Mycoscience (2005) 46:192–195
DOI 10.1007/s10267-004-0231-7



SHORT COMMUNICATION

**Takashi Nakase · Sasitorn Jindamorakot · Somjit Am-in**
**Hiroko Kawasaki · Wanchern Potacharoen**
**Morakot Tanticharoen**

# *Pichia nongkratonensis* sp. nov., a new species of ascomycetous yeast isolated from insect frass collected in Thailand

Received: October 1, 2004 / Accepted: December 21, 2004

**Abstract** A yeast strain isolated from insect frass collected in Thailand was found to represent a new species of the genus *Pichia*. It is described as *Pichia nongkratonensis* sp. nov. In the phylogenetic tree based on the D1/D2 domain sequences of 26S rDNA, this yeast constitutes a cluster with *Pichia dryadoides* with high bootstrap confidence level; however, it differs from the latter species by 5.6% base substitutions. *Pichia nongkaratonensis* resembles *P. dryadoides* also in the phenotypic characteristics but is distinguished from this species by the assimilation of several carbon and nitrogen compounds.

**Key words** New yeast from insect frass · New yeast from Thailand · *Pichia nongkratonensis* sp. nov.

In the course of a survey of yeasts in the natural environment of Thailand, an isolate of ascomycetous yeast was found to represent a new species of the genus *Pichia*. This strain, designated as ST-240, was isolated from insect frass collected in a forest in Nong Kratone, Nakhonratchasima Province, northeastern Thailand, in February 2001. Insect frass was spread on YM agar (Difco, Detroit, MI, USA) plates supplemented with chloramphenicol (100 ppm) and sodium propionate (0.2%) and incubated at 25°C. Colonies were isolated and purified by conventional streaking technique.

T. Nakase (✉) · S. Jindamorakot · S. Am-in · W. Potacharoen · M. Tanticharoen
Central Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), 113 Thailand Science Park, Phahonyothin Road, Pathumthani 12120, Thailand
Tel. +66-2-564-6700; Fax +66-2-564-6707
e-mail: nakase@biotec.or.th

T. Nakase
Department of Applied Biology and Chemistry, Faculty of Applied Biosciences, Tokyo University of Agriculture, Tokyo, Japan

H. Kawasaki
The International Center for Biotechnology, Osaka University, Osaka, Japan

Most morphological, physiological, and biochemical characteristics were examined according to Yarrow (1998). Maximum growth temperature was determined in YM broth using a metal block bath. Vitamin requirement was tested by the method of Komagata and Nakase (1967). Ubiquinones were isolated and identified according to the method of Nakase and Suzuki (1986). The DNA was isolated and purified according to Nakase and Suzuki (1985). The DNA base composition (mol% G + C) was determined by nucleoside analysis using HPLC after digesting the DNA with Nuclease P1 and phosphatase as described by Tamaoka and Komagata (1984). The D1/D2 region of 26S rDNA was directly sequenced using polymerase chain reaction (PCR) products by ABI 310 sequencer with an ABI Prism BigDye Terminator Cycle Sequence kit (Applied Biosystems, Foster City, CA, USA) as previously described (Fungsin et al. 2002). The sequence determined in this study was deposited in the DDBJ database as AB191049. The sequences were aligned with the computer program Clustal X, version 1.8 (Thompson et al. 1997). The evolutionary distances for the neighbor-joining method (Saitou and Nei 1987) were calculated according to Kimura (1980). A bootstrap analysis was conducted with 1000 replications (Felsenstein 1985).

Strain ST-240 proliferated by multilateral budding, forming globose to ellipsoidal cells, and produced hat-shaped ascospores without conjugation and fitted to those of the genus *Pichia* (Kurtzman 1998). It has Q-7 as the major component of ubiquinones and assimilated limited number of carbon compounds as found in the many typical species of the genus.

In a phylogenetic tree constructed by neighbor-joining method based on the D1/D2 domain sequences of 26S rDNA, ST-240 constituted a cluster with *Pichia dryadoides* with high bootstrap confidence level (Fig. 1). However, ST-240 differed in 27 nucleotides (5.6%) from the latter species, so that it is not so closely related to *P. dryadoides*. Undoubtedly, ST-240 is a different species from *P. dryadoides* and represents a new species of the genus *Pichia* (Kurtzman and Robnett 1998).

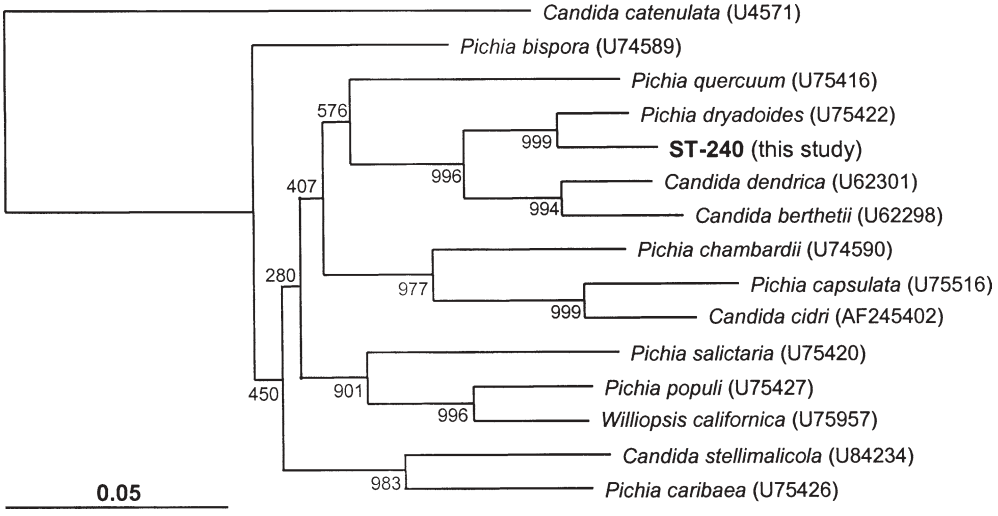


**Fig. 1.** Phylogenetic tree for *Pichia nongkratonensis* ST-240 constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. Numerals indicate values from 1000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated

ST-240 also resembles *P. dryadoides* in the phenotypic characteristics but is distinguished from the latter species by its ability to assimilate salicin (latent) and saccharic acid (latent) and by its inability to assimilate D-glucitol, 1,2-propanediol and 2,3-butanediol as carbon sources. In addition, ST-240 does not assimilate cadaverine as a nitrogen source.

## Description

***Pichia nongkratonensis*** Nakase & Jindamorakot, sp. nov. In liquido YM post 3 dies ad 25°C cellulae globosae vel subovoideae, 2.5–4.5 × 2.5–5 mm, singulae, binae vel brevi-catenatae. Pseudomycelium praesens. Annulus et sedimentum formantur. In agaro YM post unum mensem ad 20°C cultura griseo-flava vel griseo-brunnea, subnitida, mollis, margine integer. Ascosporae pileiformes, 1.5–2.3 × 2.0–3.1 mm, 2–3, vulgo 2, intra ascum.

Glucosum fermentatur (lente). Glucosum, cellobiosum (fortasse lente), ethanolum, glycerolum, D-mannitolum, salicinum (fortasse lente), glucono-δ-lactonum, acidum D-gluconicum (lente), acidum DL-lacticum (fortasse lente), acidum succinicum, acidum citricum et acidum saccharicum (lente vel nullum) assimilantur at non galactosum, L-sorbosum, sucrosum, maltosum, trehalosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, erythritolum, ribitolum, galactitolum, D-glucitolum, xylitolum, L-arabinitolum, α-methyl-D-glucosidum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, inositolum, propane 1,2-diolum, butanum 2,3-diolum nec hexadecanum. Kalium nitricum assimilatur. Maxima temperatura crescentiae: 39°–40°C. Ad crescentiam vitaminae non necessarium est. Proportio molaris guanini + cytosine in acido deoxyribonucleico 33.4 mol% (per HPLC). Ubiquinonum majus: Q-7.

Holotypus: BCC 11772 in statu lyophilo ex stirpe ST-240, cultura viva ex ligno pulvereo ab insecto efferenti, Nong Kratone, Nakhonratchasima, Thailandia isolata et in collectione culturarum in BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia conservata. Isotypus ut JCM 12550 in statu lyophilo in colletione culturarum in Japan Collection of Microorganisms (JCM), Riken, Wako, Saitama, Japonia conservatus.

Growth in YM broth: After 3 days at 25°C cells are globose to short-ovoidal, 2.5–4.5 × 2.5–5 µm, single, in pairs or in short chains (Fig. 2A). Sometimes pseudomycelia are observed. Pseudomycelial cells are elongate. An incomplete ring and a sediment are formed. After 1 month at 20°C, an incomplete ring and a sediment are present.

Growth on YM agar: After 1 month at 20°C, the streak culture is grayish-white to grayish-brown, smooth, semishining, soft, and has an entire margin.

Slide culture: Pseudomycelia are produced on YM agar (Fig. 2B,C) but not on corn meal agar.

Ascospores: Diploid vegetative cells directly transform to asci and each ascus contains one to three, usually two ascospores. Ascospores are hat shaped with prominent brims, 1.5–2.3 × 2.0–3.1 µm (Fig. 2D).

Fermentation: Glucose is slowly fermented after 3 weeks.

Assimilation of carbon compounds:

| | |
|---|---|
| Glucose | + |
| Galactose | – |
| L-Sorbose | – |
| Sucrose | – |
| Maltose | – |
| Cellobiose | + (may be latent) |
| Trehalose | – |
| Lactose | – |
| Melibiose | – |
| Raffinose | – |
| Melezitose | – |
| Inulin | – |

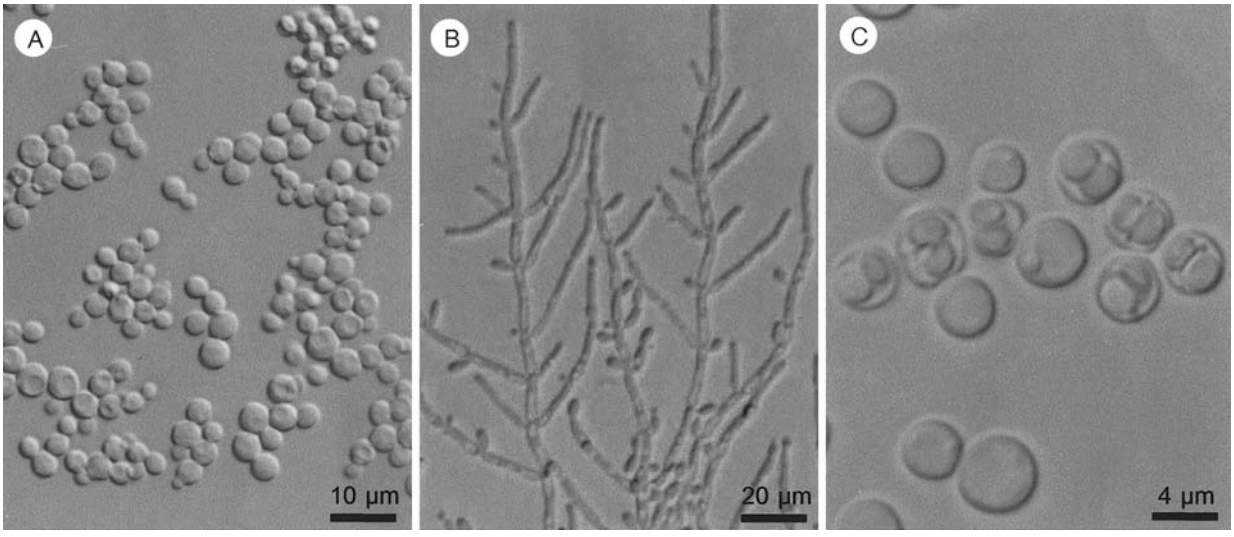


**Fig. 2.** Morphology of *Pichia nongkratonensis* ST-240. **A** Vegetative cells grown in YM broth for 3 days at 25°C. **B** Pseudomycelia produced on YM agar after 9 days at 25°C. **C** Ascospores produced on 5% malt extract agar after 8 days at 20°C

| | |
|---|---|
| Glycerol | + |
| Soluble starch | – |
| D-Xylose | – |
| L-Arabinose | – |
| D-Arabinose | – |
| D-Ribose | – |
| L-Rhamnose | – |
| D-Glucosamine | – |
| *N*-Acetyl-D-glucosamine | – |
| Methanol | – |
| Ethanol | + |
| Erythritol | – |
| Ribitol | – |
| Galactitol | – |
| D-Mannitol | + |
| D-Glucitol | – |
| Xylitol | – |
| L-Arabinitol | – |
| α-Methyl-D-glucoside | – |
| Salicin | + (may be latent) |
| Glucono-δ-lactone | + |
| D-Gluconic acid | + (latent) |
| 2-Ketogluconic acid | – |
| 5-Ketogluconic acid | – |
| DL-Lactic acid | + (may be latent) |
| Succinic acid | + |
| Citric acid | + |
| Saccharic acid | + (latent) or – |
| D-Glucuronic acid | – |
| D-Galacturonic acid | – |
| Inositol | – |
| 1,2-Propanediol | – |
| 2,3-Butanediol | – |
| Hexadecane | – |

| | |
|---|---|
| Assimilation of nitrogen compounds: | |
| Potassium nitrate | + |
| Sodium nitrite | + |
| Ethylamine | + |
| L-Lysine | + |
| Cadaverine | – |

Growth in vitamin-free medium: positive (growth is stimulated by thiamine)
Production of starchlike substances: negative
Growth on 10% NaCl/5% glucose: negative
0.1% cycloheximide resistance: weakly positive
Maximum growth temperature: 39°–40°C
Liquefaction of gelatin: negative
Acid production on chalk agar: very weakly positive
Diazonium blue B color reaction: negative
Urease: negative
G + C content of nuclear DNA: 33.4 mol% (by HPLC)
Major ubiquinone: Q-7.

Type strain: ST-240, isolated from insect frass collected in a tropical rain forest, Nong Kratone, Nakhonratchasima Prov., Thailand, in February 2001, is the type strain of this species. The lyophilized culture from the type was deposited at BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology, as BCC 11772. Isotype was deposited at Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12550, respectively. These cultures are maintained by freezing and/or lyophilization.

Etymology: The specific epithet *nongkratonensis* was derived from the place where this yeast was isolated.

**Acknowledgments** We thank Ms. Hathairat Jan-ngam, BIOTEC, who helped us with several experiments.

## References


Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fungsin B, Takashima M, Artjariyasripong S, Arunpairojana V, Hamamoto M, Nakase T (2002) *Bullera arundinariae* sp. nov., a new species of ballistoconidium-forming yeast, isolated from a plant in Thailand. Microbiol Cult Coll 18:83–90

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Komagata K, Nakase T (1967) Microbiological studies on frozen foods. V. General properties of yeasts isolated from frozen foods. J Food Hyg Soc Jpn 8:53–57

Kurtzman CP (1998) *Pichia* E. C. Hansen emend. Kurtzman. In: Kurtzman CP, Fell JW (eds) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 273–352

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73: 331–371

Nakase T, Suzuki M (1985) Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. I. Chemotaxonomic investigations. J Gen Appl Microbiol 31:49–69

Nakase T, Suzuki, M (1986) The ubiquinone system in strains of species in the ballistospore-forming yeast genera *Sporidiobolus*, *Sporobolomyces* and *Bullera*. J Gen Appl Microbiol 32:251–258

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Tamaoka M, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Lett 25:125–128

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins JD (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acid Res 24:4876–4882

Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 77–100