Short Communication

***Candida maleeae* sp. nov., a novel anamorphic yeast species in the *Ambrosiozyma* clade found in Thailand**

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In 2001, we isolated a strain of anamorphic yeast genus *Candida* from the fruit body of an unidentified wild mushroom collected in Thailand. This strain could not be assigned to any known species of the genus and was presumed to represent a hitherto undescribed species. However, we hesitated to describe this strain as a novel species because only one strain was obtained. We decided to wait for the isolation of additional strains before we proposed a novel species. In 2009, we isolated an additional strain of this species from fruit of the Salak palm (*Salacca edulis* Reinw.) in Thailand. Therefore we describe a novel species based on the two strains.

The strain ST-246^T (= BCC 15003^T = NBRC 107644^T = CBS 11900^T) was isolated from the fruit body of an unidentified wild mushroom collected in Hala Bala Wildlife Sanctuary, Narathiwat Prov., in Mar. 2001, and the strain ST-2764 (= BCC 38774 = NBRC 107645)

was found in the fruit of a Salak palm (*Salacca edulis* Reinw.), collected near Makok Waterfall, Namtok Phlio National Park, Chanthaburi Prov., in May 2009.

Most of the morphological, physiological, and biochemical characteristics were examined by the methods described by Yarrow (1998). The maximum growth temperature was determined in YM broth using a metal block bath at 1°C intervals. The ubiquinone system was analyzed according to the method described by Mikata and Yamada (1999). 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 described by Tamaoka and Komagata (1984) using the DNA-GC Kit (Yamasa Shoyu Co., Ltd., Chiba). Genomic DNA for sequencing was prepared using Dr. GenTLE for Yeast (TaKaRa Bio Inc., Otsu, Shiga, Japan). The nucleotide sequence of the D1/D2 domain of the large subunit (LSU) rRNA gene was directly determined according to the method reported by Kurtzman and Robnett (1998). The D1/D2 sequences determined in this study were deposited in the GenBank/EMBL/DDBJ database as DQ404487 and AB593187.

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The sequences were aligned with those of related species retrieved from DDBJ, using CLUSTAL X ver. 1.83 (Thompson et al., 1997). The phylogenetic trees were constructed from the evolutionary distance data of Kimura (1980) using the neighbor-joining method (Saitou and Nei, 1987) and the maximum parsimony method using the max-mini branch-and bound algorithm of MEGA ver. 4 (Nei and Kumar, 2000; Tamura et al., 2007). Sites where any gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein, 1985) were done based on 1,000 random resamplings.

The two strains examined in the present study were suggested to belong to the genus *Candida* because they proliferated by multilateral budding, did not dem-

onstrate Diazonium blue B reaction or urease activity, and did not produce ascospores in single or mixed cultures of the two strains on media commonly used for the sporulation test of yeasts, i.e., YM agar, 5% malt extract agar, Fowell's acetate agar, corn meal agar and potato dextrose agar, after 4 week's cultivation at 15°C and 25°C. Furthermore, the two strains have Q-7 as the major ubiquinone, which has not been found in any anamorphic yeast genera other than *Candida*.

The two strains have identical nucleotide sequences in the D1/D2 domain of the LSU rRNA gene. In the phylogenetic tree based on the domain sequences, the two strains are located in the *Ambrosiozyma* clade and clusters with *Candida llanquihuensis* (Ramírez and González, 1984) and an unidentified *Candida*

Fig. 1. Phylogenetic trees for *Candida maleeae* sp. nov. constructed by the neighbor-joining method (A) and maximum parsimony method (B) based on the D1/D2 domain of LSU rRNA gene sequences.

The numerals represent the percentages from 1,000 replicate bootstrap resamplings (frequencies less than 50% are not shown). Sequences were retrieved from the DDBJ databases under the accession numbers indicated.

strain JW01-7-11-1-4-y2, then connected with *Candida kashinagacola* (Endoh et al., 2008) and an unidentified strain named Fungal sp. QsPqPB-1-1, and then connected with a cluster including *Candida vanderkliftii* (Endoh et al., 2008), *Candida pseudovanderkliftii* (Endoh et al., 2008) and two unidentified strains with low bootstrap support (Fig. 1). In the trees based on the D1/D2 domain sequences, *C. llanquihuensis* is the nearest known species of the two strains but 20 nucleotides (3.5%) were substituted. In terms of nucleotide difference in this domain, *C. pseudovanderkliftii* is the nearest known species. The two strains differ from this species by 17 nucleotides (3%, 16 substitutions and 1 indel). This difference clearly suggests the difference of the two species deduced from a guideline described by Kurtzman and Robnett (1998). We concluded that the two strains represent a novel species of the genus *Candida* in the *Ambrosiozyma* clade and describe them as *Candida maleeae* sp. nov. in the present paper. The correct phylogenetic position of the novel species in the *Ambrosiozyma* clade is not clear with the molecular data compared because of low bootstrap support both in neighbor-joining and maximum parsimony trees (Fig. 1). We need the isolation of close relatives or multigene analysis for elucidating the correct phylogenetic position of *C. maleeae*.

Phenotypically, *C. maleeae* is discriminated from *C. pseudovanderkliftii* and *C. llanquihuensis* by several physiological and biochemical characteristics. In particular, *C. maleeae* is clearly discriminated from *C. pseudovanderkliftii* by good fermentation of glucose and trehalose, and from *C. llanquihuensis* by good fermentation of glucose and trehalose and the assimilation of nitrate.

The two strains of *C. maleeae* were isolated from the fruit body of an unidentified wild mushroom and fruit of the Salak palm, a tropical plant. Probably, this species has an insect-associated habitat, like many species in the *Ambrosiozyma* clade, because insects often play roles as vectors of yeasts between substrates (Ganter, 2006).

***Candida maleeae* Nakase, Jindamorakot, Am-In, Ninomiya, Kawasaki & Limtong, sp. nov.**

In liquido "YM", post dies 3 ad 25°C, cellulae ex sedimento globosae, subglobosae vel ovoideae, 3–8.5 × 3–8.5 µm, singulae, binae aut catenatae brevis, cellulae ex pelliculo globosae, subglobosae, ovoideae, or-

thogonae vel cylindratae, 2.5–4 × 3–7 µm, singulae, binae aut catenatae brevis. Sedimentum et pelliculum formantur. In agar "YM", post unum mensem ad 15°C, flavi-cinerea, glabra, subnitida, butyraceae, margine integer. Pseudomycelium formatur. Ascosporae non formantur. Glucosum et trehalosum fermentantur at non galactosum, sucrosum, maltosum, cellobiosum nec raffinose. Glucosum, L-sorbosum, trehalosum, D-xylosum (fortasse lente), D-ribosum, D-glucosaminum, N-acetyl-D-glucosaminum, ethanolum (lente), glycerolum (fortasse lente), erythritolum, ribitolum, D-mannitolum, D-glucitolum, xylitolum, L-arabinitolum, glucono-δ-lactonum (fortasse lente), acidum DL-lacticum, acidum succinicum (exiguum vel nullum) et acidum citricum assimilantur at non galactosum, sucrosum, maltosum, cellobiosum, lactosum, melibiosum, raffinose, melezitolum, inulinum, amyllum solubile, L-arabinosum, D-arabinosum, L-rhamnosum, methanolum, galactitolum, inositolum, methyl-α-D-glucosidum, arbutinum, salicinum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, propanum-1,2-diolum, butanum-2,3-diolum nec hexadecanum. Kalium nitricum assimilatur. Maxima temperatura crescentiae: 34–35°C. Ad crescentiam biotinum et thiaminum necessaria sunt. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 43.8 mol% (per HPLC). Ubiquinonum majus: Q-7.

Holotypus. Stirps ST-246^T (= BCC 15003^T = NBRC 107644^T = CBS 11900^T) in statu lyophilo, cultura viva ex fungo, Songkhla 'Hala Bala Wildlife Sanctuary', Narathiwat Prov., Thailandia, in Mar. 2001, isolata et in collectione culturarum in 'BIOTEC Culture Collection (BCC)', 'NITE Biological Resource Center (NBRC)', Kisarazu, Chiba, Japonia, et 'Centraalbureau voor Schimmelcultures (CBS)', Utrecht, Hollandia, conservata.

Growth in YM broth: After 3 days at 25°C, cells in sediment are globose, subglobose to ovoid, 3–8.5 × 3–8.5 µm, occur singly, in pairs or in chains (Fig. 2A1). Cells in pellicle are globose to subglobose, ovoid, ellipsoid, rectangular or cylindrical, 2.5–4 × 3–7 µm, occur singly, in pairs or in short chains (Fig. 2A2). A thin pellicle and sediment are formed. After 1 month at 15°C, an incomplete ring, islets and sediment are present.

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

Fig. 2. Morphology of *Candida maleeae* sp. nov. ST-246^T.

A. Vegetative cells grown in YM broth for 3 days at 25°C. A1: cells in sediment, A2: cells in pellicle. B. Pseudomycelia formed on slide culture with potato dextrose agar at 25°C. B1, 2: after 8 days, B3: after 16 days (developed into the agar). Scale bars=10 µm.

Slide culture on potato dextrose agar: Well-developed pseudomycelia are produced (Fig. 2B). Blastoconidia occur singly or in short chains on the surface of the agar (Fig. 2B1, 2), and in clusters within the agar (Fig. 2B3).

Ascospore formation: Ascospores are not produced on acetate agar, 5% malt extract agar, diluted vegetable juice agar, YM agar or cornmeal agar, at 15 and 25°C.

Fermentation: Glucose and trehalose are ferment-

ed. Galactose, sucrose, maltose, cellobiose and raffinose are not fermented.

Assimilation of carbon compounds:

Glucose	+
Galactose	–
L-Sorbose	+
Sucrose	–
Maltose	–
Cellobiose	–
Trehalose	+

Lactose	–
Melibiose	–
Raffinose	–
Melezitose	–
Inulin	–
Soluble starch	–
D-Xylose	+ (may be latent)
L-Arabinose	–
D-Arabinose	–
D-Ribose	+
L-Rhamnose	–
D-Glucosamine	+
N-Acetyl-D-glucosamine	+
Methanol	–
Ethanol	+ (latent)
Glycerol	+ (may be latent)
Erythritol	+
Ribitol	+
Galactitol	–
D-Mannitol	+
D-Glucitol	+
Xylitol	+
L-Arabinitol	+
Inositol	–
Methyl- α -D-glucoside	–
Arbutin	–
Salicin	–
Glucono- δ -lactone	+ (may be latent)
D-Gluconic acid	–
2-Ketogluconic acid	–
5-Ketogluconic acid	–
DL-Lactic acid	+
Succinic acid	+ (weak) or –
Citric acid	+
D-Glucuronic acid	–
D-Galacturonic acid	–
Propane-1,2-diol	–
Butane-2,3-diol	–
Hexadecane	–
Assimilation of nitrogen compounds:	
Potassium nitrate	+
Sodium nitrite	+
Ethylamine	+
L-Lysine	+
Cadaverine	+

Vitamins required: Biotin and thiamine.

Production of starch-like substances: Negative.

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

0.1% cycloheximide resistance: Positive.

Maximum growth temperature: 34–35°C (good growth at 33°C, slow growth at 34°C, no growth at 35°C).

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Negative.

Diazonium blue B color reaction: Negative.

Urease: Negative.

G+C content of nuclear DNA: 43.8 mol% (type strain, by HPLC).

Major ubiquinone: Q-7.

Type strain: The strain ST-246^T, isolated from an unidentified wild mushroom collected in Hala Bala Wildlife Sanctuary, Narathiwat Prov., Thailand, in Mar. 2001, was deposited at BIOTEC Culture Collection (BCC), Pathumthani, 12120 Thailand, NITE Biological Resource Center (NBRC), Kisarazu, 292-0818 Chiba Pref., Japan, and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, as BCC 15003^T, NBRC 107644^T and CBS 11900^T, respectively. The strain is maintained by freezing and/or freeze-drying in these collections.

Etymology: The specific epithet “maleeae” was chosen for this novel yeast in honor of Dr. Malee Suwana-adth for her early contribution to yeast sciences in Thailand including taxonomy and biodiversity.

References

- Endoh, R., Suzuki, M., Benno, Y., and Futai, K. (2008) *Candida kashinagacola* sp. nov., *C. pseudovanderkliftii* sp. nov. and *C. vanderkliftii* sp. nov., three new yeasts from ambrosia beetle-associated sources. *Antonie van Leeuwenhoek*, **94**, 389–402.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.
- Ganter, P. F. (2006) Yeasts and invertebrate associations. In *Biodiversity and Ecophysiology of Yeasts*, ed. by Rosa, C. A. and Péter, G., Springer, Berlin-Heidelberg-New York, pp. 303–370.
- 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.
- 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.
- 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.
- Mikata, K. and Yamada, Y. (1999) The ubiquinone system in *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno:

- A new method for identifying ubiquinone homologs from yeast cells. *IFO Res Commun.*, **19**, 41–46.
- Nei, M. and Kumar, S. (2000) *Molecular Evolution and Phylogenetics*, Oxford Univ. Press, New York, pp. 1–333.
- Ramírez, C. and González, A. (1984) Five new filamentous, glucose-fermenting *Candida* isolated from decayed wood in the evergreen rainy Valdivian forest of southern Chile. *Mycopathologia*, **88**, 83–92.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.
- Tamaoka, M. and Komagata, K. (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Lett.*, **25**, 125–128.
- Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997) CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **24**, 4876–4882.
- 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, pp. 77–100.