Full Paper

***Candida potacharoeniae* sp. nov. and *Candida spenceri* sp. nov., two novel galactose-containing ascomycetous anamorphic yeast species isolated in Thailand**

Takashi Nakase,^{1,2,*} Sasitorn Jindamorakot,² Yumi Imanishi,^{1,†} Somjit Am-In,²
Shinya Ninomiya,¹ Hiroko Kawasaki,¹ and Savitree Limtong³

¹ NITE Biological Resource Center (NBRC), Department of Biotechnology,

National Institute of Technology and Evaluation (NITE), Kisarazu, Chiba 292–0818, Japan

² Bioresources Technology Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC),
National Science and Technology Development Agency (NSTDA),

113 Thailand Science Park, Phaholyothin Road, Pathumthani 12120, Thailand

³ Department of Microbiology, Faculty of Science, Kasetsart University, 50 Phaholyothin Road, Bangkok 10900, Thailand

(Received January 7, 2010; Accepted March 1, 2010)

Fifteen strains of anamorphic yeasts isolated from various natural substrates collected in various places in Thailand were found to represent two novel species of anamorphic yeast genus *Candida* based on the sequence analysis of the D1/D2 domain of the large subunit rRNA genes, chemotaxonomic and conventional properties used for the classification of yeasts. These strains are located in the clade including *Candida etchellsii* and *Candida magnoliae*. Fourteen strains represented by ST-490^T (BCC 15176^T=NBRC 106439^T=CBS 11674^T) are closely related to *Candida sorbosivorans* in the D1/D2 sequences but 11 nucleotides (2.4%) were substituted. The remaining strain, ST-594^T (=BCC 15278^T=NBRC 106446^T=CBS 11673^T) showed a close relationship to *Candida geochares* but 21 nucleotides (4.7%) were substituted. Apparently, these strains represent two novel *Candida* species of the *Starmerella* clade. The two species are described as *Candida potacharoeniae* sp. nov. and *Candida spenceri* sp. nov. in the present paper. Like the most species of this clade, the two species contain galactose in the cells in addition to glucose and mannose and have high mol% G + C of 54.4–55.9 and 54.9, respectively.

Key Words—*Candida potacharoeniae* sp. nov.; *Candida spenceri* sp. nov.; novel anamorphic yeasts; novel yeasts from Thailand

Introduction

Ascomycetous yeasts usually have glucan-mannan type cell walls but species with glucan-galactomannan type cell walls are also found. Gorin and Spencer (1970) carried out extensive studies on mannose-containing polysaccharides of yeasts, which were alkali-extracted and purified via copper-complexes, by proton magnetic resonance spectra as in aid of identification and chemotaxonomy. In their studies, several ascomycetous yeast species have galactose in addi-

* Address reprint requests to: Dr. Takashi Nakase, NITE Biological Resource Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation (NITE), 2–5–8 Kazusakamatari, Kisarazu, Chiba 292–0818, Japan.

Tel: +81–438–20–5763 Fax: +81–438–52–2329

E-mail: nakase-takashi@nite.go.jp

† Present address: Medical Mycology Research Center (MMRC), Chiba University, 1–8–1 Inohana, Chuo-ku, Chiba, Chiba 260–8673, Japan.

tion to mannose. Suzuki and Nakase (1998) analyzed monosaccharide components of cell hydrolyzates and ubiquinones (coenzyme Q) of almost all of the known species of *Candida*. They found that, among 196 species and 9 varieties examined, 31 species and 2 varieties having Q-9 and 3 species having Q-8 had galactose in addition to mannose and glucose. Further, Suzuki et al. (1999) reported that these galactose-containing *Candida* species were located in certain phylogenetic clades mingled with galactose-lacking species.

During the course of an isolation study of yeasts from the natural substrates of Thailand, we isolated a novel galactose-containing yeast from insect frass and described it as *Candida kazuoi* (Nakase et al., 2007). In the present paper we describe additional two novel galactose-containing *Candida* species found in natural substrates collected in Thailand.

Materials and Methods

Yeast strains. Nine strains were isolated from flowers collected in Kanchanaburi Province in 2003 by direct streaking of samples on YM agar plates supplemented with chloramphenicol (100 µg ml⁻¹) and sodium propionate (0.2%, w/v), or streaking a loopful culture

broth on the plate after enrichment of yeasts in the same medium without agar. The remaining six strains were isolated from soil and the bark of trees in several places in Thailand in 2007. Sources of strains are shown in Table 1. Six strains isolated in 2007 were used for sequencing but not for the study of phenotypic characteristics.

Examination of morphological, physiological, and biochemical characteristics. Most of these characteristics were examined by the methods described in *The Yeasts, A Taxonomic Study*, 4th ed. (Yarrow, 1998). Vitamin requirement was determined as reported by Komagata and Nakase (1967). The maximum growth temperature was determined in YM broth at 1°C intervals using metal block baths.

Ubiquinone system. Cells grown in YPD broth (2% glucose, 2% peptone, 1% yeast extract) on a rotary shaker at 120 rpm at 25°C for 3 days were harvested by centrifugation. The cells were washed three times with distilled water and freeze-dried. Ubiquinones were extracted from freeze-dried cells and purified and identified according to Mikata and Yamada (1999).

DNA base composition. Nuclear DNA was isolated and purified according to Holm et al. (1986). The DNA base composition was determined by HPLC after enzymatic digestion of DNA to deoxyribonucleosides as

Table 1. Strains examined.

Strain no.	Other designation	Source	Locality	Year of isolation
<i>Candida potacharoeniae</i> sp. nov.				
ST-490 ^T	BCC 15176 ^T , NBRC 106439 ^T , CBS 11674 ^T	Flower of pink allamanda (<i>Mandevilla amoena</i> L.)	Chatuchak, Bangkok	2003
ST-525	BCC 15209, NBRC 106440	Flower of <i>Thunbergia laurifolia</i> L.	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-526	BCC 15210	Flower of <i>Thunbergia laurifolia</i> L.	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-527	BCC 15211, NBRC 106441	Flower of <i>Thunbergia laurifolia</i> L.	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-533	BCC 15217, NBRC 106442	Flower of <i>Eugenia jumbos</i>	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-534	BCC 15218, NBRC 106443	Flower of <i>Eugenia jumbos</i>	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-535	BCC 15219, NBRC 106444	Flower (unidentified)	Amphoe Tong Pha Phum, Kanchanaburi	2003
ST-536	BCC 15220, NBRC 106445	Flower (unidentified)	Amphoe Tong Pha Phum, Kanchanaburi	2003
MD15		Forest soil	Mukdahan	2007
RA4		Forest soil	Roi Et	2007
SR22		Soil	Surin	2007
UB6		Soil	Ubon Ratchathani	2007
UB7		Tree bark (unidentified tree)	Ubon Ratchathani	2007
UB12		Tree bark (unidentified tree)	Ubon Ratchathani	2007
<i>Candida spenceri</i> sp. nov.				
ST-594 ^T	BCC 15278 ^T , NBRC 106446 ^T , CBS 11673 ^T	Flower of humble-plant (<i>Mimosa pudica</i>)	Amphoe Tong Pha Phum, Kanchanaburi	2003

described by Tamaoka and Komagata (1984) using a DNA-GC Kit (Yamasa Shoyu Co., Ltd., Chiba, Japan).

Analysis of cellular monosaccharides. Cells grown in YPD broth for 3 days at 25°C under shaking at 120 rpm were harvested by centrifugation and freeze-dried. Freeze-dried cells were hydrolyzed with 4 M HCl at 100°C for 3 h, dried in vacuo, then the residue was dissolved in a small amount of water and neutralized with 6 M NaOH and/or 6 M HCl. After centrifugation the supernatant was analyzed by the method described by Mikami and Ishida (1983), using a Shimadzu model LC-10ADvp HPLC instrument equipped with a Shim-pack ISA 07/S2504 column (250 by 4 mm) and a Shimadzu model RF-10AXL spectrofluorometer.

Sequencing and phylogenetic analysis. Genomic DNA was prepared using Dr. GenTLE for Yeast (TaKaRa Bio, Inc., Otsu, Shiga, Japan) according to the manufacturer's protocol. The D1/D2 domains of the LSU rRNA gene were amplified using primers NL-1 (5'-GCA TAT CAA GCG GAG GAA AAG-3') and NL-4 (5'-GGT CCG TGT TTG AAG ACG G-3') (O'Donnell, 1993). The amplification was carried out in reaction mixture containing TaKaRa Ex Taq (TaKaRa Bio) by a Gene Amp PCR System 9700 (Applied Biosystems).

PCR was performed according to the method de-

scribed by Kurtzman and Robnett (1998). Purification of PCR products and determination of the sequence of the D1/D2 domain of the LSU rRNA gene were performed as described by Imanishi et al. (2007). The sequences determined in this study were deposited in the DDBJ database under the following accession numbers: ST-490^T, AB537430; ST-525, AB537431; ST-526, AB537438; ST-527, AB537432; ST-533, AB537433; ST-534, AB537434; ST-535, AB537435; ST-536, AB537436; ST-594^T, AB537437; MD15, AB499997; RA4, AB499036; SR22, AB500190; UB6, AB500194; UB7, AB500876; UB12, AB500878.

Reference sequences used for the phylogenetic study, shown in Fig. 1, were obtained from DNA Data Bank of Japan (DDBJ). A phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987) using a CLUSTAL X software package, ver. 1.83 (Thompson et al., 1997). Evolutionary distances were calculated according to Kimura's two-parameter model (Kimura, 1980). Bootstrap values were calculated by computer software based on Felsenstein (1985) from 1,000 random trials.

Fig. 1. Phylogenetic tree for *Candida potacharoeniae* sp. nov. and *Candida spenceri* sp. nov. constructed by neighbor-joining method based on the D1/D2 domain of LSU rRNA gene sequences.

The numerals represent the percentages from 1,000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated.

Results and Discussion

The fifteen strains examined were suggested to belong to the genus *Candida* because they proliferated by multilateral budding, contained Q-9 as a major ubiquinone, demonstrated negative Diazonium Blue B reaction and negative urease activity, and did not produce ascospores in single or mixed cultures of any possible pair on YM agar, 5% malt extract agar, Fowell's acetate agar, or corn meal agar at 15°C or 25°C.

The 15 strains are located in the *Starmerella* clade (Rosa and Lachance, 1998; Rosa et al., 2007; Teixeira et al., 2003) in a phylogenetic tree based on the D1/D2 domain sequences of the large subunit (LSU) rRNA genes but a little bit distant from known *Starmerella* species and located in a subclade including *Candida gropengiesseri*, *Candida tilneyi*, *Candida magnoliae*, *Candida sorbosivorans*, *Candida geochares*, *Candida apis* and *Candida vaccinii* (Fig. 1). Fourteen strains represented by ST-490^T had identical sequences in the D1/D2 domain and differed from *Candida sorbosivorans* (James et al., 2001), the nearest species in this domain, by 11 nucleotides (2.3%), and the remaining strain, ST-594^T, differed from *Candida geochares*, the nearest species, by 21 nucleotides (4.7%). The two groups, strain ST-594^T and the 14 strains represented by ST-490^T, differed by 28 nucleotides (6.2%) from each other. According to a guideline of Kurtzman and Robnett (1998), this nucleotide differences in the D1/D2 sequences mentioned above clearly suggested that the two groups represented respective novel species. Based on the results mentioned above, we describe *Candida potacharoeniae* sp. nov. for the 14 strains represented by ST-490^T and *Candida spenceri* sp. nov. for ST-594^T.

Among species phylogenetically related to the two novel species shown in Fig. 1, the six species, *Candida lactis-condensi*, *Candida etchellsii*, *Candida gropengiesseri*, *Candida magnoliae*, *Candida apis* and *Candida vaccinii*, were reported to have galactose in their cell hydrolyzates (Suzuki and Nakase, 1998), so we analyzed monosaccharide components of the two novel species and found that the two species contain galactose in addition to glucose and mannose in their cell hydrolyzates. The taxonomic significance of cellular galactose is not clear at present. Suzuki et al. (1999) reported that all galactose-containing *Candida* species were located in cluster II and not cluster I in the phylogenetic tree based on the small subunit rRNA

gene sequences. However, these species are mingled with galactose-lacking species in the trees. According to Sugita and Nakase (1999), many *Candida* species lacking galactose in the cells decode the codon CUG as serine but not leucine, and this property has not been reported in galactose-containing *Candida* species. These studies suggest that cellular galactose may have certain taxonomic and phylogenetic significance though it is not clear at present. Further accumulation of data is required to elucidate this problem.

Selected strains of novel species were analyzed for their mol% G+C and major ubiquinones. Mol% G+C values were found to be 54.4 for *C. potacharoeniae* ST-490^T, 55.6 for ST-525, 55.1 for ST-533 and 55.9 for ST-536, and 54.9 for *C. spenceri* ST-594^T. Ubiquinone-9 (Q-9) was found in *C. potacharoeniae* ST-490^T, ST-533 and ST-536, and *C. spenceri* ST-594^T. It is interesting that the species related to the two novel species shown in Fig. 1 have high mol% G+C from 46 to 60 except for *C. lactis-condensi* whose G+C value was reported as 42.4 mol% (Meyer et al., 1998). The G+C values of *Candida sorbosivorans* and *Candida tilneyi* were not reported at present.

In the phenotypic properties, eight strains of *C. potacharoeniae* showed characteristics similar to *C. sorbosivorans*, the nearest species in the D1/D2 sequence, but differed by the assimilation of erythritol and citric acid. *Candida spenceri* ST-594^T resembles *C. geochares*, the nearest species in the D1/D2 sequences, but is clearly differs by the ability to assimilate citric acid and inability to assimilate raffinose or L-arabinitol, and the low maximum growth temperature between 33–34°C.

Candida potacharoeniae and *C. spenceri* resemble from each other in the phenotypic properties but clearly can be discriminated by the assimilation of nitrate and nitrite as nitrogen sources, namely positive for the former and negative for the latter. Further, acids were produced by the latter species but not by the former species on chalk agar.

The two species showed similar morphological properties. *Candida spenceri* poorly produced primitive pseudomycelium on slide cultures with YM agar, potato dextrose agar and corn meal agar (Fig. 2, D) though it formed many branched chains of cells in YM broth and other liquid media commonly used for yeasts (Fig. 2, C3). *Candida potacharoeniae* usually did not produce pseudomycelium on slide cultures with YM agar, potato dextrose agar or corn meal agar but

Fig. 2. Morphology of *Candida potacharoeniae* sp. nov. and *Candida spenceri* sp. nov.

A: Vegetative cells of *C. potacharoeniae* ST-490^T grown in YM broth for 3 days at 25°C. A1, cells in sediment; A2, cells in a ring. B: Pseudomycelia of *Candida potacharoeniae* ST-526 produced on slide culture with potato dextrose agar after 20 days at 25°C. C: Vegetative cells of *C. spenceri* ST-594^T grown in YM broth for 3 days at 25°C. C1, cells in sediment; C2, cells in a ring; C3, cells in a ring forming branched chains. D: Pseudomycelia of *C. spenceri* ST-594^T produced on slide culture with potato dextrose agar after 7 days at 25°C. Scale bars=10 μm.

strains ST-526 and ST-527 poorly produced primitive pseudomycelium (Fig. 2, B).

Eight out of the 14 strains of *C. potacharoeniae* were isolated from flowers of 4 different wild plant species and *C. spenceri* was isolated from the flower of a wild plant (Table 1). Yeasts of the *Starmerella* clade are frequently found in substrates associated with insects. Yeasts in a subclade including *C. potacharoeniae* and *C. spenceri* shown in Fig. 1 were reported to be isolated from insects and/or flowers, except for *C. geochares* and *Candida* sp. MD9 (Lachance et al., 2001; Meyer et al., 1998; Tokuoaka et al., 1987). Probably, the strains of two novel species were transferred to flowers by insects who visited these flowers. *Candida potacharoeniae* were found in different places in Thailand in 2003 and 2007. This species is presumed to be widely distributed in Thailand, and probably in Southeast Asian countries.

Latin diagnosis of *Candida potacharoeniae* Nakase, Jindamorakot, Imanishi, Am-In, Ninomiya, Kawasaki & Limtong, sp. nov.

In liquido "YM", post dies 3 ad 25°C, annulus, insulae et pelliculae formantur. Cellulae ex annulo et insulis globosae, subovoideae aut ovoideae, ellipsoideae vel orthogoniae, 2–2.5 × 2–5.5 μm, singulae, binae aut

catenatae romosae, cellulae ex sedimento globosae, subovoideae aut ovoideae, ellipsoideae, interdum orthogoniae, 2–5 × 2–5.5 μm, singulae, binae, interdum catenulatae. In agar "YM", post unum mensem ad 15°C, cultura brunneo-cinereacana, glabra, hebes aut sub-nitida, mollis aut butyracea, margine integer. Pseudomycelium primitivum formatur vel non formatur. Ascosporae non formantur. Glucosum, sucrosum et cellobiosum (lente vel nulum) fermentantur at non galactosum, maltosum, trehalosum lactosum, raffinose, melibiosum, D-xylosum nec methyl-α-D-glucosidum. Glucosum, galactosum (fortasse lente), L-sorbose (fortasse lente), sucrosum, cellobiosum, raffinose (fortasse lente), melezitose (exiguum vel nullum), D-ribosum (fortasse lente), glycerolum (fortasse lente), ribitolum (lente vel nullum), D-mannitolum, D-glucitolum, xylitolum (lente), L-arabinitolum (fortasse lente), salicinum, arbutinum, glucono-δ-lactonum, acidum D-gluconicum, acidum 2-ketogluconicum (lente vel nullum) et acidum succinicum (lente vel nullum) assimulantur at non maltosum, trehalosum, lactosum, melibiosum, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, methanolum, ethanolum, erythritolum, galactitolum, methyl-α-D-glucosidum, acidum 5-ketogluconicum, acidum DL-

lacticum, acidum citricum, acidum D-glucuronicum, acidum D-galacturonicum, inositolum, propanum-1,2-diolum, butanum-2,3-diolum nec hexadecanum. Kalium nitricum assimilatur. Maxima temperatura crescentiae: 37–39°C. Ad crescentiam biotinum et thiaminum necessarium sunt. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 54.4–55.9 mol% (per HPLC). Ubiquinonum majus: Q-9.

Holotypus. BCC 15176^T in statu lyophilo ex stirpe ST-490^T, cultura viva ex flore *Mandevilla amoena*, Chatuchak, Bangkok, Thailandia, Feb. 2003, isolata et in collectione culturarum in 'BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC)', Pathumthani, Thailandia conservata. Isotypus ut NBRC 106439^T/CBS 11674^T in statu lyophilo in collectione culturarum in 'NITE Biological Resource Center (NBRC)', Kisarazu, Chiba, Japonia, et 'Centraalbureau voor Schimmelcultures (CBS)', Utrecht, The Netherlands, conservatus.

Description of *Candida potacharoeniae* Nakase, Jindamorakot, Imanishi, Am-In, Ninomiya, Kawasaki & Limtong, sp. nov.

Growth in YM broth: After 3 days at 25°C, a ring, islets and sediment are formed. Cells in ring and islets are spheroidal, short ovoid to ovoid, ellipsoidal, cocoon-shaped to rectangular with rounded ends, 2–2.5 × 2–5.5 µm, and occur singly or in pairs (Fig. 2, A2). Cells in the sediment are spheroidal, short ovoid to ovoid, ellipsoidal, sometimes cocoon-shaped to rectangular with rounded ends 2–5 × 2–5.5 µm, and occur singly, in pairs or sometimes in short chains consisting of 3–4 cells (Fig. 2, A1). After 1 month at 15°C, a ring, islets and heavy sediment are formed.

Growth on YM agar: After 1 month at 15°C, the streak culture is pale brownish gray, smooth, dull to dull-shining, soft to butyrous and has an entire margin.

Slide culture on corn meal agar: Usually, pseudomycelium is not produced but some strains produce primitive pseudomycelium (Fig. 2, B).

Ascospore formation: Not observed.

Fermentation: Glucose and sucrose are fermented. Cellobiose is slowly fermented or not fermented. Galactose, maltose, trehalose, lactose, raffinose, melibiose, D-xylose and methyl-α-glucoside are not fermented.

Assimilation of carbon compounds:

Glucose +

Galactose	+ (may be latent)
L-Sorbose	+ (may be weak)
Sucrose	+
Maltose	–
Cellobiose	+
Trehalose	–
Lactose	–
Melibiose	–
Raffinose	+ (may be latent)
Melezitose	+ (weak) or –
Inulin	–
Soluble starch	–
D-Xylose	–
L-Arabinose	–
D-Arabinose	–
D-Ribose	+ (may be latent)
L-Rhamnose	–
D-Glucosamine	–
N-Acetyl-D-glucosamine	–
Methanol	–
Ethanol	–
Glycerol	+ (may be latent)
Erythritol	–
Ribitol	+ (latent) or –
Galactitol	–
D-Mannitol	+
D-Glucitol	+
Xylitol	+ (latent)
L-Arabinitol	+ (may be latent)
Methyl-α-D-glucoside	–
Salicin	+
Arbutin	+
Glucono-δ-lactone	+
D-Gluconic acid	+
2-Ketogluconic acid	+ (latent) or –
5-Ketogluconic acid	–
DL-Lactic acid	–
Succinic acid	+ (latent) or –
Citric 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	+
L-Lysine	+

Ethylamine +

Cadaverine +

Vitamin required: Biotin, thiamine (stimulative).

Production of starch-like substances: Negative.

Growth in 10% NaCl + 5% glucose: Positive.

0.1% cycloheximide resistance: Negative.

Maximum growth temperature: 37–39°C (All strains grow at 39°C, some strains grow and others not at 38°C).

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Negative.

G+C content of nuclear DNA: 54.4–55.9 mol% (by HPLC).

Major ubiquinone: Q-9.

Monosaccharide components in cell hydrolyzate: Glucose, mannose and galactose.

Type strain: ST-490^T, isolated from flower of pink allamanda (*Mandevilla amoena*, Apocynaceae), Chatchak, Bangkok, Thailand, in Mar. 2003, is the holotype strain of this species. It was deposited at BIOTEC Culture Collection (BCC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand, as BCC 25963^T, NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Kisarazu, Chiba 292-0818, Japan, and Centraalbureau voor Schimmelcultures (CBS), The Netherlands, NBRC 106439^T and CBS 11674, respectively. The strain is maintained by freezing and lyophilization in these culture collections.

Etyymology: The specific epithet "*potacharoeniae*" was chosen in honor of Ms. Wanchern Potacharoen who established a yeast research group in Thailand and strongly supported the study of yeast biodiversity in the natural substrates in Thailand.

Distribution: *Candida potacharoeniae* was isolated from various substrates and various places in Thailand (Table 1). This species are presumed to be common species in the natural environment of Thailand, and probably in Southeast Asia where the climate is similar to Thailand's.

Latin diagnosis of *Candida spenceri* Nakase, Jindamorakot, Imanishi, Am-In, Ninomiya, Kawasaki & Limtong, sp. nov.

In liquido "YM", post dies 3 ad 25°C, annulus et sedimentum formantur. Cellulae ex annulo subovoideae aut ovoideae, ellipsoideae vel orthogonia, 2–3 × 2.5–5 µm, singulae aut binae vel catenatae romosae, cellulae ex sedimento subovoideae aut ovoideae vel

ellipsoideae, 2–4.5 × 2.5–7 µm, singulae, binae, catenatae ramosae. In agaro "YM", post unum mensem ad 15°C, cultura brunneo-cinerea, glabra, subnitida, butyracea et margine integer. Pseudomycelium primitivum formatur. Ascosporae non formantur.

Glucosum et sucrosum fermentantur at non galactosum, maltosum, lactosum, cellobiosum, trehalosum, raffinsum, melibiosum, D-xylosum nec methyl-α-D-glucosidum. Glucosum, galactosum (lente vel nullum), L-sorbosum (lente), sucrosum, cellobiosum, trehalosum (lente et exiguum, vel nullum), raffinsum (lente), D-ribosum (lente vel nullum), glycerolum (lente), D-mannitolium, D-glucitolium, xylitolium (lente et exiguum, vel nullum), L-arabinitolum (lente), salicinum, arbutinum, glucono-δ-lactonum, acidum D-gluconicum et acidum 2-ketogluconicum (lente) assimilantur at non maltosum, lactosum, melibiosum, melezitolum, inulinum, amyllum solubile, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, methanolium, ethanolium, erythritolum, ribitolium, galactitolium, methyl-α-D-glucosidum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, acidum D-glucuronicum, acidum D-galacturonicum, inositolium, propanum-1,2-diolium, butanum-2,3-diolium nec hexadecanum Kalium nitricum non assimilatur. Maxima temperatura crescentiae: 33–34°C. Ad crescentiam biotinae et thiaminae necessarium sunt. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 54.9 mol% (per HPLC). Ubiquinonum majus: Q-9.

Holotypus. BCC 15278^T in statu lyophilo ex stirpe ST-594^T, cultura viva ex *Mimosa pudica*, Amphoe Tong Pha Phum, Kanchanaburi Prov., Thailandia, isolata et in collectione culturarum in 'BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC)', Pathumthani, Thailandia conservata. Isotypus ut NBRC 106446^T/CBS 11673^T in statu lyophilo in collectione culturarum in 'NITE Biological Resource Center (NBRC)', Kisarazu, Chiba, Japonia, et 'Centraalbureau voor Schimmelcultures (CBS)', Utrecht, The Netherlands, conservatus.

Description of *Candida spenceri* Nakase, Jindamorakot, Imanishi, Am-In, Ninomiya, Kawasaki & Limtong, sp. nov.

Growth in YM broth: After 3 days at 25°C, a ring and sediment are formed. Cells in ring are short ovoid to ovoid, ellipsoidal or rectangular with rounded ends, 2–3 × 2.5–5 µm, and occur singly, in pairs or in

branched chains (Fig. 2, C2, 3). Cells in the sediment are short ovoid to ovoid or ellipsoidal, $2\text{--}4.5 \times 2.5\text{--}7\text{ }\mu\text{m}$, and occur singly, in pairs or in branched chains (Fig. 2, C1). After 1 month at 15°C , a ring, islets and heavy sediment are present.

Growth on YM agar: After 1 month at 15°C , the streak culture is pale brownish gray, smooth, dull-shining, butyrous and has an entire margin.

Slide culture: Primitive pseudomycelia are produced on potato dextrose agar, YM agar and corn meal agar (Fig. 2, D).

Ascospore formation: Not observed.

Fermentation: Glucose and sucrose are fermented. Galactose, maltose, lactose, cellobiose, trehalose, raffinose, melibiose, D-xylose and methyl- α -glucoside are not fermented.

Assimilation of carbon compounds:

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

Arbutin	+
Glucono- δ -lactone	+
D-Gluconic acid	+
2-Ketogluconic acid	+ (latent)
5-Ketogluconic acid	–
DL-Lactic acid	–
Succinic acid	–
Citric 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	–
L-Lysine	+
Ethylamine	+
Cadaverine	+

Vitamins required: Biotin and thiamine.

Production of starch-like substances: Negative.

Growth in 10% NaCl + 5% glucose: Positive.

0.1% cycloheximide resistance: Negative.

Maximum growth temperature: $33\text{--}34^{\circ}\text{C}$ (Growth at 33°C , no growth at 34°C).

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Positive (slow).

G+C content of nuclear DNA: 54.9 (type strain, by HPLC).

Major ubiquinone: Q-9.

Monosaccharide components in cell hydrolyzate: Glucose, mannose and galactose.

Type strain: ST-594^T, isolated from the flower of the humble-plant (*Mimosa pudica*, Leguminosae), Amphoe Tong Pha Phum, Kanchanaburi Prov., Thailand, in Nov. 2003, is the holotype strain of this species. It was deposited at BIOTEC Culture Collection (BCC), National Science and Technology Development Agency (NSTDA), Pathumthani, Thailand, NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Kisarazu, Chiba 292-0818, Japan, and Centraalbureau voor Schimmelcultures (CBS), The Netherlands, as BCC 15278^T, NBRC 106446^T and CBS 11673^T, respectively. The strain is maintained by freezing and lyophilization in these culture collections.

Etymology: The specific epithet “*spenceri*” was chosen in honor of Dr. J. F. T. Spencer for his many

contributions to yeast sciences including taxonomy.

References

- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.
- Gorin, P. A. J. and Spencer, J. F. T. (1970) Proton magnetic resonance spectroscopy—An aid in identification and chemotaxonomy of yeasts. *Adv. Appl. Microbiol.*, **13**, 25–89.
- Holm, C., Meeks-Wagner, D. W., Fangman, W. L., and Botstein, D. (1986) A rapid, efficient method for isolating DNA from yeast. *Gene*, **42**, 169–173.
- Imanishi, Y., Ueda-Nishimura, K., and Mikata, K. (2007) Two new species of *Kazachstania* that form ascospores connected by belt-like intersporal body: *Kazachstania zonata* and *Kazachstania gamospora*. *FEMS Yeast Res.*, **7**, 330–338.
- James, S. A., Bond, C. J., and Roberts, I. (2001) *Candida sorbo-sivorans* sp. nov., a new member of the genus *Candida* Berkhout. *Int. J. Syst. Evol. Microbiol.*, **51**, 1215–1219.
- 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. and 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, C. P. and Robnett, C. J. (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek*, **73**, 331–371.
- Lachance, M.-A., Bowles, J. M., Chavarria Diaz, M. M., and Janzen, D. H. (2001) *Candida cleridarum*, *Candida tilneyi* and *Candida powellii*, three new yeast species isolated from insects associated with flowers. *Int. J. Syst. Evol. Microbiol.*, **51**, 1201–1207.
- Meyer, S. A. (1998) *Candida* Berkhout. In *The Yeasts, A Taxonomic Study*, 4th ed., ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, The Netherlands, pp. 454–573.
- Mikami, H. and Ishida, Y. (1983) Post-column fluorometric detection of reducing sugars in high-performance liquid chromatography using arginine. *Bunseki Kagaku*, **32**, E207–E210.
- Mikata, K. and Yamada, Y. (1999) The ubiquinone system in the *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno: A new method for identify ubiquinone homologs from yeast cells. *IFO Res. Commun.*, **19**, 41–46.
- Nakase, T., Jindamorakot, S., Limtong, S., Am-In, S., Kawasaki, H., Imanishi, Y., Potacharoen, W., and Tanticharoen, M. (2007) *Candida kazuoi* sp. nov. and *Candida hasegawae* sp. nov., two new species of ascomycetous anamorphic yeasts isolated from insect frass in Thailand. *J. Gen. Appl. Microbiol.*, **53**, 239–245.
- O'Donnel, K. (1993) *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*, ed. by Reynolds, D. R. and Taylor, J. W., CAB International, Wallingford, pp. 225–233.
- Rosa, C. A. and Lachance, M.-A. (1998) The yeast genus *Starmerella* gen. nov. and sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. *Int. J. Syst. Bacteriol.*, **48**, 1413–1417.
- Rosa, C. A., Pagnocca, F. C., Lachance, M.-A., Ruivo, C. C. C., Medeiros, A. O., Pimentel, M. R. C., Fontenelle, J. C. R., and Martins, R. P. (2007) *Candida floscolorum* sp. nov. and *Candida floris* sp. nov., two new species associated with tropical flowers. *Int. J. Syst. Evol. Microbiol.*, **57**, 2970–2974.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.
- Sugita, T. and Nakase, T. (1999) Non-universal usage of the leucine CUG codon and the molecular phylogeny of the genus *Candida*. *Syst. Appl. Microbiol.*, **22**, 79–86.
- Suzuki, M. and Nakase, T. (1998) Cellular neutral sugar compositions and ubiquinone systems of the genus *Candida*. *Microbiol. Cult. Coll.*, **14**, 49–62.
- Suzuki, M., Suh, S.-O., Sugita, T., and Nakase, T. (1999) A phylogenetic study on galactose-containing *Candida* species based on 18S ribosomal DNA sequences. *J. Gen. Appl. Microbiol.*, **45**, 229–238.
- Tamaoka, M. and Komagata, K. (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol. Lett.*, **25**, 125–128.
- Teixeira, A. C. P., Marini, M. M., Nicoli, J. R., Antonini, Y., Martins, R. P., Lachance, M.-A., and Rosa, C. A. (2003) *Starmerella meliponinorum* sp. nov., a novel ascomycetous yeast species associated with stingless bees. *Int. J. Syst. Evol. Microbiol.*, **53**, 339–343.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) The Clustal X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **24**, 4876–4882.
- Tokuoka, K., Ishitani, T., Goto, S., and Komagata, K. (1987) Four new yeast species belonging to the genus *Candida*. *J. Gen. Appl. Microbiol.*, **33**, 1–10.
- Yarrow, D. (1998) Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, A Taxonomic Study*, 4th ed., ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, The Netherlands, pp. 77–100.