuronic acid	+
D-Galacturonic acid	+
Inositol	+
Propane 1,2-diol	+
Butane 2,3-diol	+
Hexadecane	–
Assimilation of nitrogen compounds:	
Potassium nitrate	–
Sodium nitrite	+
Ethylamine	+
L-Lysine	+
Cadaverine	+

Maximum growth temperature: 35°–36°C.
Vitamins required: Thiamine.
Production of starchlike compounds: Negative.
Growth on 50% (w/w) glucose yeast extract agar: Negative.
Urease: Positive.
Growth in 0.1% cycloheximide: Negative.
Liquefaction of gelatin: Negative.
Diazonium blue B reaction: Positive.
Mol% G+C: 63.4 (by HPLC).
Major ubiquinone: Q-9.
Xylose in the whole cell hydrolysates: Positive.

Holotype: BCC 11797, which was originally isolated as ST-318 from insect frass collected in Khao Yaow, Pattani Province, Thailand, in March 2001, by Sasitorn Jindamorakot, is the holotype of this species. Lyophilized cultures are deposited as holotype at BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Pathumthani 12120, Thailand. Isotype is maintained at TISTR Culture Collection, Thailand Institute of Scientific and Technological Research (TISTR), Pathumthani 12120, Thailand, as TISTR 5823, and Japan Collection of Microorganisms (JCM), RIKEN, Wako, Saitama 351-0198, Japan, as JCM 12478. These cultures are maintained by lyophilization and/or freezing.

Etymology: The specific epithet of this species was derived from Siam, the old name of Thailand where this yeast was isolated.

In the phenotypic characteristics, *T. siamense* resembles species in the same cluster, especially *T. montevideense*. However, *T. siamense* is clearly discriminated from the latter species by its ability to assimilate L-sorbose, L-rhamnose, and sodium nitrite, its inability to assimilate galactitol and salicin, and the lack of growth on 50% glucose yeast extract agar.

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