Yamadazyma siamensis *sp. nov.*, Yamadazyma phyllophila *sp. nov. and* Yamadazyma paraphyllophila *sp. nov.*, *three novel yeast species isolated from phylloplane in Thailand and Taiwan*

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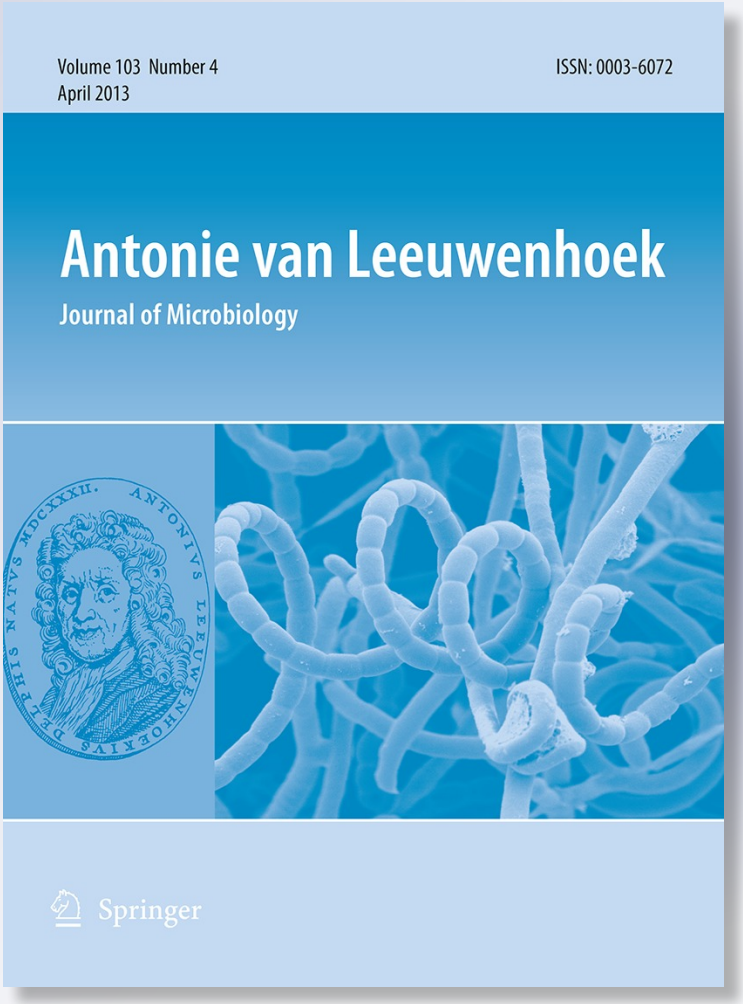

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ORIGINAL PAPER

Yamadazyma siamensis sp. nov., *Yamadazyma phyllophila* sp. nov. and *Yamadazyma paraphyllophila* sp. nov., three novel yeast species isolated from phylloplane in Thailand and Taiwan

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Abstract Four strains representing three novel anamorphic yeast species were isolated from the external surface of sugarcane leaves (DMKU-RK254^T), corn leaves (DMKU-RK548^T), bean leaves (K129) in Thailand and hengchun pencilwood leaves (TrB1-1^T) in Taiwan. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, the sequence analysis of the D1/D2 region of the large subunit (LSU) rRNA gene, the internal transcribed spacer (ITS) region, the actin gene (ACT1) and the elongation factor 2 gene (EF2), the four strains were determined to represent novel *Yamadazyma* species although formation of ascospores was not observed. Strain DMKU-RK254^T was determined to be related to *Candida diddensiae*, *Candida naeodendra* and *Candida kanchanaburiensis* but with 1.8, 1.8 and 2.0 % nucleotide substitutions in the D1/D2 region of the LSU rRNA gene, respectively. It was assigned to *Yamadazyma siamensis* sp. nov. (type strain DMKU-RK254^T = BCC 50730^T = NBRC 108901^T = CBS 12573^T). The sequences of the D1/D2 region of the LSU rRNA gene, the ITS region, ACT1 gene and EF2 gene of two strains (DMKU-RK548^T and K129) were identical but differed from that of strain TrB1-1^T by 0.6, 1.0, 3.3 and 5.9 % nucleotide substitutions, respectively. Therefore, the two strains (DMKU-RK548^T and K129) and strain TrB1-1^T were assigned to be two separate species. The closest species in terms of pairwise sequences similarity of the D1/D2 region to the two novel species was *Yamadazyma philogaea* but with 1.1–1.7 % nucleotide substitutions. The two strains (DMKU-RK548^T and K129) were assigned to *Yamadazyma phyllophila* sp. nov. (type strain DMKU-RK548^T = BCC 50736^T = NBRC 108906^T = CBS 12572^T) and the strain TrB1-1^T was named *Yamadazyma paraphyllophila* sp. nov. (type strain TrB1-1^T = BCRC 23030^T = CCTCC AY 204005^T = CBS 9928^T).

Keywords *Yamadazyma siamensis* sp. nov. · *Yamadazyma phyllophila* sp. nov. · *Yamadazyma paraphyllophila* sp. nov. · Phylloplane yeast · Ascomycete yeast · New yeast species · Thailand · Taiwan

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Introduction

The genus *Yamadazyma* was described by Billon-Grand (1989) to accommodate species that had previously been assigned to the genus *Pichia* and that formed CoQ-9 as their major ubiquinone and formed hat-shaped ascospores (Kurtzman and Robnett 1998). Analysis of the D1/D2 region of the large subunit (LSU) rRNA gene by Kurtzman and Robnett (1998) showed that species assigned to the genus *Yamadazyma* are placed in several clade. Later some of the species that had been assigned to this genus by Billon-Grand (1989) were transferred to the newly described genera *Babjeviella*, *Meyerozyma*, *Millerozyma* and *Priceomyces* based on phylogenetic analysis of the D1/D2 region of the LSU rRNA gene and the nearly complete small subunit rRNA gene (Kurtzman and Suzuki 2010). Then after the genus *Yamadazyma* became a well-support clade (Kurtzman and Suzuki 2010) and a generally accepted genus in the Family Debaryomycetaceae of Order Saccharomycetales (Kurtzman 2011) in The Yeast, a taxonomic study 5th edition *Y. philogaea*, the type species, as well as *Y. akitaensis*, *Y. mexicana*, *Y. nakazawae*, *Y. scolyti*, *Y. triangularis* and 23 *Candida* species are placed in the *Yamadazyma* clade (Kurtzman 2011; Lachance et al. 2011). More recently, a few novel *Candida* species in this clade have been described i.e. *Candida kanchanaburiensis*, which was isolated from the fruit body of a mushroom and rotted fruit of guava collected in the western Thailand (Nakase et al. 2008), *C. khaothaluensis*, which was isolated from unidentified tree in Thailand, *C. vaughaniae* and *C. tallmaniae*, which were isolated from flowers of unidentified plants in French Guiana (Groenewald et al. 2011), and *C. oceani*, which was obtained from an unidentified deep-sea coral, water samples and the stomach of a marine fish on the Mid-Atlantic Ridge (Burgaud et al. 2011).

The external surface of plant leaves is usually referred to as the phylloplane, or phyllosphere (Fonseca and Inacio 2006; Phaff and Starmer 1987). The phylloplane has been found to be colonized by members of both basidiomycete and ascomycete yeasts (Fonseca and Inacio 2006; Glushakova et al. 2007; Landell et al. 2010; Nakase et al. 2001; Slavikova et al. 2009). Although most common phylloplane yeasts are yeast species in basidiomycete genera such as *Cryptococcus*, *Rhodotorula*, *Sporobolomyces* and *Trichosporon* (de Azeredo et al. 1998; Fonseca and Inacio 2006; Glushakova and Chernov 2010; Nakase et al. 2001; Slavikova et al. 2009), various ascomycete yeast species have also been detected on the phylloplane i.e. *Debaryomyces hansenii*, *Hanseniaspora uvarum, Kazachstania barnetii*, *Metschnikowia lopburiensis, Metschnikowia pulcherrima*, *Metschnikowia saccharicola, Pichia membranifaciens*, *Saccharomyces cerevisiae* and various *Candida* species including *Candida aechmeae*, *Candida olephila*, *Candida chumphonensis, Candida mattranensis* and *Candida vrieseae* (Glushakova et al. 2007; Glushakova and Chernov 2010; Kaewwichian et al. 2012; Koowadjanakul et al. 2011; Landell et al. 2010; Slavikova et al. 2009).

During an investigation of yeasts on the external leaf surfaces of plants in Thailand and Taiwan, four strains representing three novel species of *Yamadazyma* were isolated from the phylloplane of sugarcane, corn, bean and hengchun pencilwood. In this paper, one strain (DMKU-RK254^T) is described as *Yamadazyma siamensis* sp. nov., two strains (DMKU-RK548^{T}and K129) are described as *Yamadazyma phyllophila* sp. nov. and one strain (TrB1-1^T) is described as *Yamadazyma paraphyllophila* sp. nov.

Materials and methods

Yeast isolation

Strain DMKU-RK254^T, DMKU-RK548^T and K129 were isolated from leaf surfaces of sugarcane, corn and bean collected in Thailand (Table 1) by an enrichment technique using acidified yeast extract malt extract (YM) broth (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone and 1 % glucose) supplemented with 0.025 % sodium propionate and 0.02 % chloramphenicol (Limtong et al. 2007). 3 g of cut leaves were aseptically placed in 250 ml Erlenmeyer flask, containing 50 ml of acidified YM broth and incubated on a rotary shaker at room temperature (27 ± 3 °C) for 3 days. The enrichment culture was then spread on YM agar supplemented with 0.025 % sodium propionate and 0.02 % chloramphenicol and incubated at room temperature until yeast colonies appeared. Yeast colonies of different morphologies were picked and purified by cross streaking on YM agar. Strain TrB1-1^T

Table 1 Strains of *Yamadazyma siamensis* sp. nov., *Yamadazyma phyllophila* sp. nov. and *Yamadazyma paraphyllophila* sp. nov. isolated from surface of plant leaves used in this study

Strain	Accession number	GenBank accession number				Sample for isolation		
		D1/D2	ITS	ACT1	EF2	Leaves	ocality	Date
Y. siamensis sp. nov.								
DMKU-RK254^T	BCC 50730^T NBRC 108901^T CBS 12573^T	AB734046	AB734049	–	–	Sugarcane (*Saccharum officinarum*)	Bueng Sam Phan district, Phetchabun province, Thailand	23 Mar 2010
Y. phyllophila sp. nov.								
DMKU-RK548^T	BCC 50736^T NBRC 108906^T CBS 12572^T	AB734047	AB734050	AB761313	AB761316	Corn (*Zea mays*)	Pak Chong district, Nakhon Ratchasima province, Thailand	6 May 2010
K129	–	AB734048	AB734051	AB761312	AB761315	Bean (*Phaseolus vulgaris*)	Kanjanaburi province, Thailand	15 Nov 2009
Y. paraphyllophila sp. nov.								
TrB1-1^T	BCRC 23030^T CCTCC AY 204005^T CBS 9928^T	AY562397	AY559447	AB761314	AB761317	Hengchun pencilwood (*Dysoxylum hongkongense*)	NanJen-Shan Natural Reserve on Pindung, Taiwan	2004

BCC BIOTEC Culture Collection, *NBRC* NITE Biological Resources Center, *CBS* Centraalbureau voor Schimmelcultures, *BCRC* Bioresource Collection and Research Center, *CCTCC* China Center for Type Culture Collection

was isolated from hengchun pencilwood leaves collected in Taiwan (Table 1) according to the procedures described by Rosa et al. (1995) with slight modification. Leaves were collected in clean polyethylene plastic bags, transported to the laboratory and washed with 30–40 ml of sterile distilled water, vortexed for 30 min, an aliquot of 0.1 ml was spread on acidified YM agar supplemented with 0.034 % chloramphenicol adjusted to pH 3.7 by 1 N HCl and incubated at 26 °C. After 2 days, yeast colonies were picked and purified. Purified yeast strains were suspended in YM broth supplemented with 10 % glycerol and maintained at −80 °C.

DNA sequencing and phylogenetic analysis

The sequences of the D1/D2 region of the LSU rRNA gene, the internal transcribed spacer (ITS) region, the actin gene (ACT1) and the elongation factor 2 gene (EF2) were determined from PCR products amplified from genomic DNA. The D1/D2 region of the LSU rRNA gene, the ITS region, the ACT1 gene and EF2 gene were amplified and sequenced with primers, NL1 and NL4, ITS1 and ITS4 or ITS5 and ITS4, ACT1 and ACT2, and EFIIF1 and EFIIR2 (Table 2). Methods for DNA extraction and amplification of the D1/D2 region of the LSU rRNA gene, ITS region, and ACT1 gene and EF2 gene of strain DMKU-RK548^T, K129 and TrB1-1^T were as described previously (Limtong et al. 2007). The PCR products were checked by agarose gel electrophoresis and purified by using the QIAquick purification kit (Qiagen, Germany). The purified products were submitted to Macrogen Inc. (Korea) for sequencing. The DNA was extracted for the amplification of the D1/D2 region of the LSU rRNA gene and the ITS region of strain TrB1-1^T by using a variation of a CTAB method (Doyle and Doyle 1990). Dichloromethane was substituted for chloroform in the extraction procedures. Yeast cells were suspended in 500 µl of CTAB extraction buffer [1 ± 4 M NaCl, 100 mM Tris–HCl, pH 8 ± 0, 20 mM EDTA, 2 % w/v PVP-40, 20 mM cetyl trimethyl ammonium bromide (CTAB), and 0 ± 5 % v/v 2-mercaptoethanol] in an eppendorf and placed in a dry bath at 95 °C for 30 min and then in a water bath at 60 °C for 30 min with occasional gentle mixing. An equal volume of dichloromethane:isoamyl alcohol (24:1) was added, mixed by gentle inversion and centrifuged at 1,500×*g* for 4 min at 20 °C. The upper aqueous phase was recovered and DNA was precipitated by the addition of 0.6 volume of isopropanol. The tube was inverted gently and then centrifuged at 460×*g* for 2 min. The DNA pellet was rinsed with 500 µl wash buffer (76 % ethanol and 10 mM ammonium acetate) for 2 min, and rinsed again with wash buffer. The tube was spun at 1,500×*g* for 10 min and the supernatant was decanted. The tube was inverted and air-dried for 10–30 min and then the pellet was resuspended in 20 µl SDW at 37 °C for 30 min. DNA was quantified with a fluorimeter, according to the manufacturer's instructions, and stored at −20 °C. The PCR product was sequenced according to the manufacturer's instructions (ABI prism 3100 Genetic Analyzer, PE Applied Biosystems, USA). The sequences were compared pairwise using a BLAST search (Altschul et al. 1997) and were aligned with the sequences of related species retrieved from GenBank using the multiple alignment program CLUSTAL_X version 1.81 (Thompson et al. 1997).

Table 2 Primers used for PCR amplification and sequencing

Region/gene	Primer	Sequence	Reference
D1/D2	NL1 (forward)	5′ GCATATCAATAAGCGGAGGAAAAG 3′	Kurtzman and Robnett (1998)
	NL4 (reverse)	5′ GGTCCGTGTTTCAAGACGG 3′	
ITS	ITS1 (forward)	5′ TCCGTAGGTGAACCTGCGG 3′	White et al. (1990)
	ITS5 (forward)	5′ GGAAGTAAAAGTCGTAACAACG 3′	
	ITS4 (reverse)	5′ TCCTCCGCTTATTGATATGC 3′	
ACT1	ACT1 (forward)	5′ TACCCAATTGAACACGGTAT 3′	Diezmann et al. (2004)
	ACT2 (reverse)	5′ TCTGAATCTTTCGTTACCAAT 3′	
EF2	EFIIF1 (forward)	5′ AAGTCTCCAAACAAAGCATAAC 3′	Diezmann et al. (2004)
	EFIIR2 (reverse)	5′ GGGAAAGCTTGACCACCAGTAGC 3′	

ITS5 (forward primer) was used for strain TrB1-1

A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura 1980), using the neighbor joining method (Saitou and Nei 1987) by MEGA software version 4.0 (Tamura et al. 2007). Confidence levels of the clades were estimated from bootstrap analysis (1,000 replicates) (Felsenstein 1985).

Examination of taxonomic characteristics

The strains were characterized morphologically, biochemically, and physiologically according to the standard methods described by Yarrow (1998). Mycelium formation was investigated on potato dextrose agar (PDA) in slide culture at 25 °C for up to 7 days. Ascospores formation was investigated individually or in pairs on 5 % malt extract agar, McClary's acetate agar, Gorodkowa agar, corn meal agar, V8 agar and yeast carbon base supplemented with 0.01 % ammonium sulfate (YCBAS) agar at 15 and 25 °C for up to 4 weeks. Carbon assimilation tests were conducted in liquid medium according to the method described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula following the method of Nakase and Suzuki (1986). Growth at various temperatures was determined by cultivation in YM broth. Ubiquinones were extracted from cells cultivated in a 500 ml Erlenmeyer flask containing 250 ml of yeast extract peptone dextrose (YPD) broth (1 % yeast extract, 2 % peptone and 2 % dextrose) on a rotary shaker at 28 °C for 24–48 h and purified according to the method described by Yamada and Kondo (1973) and Kuraishi et al. (1985). Isoprenologues were identified by HPLC as described previously (Limtong et al. 2007). The DNA base composition was determined by HPLC as described by Tamaoka and Komagata (1984) using the DNA-GC kit (Yamasa Shoyu Co., Japan).

Results and discussion

Novel species delineation and identification

Analysis of the D1/D2 region of the LSU rRNA gene sequence revealed that the sequence of strain DMKU-RK254^T was closely related to *Candida diddensiae* and *Candida naeodendra* with 1.8 % nucleotide substitutions (9 nucleotide substitutions out of 493 nt) and to *Candida kanchanaburiensis* with 2.0 % nucleotide substitutions (10 nucleotide substitutions out of 493 nt). The sequences of the ITS region of strain DMKU-RK254^T differed by 6.6 % nucleotide substitutions (36 nucleotide substitutions and 13 gaps out of 545 nt) from *C. diddensiae* by 6.6 % nucleotide substitutions (39 nucleotide substitutions and 30 gaps out of 589 nt) from *C. naeodendra* and by 5.8 % nucleotide substitutions (34 nucleotide substitutions and 11 gaps out of 585 nt) from *C. kanchanaburiensis*.

The D1/D2 region of the LSU rRNA gene sequences of the two strains (DMKU-RK548^T and K129) were identical and differed by three nucleotide substitutions from strain TrB1-1^T. The closest known species to the three strains was *Yamadazyma philogaea*; the two strains (DMKU-RK548^T and K129) differed by 1.1 % nucleotide substitutions (6 nucleotide substitutions out of 530 nt) while strain TrB1-1^T differed by 1.7 % nucleotide substitutions (9 nucleotide substitutions out of 530 nt). The sequences of ITS region of the two strains (DMKU-RK548^T and K129) were identical and differed from strain TrB1-1^T by six nucleotide substitutions and one gap out of 582 nt. The two strains (DMKU-RK548^{T}and K129) differed by 5.2 % nucleotide substitutions (30 nucleotide substitutions and five gaps out of 582 nt) while strain TrB1-1^T differed by 5.5 % nucleotide substitutions (32 nucleotide substitutions and 6 gaps out of 582 nt) from *Y. philogaea*. Moreover, the sequences of the ACT1 gene and the EF2 gene of the two strains (DMKU-RK548^T and K129) were identical and differed from strain TrB1-1^T by 17 nucleotide substitutions out of 523 nt (3.3 % nucleotide substitutions) and 36 nucleotide substitutions out of 606 nt (5.9 % nucleotide substitutions), respectively. The difference in the ACT1 and EF2 genes of the two strains (DMKU-RK548^T and K129) from strain TrB1-1^T is high enough to justify them as two separate species as report for the ACT1 gene in *Clavispora* spp. by Lachance et al. (2003) and for EF2 gene in *Wickerhamomyces* spp. by Imanishi et al. (2009).

The phylogenetic tree based on the sequences of the D1/D2 region of the LSU rRNA gene further demonstrated that the four strains of the three novel species are in *Yamadazyma* clade. Strain DMKU-RK254^T connects to *Candida kanchanaburiensis* and is placed in the same subclade that contains *C. naeodendra*, *C. diddensiae* and the three strains of another two novel species (Fig. 1). Strains DMKU-RK548^T and

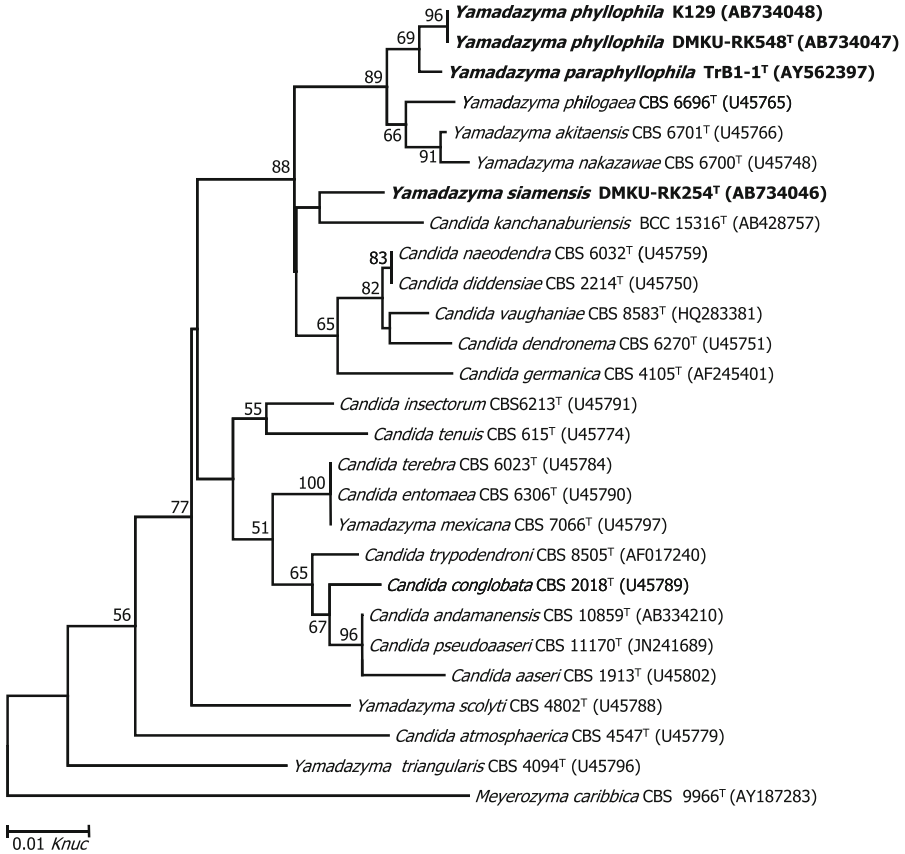

Fig. 1 Phylogenetic tree based on the sequences of the D1/D2 region of the LSU rRNA gene, showing positions of *Yamadazyma siamensis* sp. nov. (DMKU-RK254^T), *Yamadazyma phyllophila* sp. nov. (DMKU-RK548^T and K129) and *Yamadazyma paraphyllophila* sp. nov. (TrB1-1^T), with respect to closely related species. The phylogenetic tree was constructed from evolutionary distance data with Kimura's two-parameter correction, using the neighbor joining method by MEGA software version 4.0. Numbers indicate percentages of bootstrap sampling, derived from 1,000 samples. The *numbers* in *parentheses* are GenBank accession numbers. *Meyerozyma caribbica* was the outgroup in the analysis

K129 are located in the same position and connect to strain TrB1-1^T. They cluster with *Yamadazyma philogaea*, the closest known species, and *Yamadazyma nakazawae* and *Yamadazyma akitaensis* with high bootstrap support (Fig. 1).

On the basis of morphological, biochemical, physiological, chemotaxonomic characteristics, and the sequence analysis of the D1/D2 region of the LSU rRNA gene, the ITS region, the ACT1 gene and the EF2 gene, we concluded that the four strains represent three novel *Yamadazyma* species although formation of ascospores was not observed. The name *Yamadazyma siamensis* sp. nov. (MB801056) is proposed for the strain DMKU-RK254^T, the name *Yamadazyma phyllophila* sp. nov. (MB801057) is assigned for the two strains (DMKU-RK548^{T}and K129) and the name *Yamadazyma paraphyllophila* sp. nov. (MB802274) is proposed for the strain TrB1-1^T.

In practice, *Yamadazyma siamensis* sp. nov., *Y. phyllophila* sp. nov. and *Y. paraphyllophila* sp. nov. can be distinguished from their close related species *C. diddensiae*, *C. naeodendra* and *C. kanchanaburiensis*, and *Y. philogaea*, respectively, not only on the basis of the sequences of the D1/D2 region of the LSU rRNA gene and the ITS region but also several phenotypic characteristics as shown in Table 3. *Y. phyllophila* sp. nov. can be distinguished from *Y. paraphyllophila* sp. nov. only by the different in genotypic characteristics while the phenotypic characteristics were the same.

Most of the members of the *Yamadazyma* clade such as *C. michaelii*, *C. gorgasii*, *C. lessepsii*, *C. cerambycidarum*, C. *endomychidarum*, *C. amphixiae*, *C. diddensiae*, *C. naeodendra* and *C. dendronema* exhibit an association with the gut of beetles and other insects (Lachance et al. 2011). However, some strains of the recognized species were reported to have been isolated from plants i.e. a strain of *Yamadazyma akitaensis* (CBS 6701^T) that was isolated from exudate of a willow, two strains of *Yamadazyma mexicana* (CBS 7066^T and CBS 7067) that were isolated from cactus, *Candida diospyri* (CBS 9769^T) that was isolated from a kaki fruit and *Candida buinensis*

Table 3 Phenotypic characteristics that differentiate *Yamadazyma siamensis* sp. nov., *Yamadazyma phyllophila* sp. nov. and *Yamadazyma paraphyllophila* sp. nov. from their closest species, *Candida diddensiae*, *C. naeodendra*, *C. kanchanaburiensis* and *Yamadazyma philogaea*, respectively

Characteristics	Species						
	1	2[a]	3[b]	4[c]	5	6	7[d]
Fermentation							
Glucose	+	+	+	w	+	+	+
Galactose	w	+	s	w	+	+	w
Sucrose	−	v	−	−	−	−	−
Maltose	−	v	−	−	−	−	v
Lactose	−	−	−	n	−	−	−
Raffinose	−	−	−	n	−	−	−
Trehalose	−	+	+	−	−	−	+
Cellobiose	+	n	n	−	−	−	n
Xylose	−	n	n	−	−	−	n
Assimilation of carbon/nitrogen compounds							
D-Glucose	+	+	+	+	+	+	+
D-Galactose	+	+	+	+	+	+	+
L-Sorbose	+	v	s	l	−	−	+
Sucrose	+	+	−	+	+	+	+
Maltose	+	+	−	+	+	+	+
Cellobiose	+	+	+	+	+	+	+
Trehalose	+	v	+	+	+	+	+
Lactose	−	−	−	−	+	+	−
Melibiose	−	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−	−
Melezitose	+	+	+	+	+	+	+
Inulin	−	−	−	−	−	−	−
Soluble starch	−	−	+	−	−	−	−
D-Xylose	+	+	+	+	+	+	+
L-Arabinose	+	v	+	+	w	w	+
D-Arabinose	s	v	s	v	−	−	+
D-Ribose	+	v	+	v	−	−	+
L-Rhamnose	+	−	s	l	+	+	−
Ethanol	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+
Erythritol	+	+	+	+	+	+	+
Ribitol	+	+	+	+	+	+	+
Galactitol	−	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	+
D-Glucitol	+	+	+	+	+	+	+
α-Methyl-D-glucoside	+	v	+	+	+	+	+
Salicin	+	v	+	+	+	+	−
DL-Lactic acid	w	−	−	−	−	−	−
Succinic acid	+	v	+	+	+	+	+
Citric acid	+	v	+	+	−	−	+
Inositol	−	−	−	−	−	−	−
Arbutin	+	n	n	+	−	−	n
D-Gluconic acid	+	+	+	+	+	+	+
D-Glucosamine	+	v	+	s	−	−	+
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+
2-Keto-D-gluconic acid	−	v	−	−	−	−	+
5-Keto-D-gluconic acid	−	n	n	−	−	−	−
Hexeadecane	−	w	+	−	−	−	+
Methanol	−	−	−	−	−	−	−
Propane-1,2-diol	−	n	n	l	−	−	n
Butane-2,3-diol	−	n	n	−	−	−	n
D-Glucono-δ-lactone	−	+	−	−	w	w	n
Xylitol	+	v	+	+	+	+	n
D-Glucuronic acid	−	−	−	−	−	−	n
D-Galacturonic acid	−	n	n	−	−	−	n
Arabinitol	+	n	n	+	−	−	n
Nitrate	−	−	−	−	−	−	−
Nitrite	−	−	−	−	−	−	n
Ethylamine	+	+	+	+	+	+	n
L-Lysine	+	+	+	w	+	+	+
Cadaverine	+	+	+	+	+	+	n
Other growth characteristics							
Vitamin free medium	w	−	−	n	−	−	−
50 % Glucose	+	v	+	n	+	+	n
60 % Glucose	+	n	n	n	+	+	n
10 % NaCl + 5 % glucose	+	+	−	v	w	w	+
16 % NaCl + 5 % glucose	−	n	n	n	−	−	n
0.01 % Cycloheximide	w	−	−	n	−	−	−
0.1 % Cycloheximide	−	−	−	−	−	−	n
Growth at 25 °C	+	+	+	+	+	+	+
Growth at 30 °C	+	+	+	+	+	+	+
Growth at 35 °C	+	+	+	+	+	+	+
Growth at 37 °C	+	+	+	+	+	+	w
Growth at 40 °C	+	n	n	n	+	+	n
Growth at 42 °C	−	n	n	n	−	−	n
Growth at 45 °C	−	n	n	n	−	−	n
Starch production	−	−	−	−	−	−	−

Table 3 continued

Characteristics	Species						
	1	2[a]	3[b]	4[c]	5	6	7[d]
Diazonium blue B	–	–	–	n	–	–	–
Urease	–	n	n	n	–	–	n
Major ubiquinone	Q9	Q9	Q9	Q9	Q9	Q9	Q9

+, positive; −, negative; l, latent positive; s, slow positive; w, weakly positive; v, variable; n, no data

1 Yamadazyma siamensis sp. nov., *2 Candida diddensiae*, *3 C. naeodendra*, *4 C. kanchanaburiensis*, *5 Y. phyllophila* sp. nov., *6 Y. paraphyllophila* sp. nov., *7 Y. philogaea*

[a,b] Data from Lachance et al. (2011)

[c] Data from Nakase et al. (2008)

[d] Data from Kurtzman (2011)

(CBS 6796^T) that was isolated from the gelatinous exudate of a tree fern (Kurtzman 2011; Lachance et al. 2011). In Thailand few *Candida* species in the *Yamadazyma* clade have been isolated i.e. *Candida khaothaluensis*, which was isolated from an unidentified tree (Groenewald et al. 2011) and *Candida kanchanaburiensis*, which was isolated from a fruit body of a unidentified mushroom (*Coprinus* sp.) and a rotted guava fruit (Nakase et al. 2008). Until the present, no members of this clade have been reported to have been isolated from the phylloplane. In this study, four strains of the two novel *Yamadazyma* species are proposed, but from limited number of strains derived in this study, we could not conclude that the phylloplane is one of the habitats of yeast in this clade. However, the phylloplane is interesting as the subject for further investigation of yeast in this clade.

Description of *Yamadazyma siamensis* Kaewwichian, Yongmanitchai, Kawasaki, Wang, Yang & Limtong sp. nov.

Growth in yeast extract malt extract (YM) broth: After 3 days at 25 °C, cells are globose, subglobose to ovoid (2–7 × 3–8 μm) and occur singly, in pairs or in groups (Fig. 2a). Budding is multilateral. Growth on YM agar: after 3 days at 25 °C, the streak culture is grayish white, smooth, soft to butyrous and has an entire margin. Pseudohyphae are formed but true hyphae are not formed in slide culture on PDA after 3 days at 25 °C (Fig. 2b). Ascospores were not produced on YM agar, YPD agar, 5 % malt extract agar, McClary's acetate agar, corn meal agar, Gorodkowa agar, V8 agar and YCBAS agar after 4 weeks

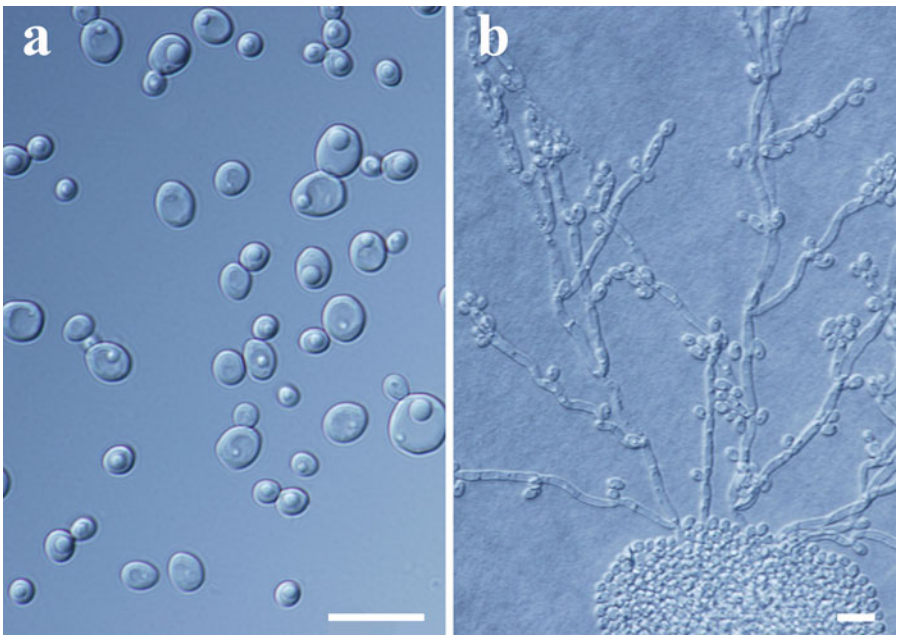

Fig. 2 *Yamadazyma siamensis* sp. nov. (DMKU-RK254^T) **a** budding cells in YM broth after 3 days at 25 °C **b** pseudohyphae formed on PDA after 3 days at 25 °C. *Scale bar*, 10 μm

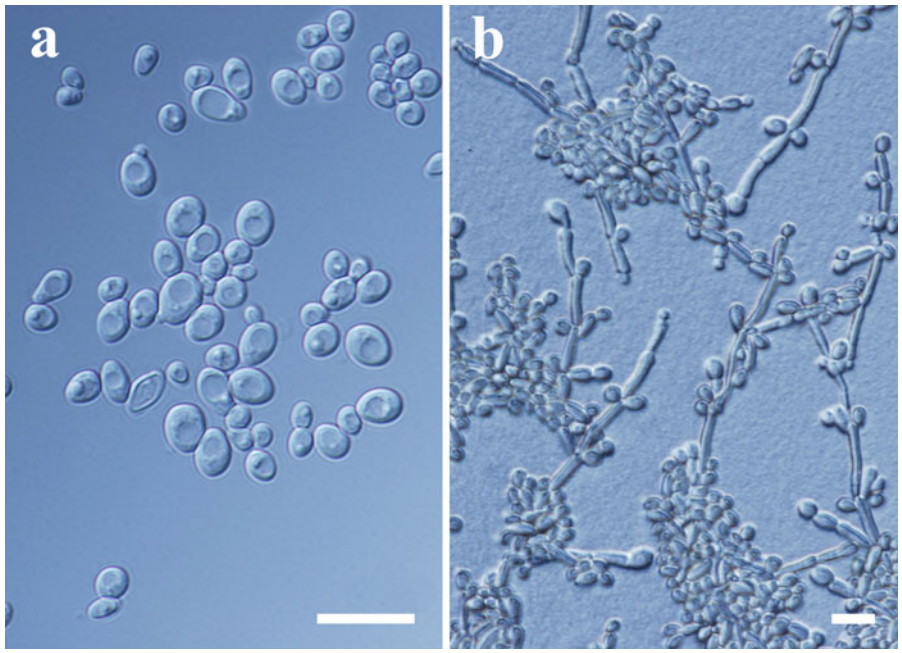

Fig. 3 *Yamadazyma phyllophila* sp. nov. (DMKU-RK548^T) **a** budding cells in YM broth after 3 days at 25 °C **b** pseudohyphae formed on PDA after 3 days at 25 °C. *Scale bar*, 10 μm

at 15 and 25 °C. DNA G+C content is 46.2 mol %. The other phenotypic characteristics of the species are shown in Table 3.

Holotype

DMKU-RK254^T is the holotype of *Yamadazyma siamensis* (MB801056). The strain was isolated from phylloplane of sugarcane (*Saccharum officinarum*) collected from Phetchabun province, Thailand. The living culture from type was deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 50730^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 108901^T and CBS-KNAW Fungal Biodiversity Center (CBS), Utrecht, The Netherlands as CBS 12573^T.

Etymology

The species epithet *siamensis* refers to Siam the old name of Thailand, where the strain was isolated.

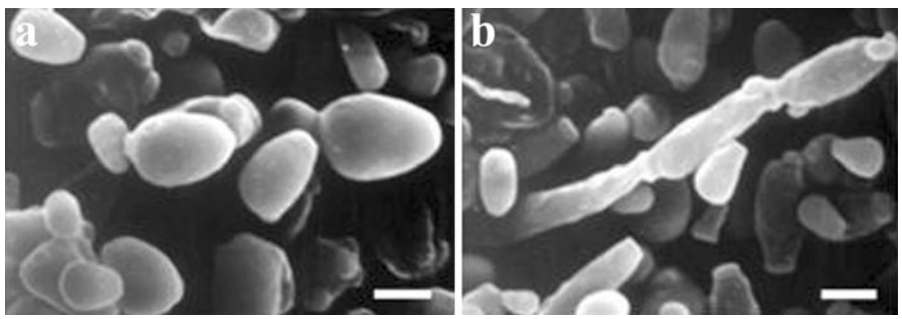

Fig. 4 *Yamadazyma paraphyllophila* sp. nov. (TrB1-1^T) **a** budding cells in YM broth after 3 days at 25 °C **b** pseudohyphae formed on PDA after 3 days at 25 °C. *Scale bar*, 1 μm. Scanning electron microscopy was prepared according to the procedures described by Lee et al. (1994)

Description of *Yamadazyma phyllophila* Kaewwichian, Yongmanitchai, Kawasaki, Wang, Yang & Limtong sp. nov.

Growth in yeast extract malt extract (YM) broth: after 3 days at 25 °C, cells are ovoid to ellipsoid (2–4 × 3–6 µm) and occur singly, in pairs or in groups (Fig. 3a). Budding is multilateral. Growth on YM agar after 3 days at 25 °C, the streak culture is white, smooth and has an entire margin. Pseudohyphae are formed but true hyphae are not formed in slide culture on PDA after 3 days at 25 °C (Fig. 3b). Ascospores were not produced by any of the strains either individually or when paired on YM agar, YPD agar, 5 % malt extract agar, McClary's acetate agar, corn meal agar, Gorodkowa agar, V8 agar and YCBAS agar after 4 weeks at 15 and 25 °C. DNA G+C content is 38.7 mol %. The other phenotypic characteristics of the species are shown in Table 3.

Holotype

DMKU-RK548^T is the holotype of *Yamadazyma phyllophila* sp. nov. (MB801057). The strain was isolated from phylloplane of corn (*Zea mays*) collected from Nakhon Ratchasima province, Thailand. The living culture from type was deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 50736^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 108906^T and CBS-KNAW Fungal Biodiversity Center (CBS), Utrecht, The Netherlands as CBS 12572^T.

Etymology

The species epithet *phyllophila* refers to phylloplane where the three strains of this species were isolated.

Description of *Yamadazyma paraphyllophila* Wang, Yang, Kaewwichian, Yongmanitchai, Kawasaki & Limtong sp. nov.

Growth in yeast extract malt extract (YM) broth: after 3 days at 25 °C, cells are ovoid to ellipsoid (2–3 × 3–5 µm) and occur singly, in pairs or in groups (Fig. 4a). Budding is multilateral. Growth on YM agar after 3 days at 25 °C, the streak culture is white, smooth and has an entire margin. Pseudohyphae are formed but true hyphae are not formed in slide culture on PDA after 3 days at 25 °C (Fig. 4b). Ascospores were not produced on YM agar, YPD agar, 5 % malt extract agar, McClary's acetate agar, corn meal agar, Gorodkowa agar, V8 agar and YCBAS agar after 4 weeks at 15 and 25 °C. The other phenotypic characteristics of the species are shown in Table 3.

Holotype

TrB1-1^T is the holotype of *Yamadazyma paraphyllophila* sp. nov. (MB802274). The strain was isolated from phylloplane of hengchun pencilwood (*Dysoxylum hongkongense*) collected from NanJen-Shan Natural Reserve on Pindung, Taiwan. The living culture from type was deposited at the Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan R.O.C., as BCRC 23030^T; China Center for Type Culture Collection, Wuhan, Hubei, China, as CCTCC AY 204005^T and CBS-KNAW Fungal Biodiversity Center (CBS), Utrecht, The Netherlands as CBS 9928^T.

Etymology

The species epithet *paraphyllophila* indicating affinity with *phyllophila* species.

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