

# *Ogataea kanchanaburiensis* sp. nov. and *Ogataea wangdongensis* sp. nov., two novel methylotrophic yeast species from phylloplane in Thailand

Savitree Limtong · Rungluk Kaewwichian ·  
Marizeth Groenewald

Received: 31 August 2012 / Accepted: 18 October 2012 / Published online: 27 October 2012  
© Springer Science+Business Media Dordrecht 2012

**Abstract** Three strains (KM03<sup>T</sup>, KM13<sup>T</sup> and KM15) representing two novel methylotrophic yeast species were isolated from the external surface of plant leaves, which were collected from Kanchanaburi province, Thailand, by three-consecutive enrichments in methanol broth. Strain KM03<sup>T</sup> was isolated from phylloplane of a mango tree (*Mangifera indica*) and two strains, KM13<sup>T</sup> and KM15, were obtained from phylloplane of different wine grapes (*Vitis vinifera*). The sequences of the D1/D2 region of the large subunit (LSU) rRNA gene of the two strains (KM13<sup>T</sup> and KM15) were identical and differed markedly from that of strain KM03<sup>T</sup>. In terms of pairwise sequence similarity of the D1/D2 region the closest species to the strains KM13<sup>T</sup> and KM15 were *Candida suzukii* (CBS 9253<sup>T</sup>) and *Candida nitratophila* (CBS 2027<sup>T</sup>) but with 2.1 % nucleotide substitutions. Strain KM03<sup>T</sup> differed from *Ogataea wickerhamii* (CBS 4307<sup>T</sup>), its closest relative, by 2.3 % nucleotide substitutions. Phylogenetic analysis based on

the D1/D2 sequences placed the three strains in the *Ogataea* clade. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, the sequence analyses of the D1/D2 and the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene (nrRNA) operon, the three strains represent two novel *Ogataea* species although formation of ascospores was not observed. *Ogataea kanchanaburiensis* sp. nov. is proposed for strain KM03<sup>T</sup> (=BCC 47626<sup>T</sup> = NBRC 108603<sup>T</sup> = CBS 12673<sup>T</sup>). Two strains, KM13<sup>T</sup> and KM15, were assigned to *Ogataea wangdongensis* sp. nov. (type strain KM13<sup>T</sup> = BCC 42664<sup>T</sup> = NBRC 107778<sup>T</sup> = CBS 12674<sup>T</sup>). GenBank/EMBL/DDJB accession numbers for the sequences of the D1/D2 and the ITS regions of *O. kanchanaburiensis* KM03<sup>T</sup> are AB734090 and AB734093, respectively, of *O. wangdongensis* KM13<sup>T</sup> are AB734091 and AB734094, respectively, and of *O. wangdongensis* KM15 are AB734092 and AB734095, respectively.

S. Limtong (✉) · R. Kaewwichian  
Department of Microbiology, Faculty of Science,  
Kasetsart University, Bangkok 10900, Thailand  
e-mail: fscistl@ku.ac.th

S. Limtong  
Center for Advanced Studies in Tropical Natural  
Resources, National Research University,  
Kasetsart University, Bangkok 10900, Thailand

M. Groenewald  
CBS-KNAW Fungal Biodiversity Center, Uppsalalaan,  
3584 CT Utrecht, The Netherlands

**Keywords** *Ogataea kanchanaburiensis* sp. nov. ·  
*Ogataea wangdongensis* sp. nov. ·  
Methylotrophic yeast · Phylloplane · Thailand

## Introduction

Methylotrophic yeasts, the yeasts that can utilize methanol as a sole source of carbon and energy, represent a relative small proportion of yeasts and

belong to a limited number of yeast genera, including *Ogataea* (Yamada et al. 1994; Mikata and Yamada 1995; Suh et al. 2006), *Komagataella* (Yamada et al. 1995; Dlauchy et al. 2003; Kurtzman 2005), *Kuraishia* (Yamada et al. 1994; Péter et al. 2005), and related *Candida* species in these three groups (Lachance et al. 2011). In the recent years members of *Ogataea* increased rapidly, not only because of the transfer of many *Pichia* species to this genus after emendation of the genus description for nitrate assimilation, ascospores shape, ascospores number and multi-gene phylogenetic analyses, but also due to the discovery of many novel species in the past few years (Glushakova et al. 2010; Kurtzman and Robnett 2010; Limtong et al. 2008; Nagatsuka et al. 2008; Péter et al. 2008, 2009; Suh and Zhou 2010). In the yeasts, a taxonomic study, 5th edition 31 species are accepted in the genus *Ogataea* and 22 *Candida* species are placed in the *Ogataea* clade (Kurtzman et al. 2011b; Lachance et al. 2011). Subsequently, several additional members of the *Ogataea* clade have been proposed such as *O. phyllophila*, *Candida chumphonensis*, *C. matranensis* (Koowadjanakul et al. 2011), *C. rishirensis* (Nakase et al. 2010), *O. parapolyomorpha* (Kurtzman 2011a) and *O. saltuana* (Péter et al. 2011).

The external surface of plant leaves, which is usually referred to as the phylloplane or phyllosphere, has been recognized as an important habitat for epiphytic microorganisms (Fonseca and Inacio 2006; Phaff and Starmer 1987). Phylloplane are found to be colonized by members of both basidiomycetous and ascomycetous 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 basidiomycetous species (Fonseca and Inacio 2006; Nakase et al. 2001) the occurrence of methylotrophic ascomycetous species were reported on leaves in Hungary (Péter et al. 2007) and Thailand (Koowadjanakul et al. 2011). This may be due to the fact that methanol on phylloplane derives by pectin demethylation inside the leaves and emitted to the surface through stomata during transpiration (Nemecek-Marshall et al. 1995) and methylotrophic yeasts utilize it for their growth (Péter et al. 2007).

During the investigation of methylotrophic yeasts on phylloplane of wine and table grapes and other plant species cultivated in Kanchanaburi province, Thailand, three strains that represent two novel species

were isolated. Detailed analyses demonstrated that they belong to the genus *Ogataea*. In this report two novel *Ogataea* species are described.

## Materials and methods

### Yeast isolation

Green and undamaged leaves of wine and table grapes (23 samples) and other plant species (12 samples) were collected from fields in Kanchanaburi Research Station, Agro-Ecological System Research and Development Institute (N14°07'15.1" E099°19'05.6") of Kasetsart University located in Wang Dong sub-district, Mueang district, Kanchanaburi province, Thailand on 2 July 2009. Strain KM03<sup>T</sup> was isolated from the surface of a leaf from a mango tree (*Mangifera indica*, Family: Anacardiaceae) and strains KM13<sup>T</sup> and KM15 were obtained from the surface of leaves of two wine grapes (*Vitis vinifera*, Family: Vitaceae).

Methylotrophic yeasts were isolated from leaf surface by a technique involving three consecutive methanol enrichments as described by Limtong et al. (2004) but using 0.5 % (v/v) methanol-yeast nitrogen base (YNB) broth (0.67 % Difco yeast nitrogen base and 0.5 % (v/v) methanol) instead of 1 % (v/v) methanol-YNB broth. Three grams of cut leaves were aseptically placed in a 250 ml Erlenmeyer flask containing 50 ml enrichment broth and incubated on a rotary shaker at room temperature (27 ± 3 °C) for 4–5 days. After three consecutive enrichment cultivation a loopful of the enriched culture was streaked on 0.5 % (v/v) methanol-YNB agar and incubated at room temperature until yeast colonies appeared. Yeast colonies of different morphologies were picked and purified by cross streaking on yeast extract malt extract (YM) agar (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1 % glucose and 2 % agar). Purified yeast strains were suspended in YM broth supplemented with 10 % glycerol and maintained at –80 °C.

Yeast strains obtained were tested for growth on 0.5 % (v/v) methanol-YNB agar at room temperature (27 ± 3 °C).

### DNA sequencing and phylogenetic analyses

The sequences of the D1/D2 and the ITS regions were determined from PCR products amplified from

genomic DNA. Methods for DNA extraction and amplification of the D1/D2 region were described previously (Limtong et al. 2007). The ITS region was amplified with primers, ITS1 and ITS4, following the method of White et al. (1990). The PCR products were checked by agarose gel electrophoresis and purified by using the QIA quick purification kit (Qiagen, Germany). The purified products were sequenced commercially by Macrogen Inc. (Seoul, Korea) using primers, NL1 and NL4 for the D1/D2 region and ITS1 and ITS4 for the ITS region. 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). The phylogenetic trees were constructed with the neighbor-joining, maximum-parsimony and maximum-likelihood methods using MEGA version 5 (Tamura et al. 2011). 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 corn meal agar in slide culture at 25 °C for up to 7 days. Ascospore formation was investigated for individual strains or strain pairs on 5 % malt extract agar, Gorodkova agar, Fowell's acetate agar and corn meal agar at 15 and 25 °C for up to 4 weeks. Carbon assimilation tests were conducted in liquid medium as 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 of the strains in YM broth. Ubiquinones were extracted from cells that were cultivated in 500 ml Erlenmeyer flasks 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 methods described by Yamada and Kondo (1973) and Kuraishi et al. (1985). Isoprenologues were identified by HPLC as described previously (Limtong et al. 2007).

## Results and discussion

### Yeasts isolation, identification and novel species delineation

A total of 13 yeast strains were isolated from 35 leaf samples and seven strains grew well on 0.5 % (v/v) methanol-YNB agar at room temperature. Identification on the basis of similarities of the D1/D2 sequences revealed three strains (KM03<sup>T</sup>, KM13<sup>T</sup> and KM15) that represent novel species. Two strains were identified to be *O. philodendri* and the other two strains were closest to *Candida* sp. NRRL Y-12764, an undescribed species, but with 1 nucleotide substitution.

The sequences of the D1/D2 regions of KM13<sup>T</sup> and KM15 were identical and differed markedly from that of KM03<sup>T</sup>. In terms of pairwise sequence similarity the closest relative to KM03<sup>T</sup> was *O. wickerhamii* (GenBank EU011612) with 2.3 % nucleotide substitutions (13 nucleotide substitutions out of 566 nt). The two strains, KM13<sup>T</sup> and KM15, were closest to *Candida* sp. NRRL YB-1238 (CBS 12667; GenBank EU011607) with 1.9 % nucleotide substitutions (11 nucleotide substitutions out of 566 nt), differed from *Candida suzukii* (GenBank EU011610) and *Candida nitratophila* (GenBank EU011606) with 2.1 % nucleotide substitutions (12 nucleotide substitutions out of 566 nt), and differed from *C. nanaspora* (GenBank EU011602) with 2.4 % (13 substitutions and 1 indel out of 566 nt).

The nucleotide sequences of the ITS regions of these strains were also analyzed, and the sequences of KM03<sup>T</sup>, KM13<sup>T</sup>, KM15, *O. wickerhamii* CBS 4307<sup>T</sup>, *Candida suzukii* CBS 9253<sup>T</sup>, *Candida nitratophila* CBS 2027<sup>T</sup>, *C. nanaspora* CBS 7200<sup>T</sup> and *Candida* sp. NRRL YB-1238 (CBS 12667) were deposited as AB734093, AB734094, AB734095, JX546582, JX965187, JX965188, JX546583 and JX546581, respectively. In the ITS regions the sequences of KM03<sup>T</sup> exhibited 17 % nucleotide differences (91 nucleotide substitutions and 33 indels out of 722 nt) with *O. wickerhamii* (GenBank JX546582). Strains KM13<sup>T</sup> and KM15 that showed identical sequences differed by 8.5 % (43 nucleotide substitutions and 9 indels out of 617 nt) from *Candida* sp. NRRL YB-1238 (GenBank JX546581), 9.8 % (42 nucleotide substitutions and 22 indels out of 662 nt) from *Candida suzukii* (GenBank JX965187), 7.3 % (37 nucleotide substitutions and 11 indels out of 655 nt) from *Candida nitratophila* (GenBank JX965188) and

9.2 % (46 nucleotide substitutions and 12 indels out of 631nt) from *C. nanspora* (GenBank JX546583).

The phylogenetic trees based on the sequence of the D1/D2 region of the LSU rRNA gene constructed by the three methods namely neighbor-joining, maximum-parsimony and maximum-likelihood methods showed only slight differences in the topology of the closely related species but that the main topology of the clusters was the same (data not shown for maximum-parsimony and maximum-likelihood trees). They demonstrated that the three strains were in two separate clusters in the *Ogataea* clade (Fig. 1). Strain KM03<sup>T</sup> clustered with *O. wickerhamii*, the closest species in term of pairwise sequence similarities, and placed in a cluster that contains *O. minuta*, the type species of the genus. Strains KM13<sup>T</sup> and KM15 are located in the same position and form a cluster with *C. suzukii*, *C. nitratophila*, *C. nanspora*, *O. ramenticola* and *Candida* sp. NRRL YB-1238 (CBS 12667).

On the basis of the evidence from the molecular and other taxonomic criteria obtained during this study, we concluded that the three strains represent two novel *Ogataea* species although formation of ascospores was not observed in order to follow International Code of Nomenclature for algae, fungi, and plants concerning with the nomenclatural rules for fungi that the most important is the adoption of “one fungus, one name” which was enacted at the 18th International Botanical Congress in Melbourne, Australia in July 2011 (Miller et al. 2011). The name *O. kanchanaburiensis* sp. nov. (MB800996) is proposed for strain KM03<sup>T</sup> and the name *O. wangdongensis* sp. nov. (MB800997) is assigned to strains KM13<sup>T</sup> and KM15.

The novel species *O. kanchanaburiensis* sp. nov. and *O. wangdongensis* sp. nov. can be distinguished from each other and from their closely related recognized species, *O. wickerhamii*, and *C. suzukii*, *C. nitratophila* and *C. nanspora*, respectively, not only on the basis of the sequences of the D1/D2 and the ITS regions but also by several phenotypic characteristics as shown in Table 1.

Members of the genus *Ogataea* have been found associated with plant material including tree bark, tree exudate, flower, leaf, gall on leaf, rotten wood insect and frass (Glushakova et al. 2010; Koowadjanakul et al. 2011; Kurtzman 2011a; Morais et al. 2004). Among these a few strains of the recognized species were reported to be isolated from plant leaves i.e. *O. allantospora*, *O. nitrataversa*, *O. phyllophila* and *O.*

*saltuana* (Koowadjanakul et al. 2011; Kurtzman 2011a; Péter et al. 2011). This study increases the number of *Ogataea* species isolated from phylloplane.

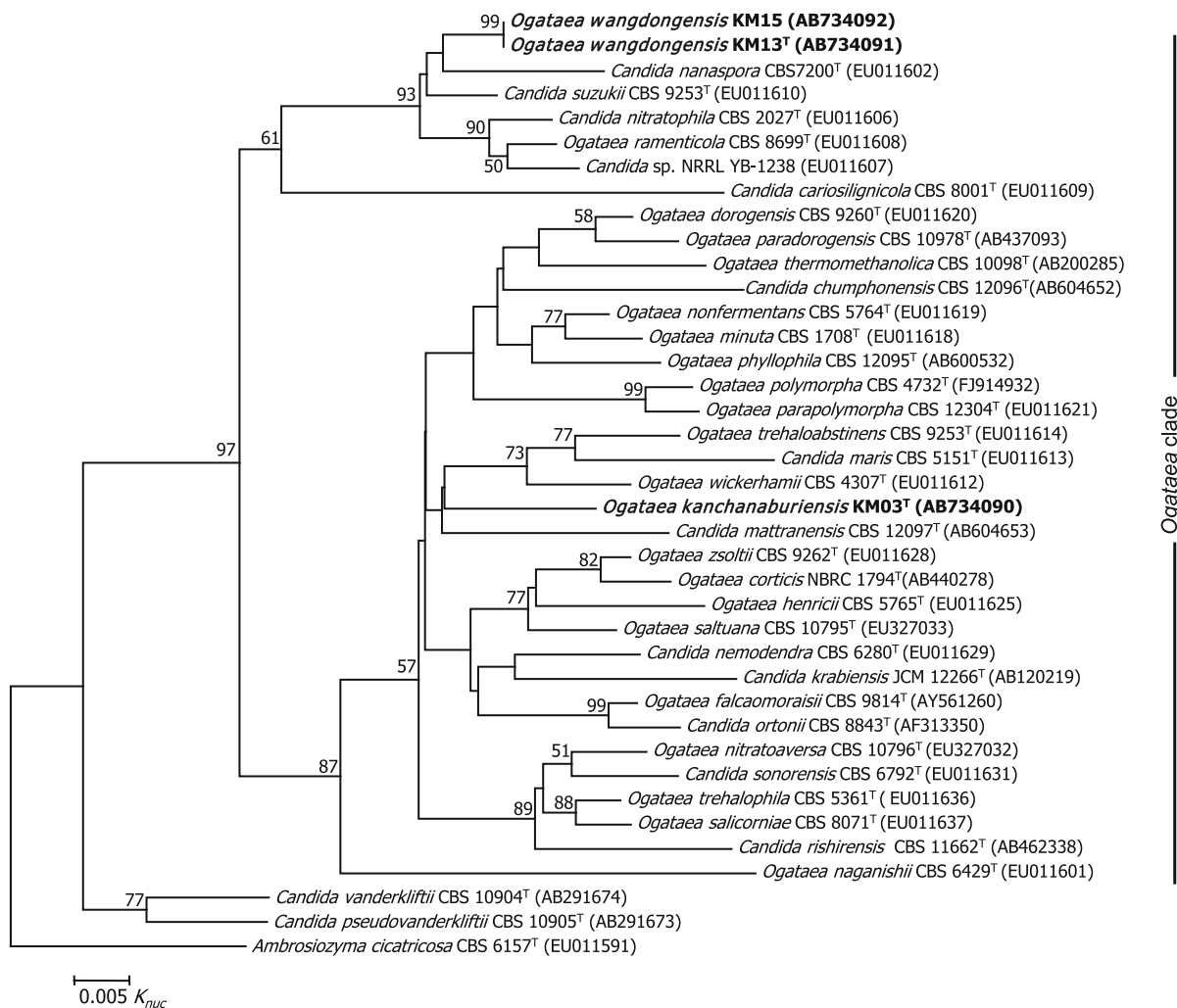
### Description of *Ogataea kanchanaburiensis* Limtong, Kaewwichian and M. Groenewald sp. nov.

Growth in YM broth: After 3 days at 25 °C, cells are spherical (2.2–5.0 × 2.5–5.0 μm), and occur singly or in pairs (Fig. 2a). Budding is multilateral. Growth on YM agar: After 3 days at 25 °C, the streak culture is butyrous, cream-coloured, with a smooth surface and has an entire margin. Pseudohyphae and true hyphae are not formed in slide culture on corn meal agar after 7 days at 25 °C. Ascospores were not produced on 5 % malt extract agar, Fowell’s acetate agar, Gorodkova agar and corn meal agar at 15 and 25 °C up to 6 weeks.

Fermentation of all carbon sources is negative. D-glucose, D-galactose, L-sorbose, α-α-trehalose, D-mannitol, D-glucono-1,5-lactone, succinate (weak), methanol, ethylamine HCl, L-lysine HCl and cadaverine are assimilated, but N-acetyl-D-glucosamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, glycerol, erythritol, galactitol, myo-inositol, 2-ketogluconate, 5-ketogluconate, D-gluconate, D-glucuronate, D-galacturonate, ribitol, D-glucitol, DL-lactate, citrate, ethanol, potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin free medium is positive (slow). Growth on medium containing 50 % (w/v) glucose is positive but on medium containing 60 % (w/v) glucose or 10 % (w/v) sodium chloride/5 % (w/v) glucose are negative. Growth with 0.01 % cycloheximide and 0.1 % cycloheximide are negative. Growth at 37 °C (slow) is positive, but at 40 °C is negative. Starch like compounds are not produced. Diazonium blue B color and urease reactions are negative. The major ubiquinone is Q-7.

### Holotype

KM03<sup>T</sup> is the holotype of *O. kanchanaburiensis* (MB800996). The strain was isolated from leaves of mango tree (*Mangifera indica* Linn.), Kanchanaburi



**Fig. 1** Phylogenetic tree based on the sequences of the D1/D2 region of the LSU rRNA gene, showing the clustering of *Ogataea kanchanaburiensis* sp. nov. (KM03<sup>T</sup>) and *Ogataea wangdongensis* sp. nov. (KM13<sup>T</sup> and KM15), with respect to their closely related species. The phylogenetic trees were constructed with the neighbor-joining method using MEGA

version 5 (Tamura et al. 2011). Numbers indicate percentages of bootstrap sampling, derived from 1,000 samples. The numbers in parentheses are GenBank accession numbers. *Ambrosiozyma cicatricosa* was the outgroup species in the analysis. Bar = 0.01 *K*<sub>nuc</sub>

Research Station (N14°07'15.1"E099°19'05.6"), Wang Dong sub district, Mueang District, Kanchanaburi province collected on 2 July 2009. A living culture from the type strain was deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 47626<sup>T</sup>; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 108603<sup>T</sup> and CBS-KNAW Fungal Biodiversity Center (CBS), Utrecht, The Netherlands as CBS 12673<sup>T</sup>.

### Etymology

The species epithet *kanchanaburiensis* refers to Kanchanaburi province, Thailand, where the strain was isolated.

### Description of *Ogataea wangdongensis* Limtong, Kaewwichian and M. Groenewald sp. nov.

Growth in YM broth: After 3 days at 25 °C, cells are spherical (2–4 × 2–4 μm), and occur singly or in



**Table 1** Phenotypic characteristics that differentiate *Ogataea kanchanaburiensis* sp. nov. and *Ogataea wangdongensis* sp. nov. from the closely related species

Characteristics	1	2 <sup>a</sup>	3	4 <sup>b</sup>	5 <sup>b</sup>	6 <sup>b</sup>
<b>Fermentation</b>						
Glucose	–	–	s	–	s	+
Galactose	–	n	–		s/–	+
Trehalose	–	n	–	n	+	+
<b>Assimilation</b>						
L-Sorbose	+	+	+	+	–	–
N-Acetyl-D-glucosamine	–	–	+	+	+	+
D-Ribose	–	+	–	+	+	+
D-Xylose	–	n	+	+	+	+
L-Arabinose	–	v	+	+	+	+
D-Arabinose	–	+	–	–	–	v
L-Rhamnose	–	+	+	+	+	+
α-α-Trehalose	+	s	+	+	+	+
Glycerol	–	+	+	+	+	+
Erythritol	–	+	+	+	–	+
Ribitol	–	+	+	+	+	+
D-Glucitol	–	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+
D-Glucono-1,5-lactone	+	n	+	n	+	+
DL-Lactate	–	–	+	+	–	–
Succinate	w	w/–	w	+	s	+
Ethanol	–	+	+/s	+	+	+
Xylitol	n	n	n	+	+	+
Potassium nitrate	–	+	+	+	+	+
Sodium nitrite	–	n	+	+	+	+
Ethylamine HCl	+		+	+	+	+
<b>Other growth characteristics</b>						
Growth w/o vitamins	s	–	s	–	–	–
Growth at 35 °C	+		+	–	n	n
Growth at 37 °C	s	–	s	n	–	+
0.01 % cycloheximide	–	n	–	n	+	+
0.1 % cycloheximide	–	n	–	+	+	+
50 % glucose	+	n	+	–	+	–
10 % NaCl + 5 % glucose	–	–	+	–	–	–

Species 1 *O. kanchanaburiensis* sp. nov., 2 *O. wickerhamii*, 3 *O. wangdongensis* sp. nov., 4 *C. suzukii*, 5 *C. nitratophila*, 5 *C. nanasporea*

+ positive, s slow positive, w weak positive, v variable, – negative, n no data

<sup>a</sup> Data from Kurtzman et al. (2011b)

<sup>b</sup> Data from Lachance et al. (2011)

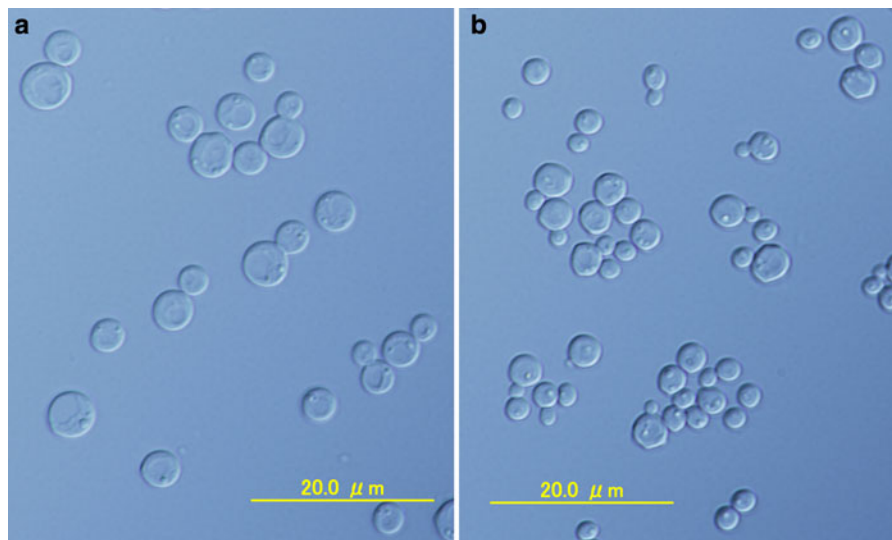
pairs or in groups (Fig. 2b). Budding is multilateral. Growth on YM agar: After 3 days at 25 °C, the streak culture is butyrous, white to cream-coloured, with an

umbonate surface and has an undulate margin. Pseudohyphae and true hyphae are not formed in slide culture on corn meal agar after 7 days at 25 °C. Ascospores were not produced by individual strains or strains paired on 5 % malt extract agar, Fowell's acetate agar, Gorodkova agar and corn meal agar at 15 and 25 °C up to 6 weeks.

Fermentation of D-glucose is positive (slow) but negative for D-galactose, sucrose, maltose, lactose, trehalose and raffinose. Assimilation of D-glucose, D-galactose, L-sorbose, N-acetyl-D-glucosamine, D-xylose, L-arabinose, L-rhamnose, α-α-trehalose, glycerol, erythritol, ribitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, DL-lactate, succinate (weak), methanol, ethanol (positive/slow), potassium nitrate, sodium nitrite, ethylamine HCl, L-lysine HCl, and cadaverine are positive, but D-ribose, D-arabinose, sucrose, maltose, methyl-α-D-glucoside, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, galactitol, myo-inositol, 2-ketogluconate, 5-ketogluconate, D-gluconate, D-glucuronate, D-galacturonate and citrate are not assimilated. Growth in vitamin free medium is positive (slow). Growth on media containing 50 % (w/v) glucose, 60 % (w/v) glucose or 10 % (w/v) sodium chloride/5 % (w/v) glucose is positive. Growth with 0.01 % cycloheximide and 0.1 % cycloheximide is negative. Growth at 37 °C (slow) is positive, but at 40 °C is negative. Starch like compounds are not produced. Diazonium blue B color and urease reactions are negative. The major ubiquinone is Q-7.

### Holotype

KM13<sup>T</sup> is the holotype of *O. wangdongensis* (MB800997). The strain was isolated from leaves of wine grape (*Vitis vinifera*), Kanchanaburi Research Station (N14°07'15.1" E099°19'05.6"), Wang Dong sub district, Mueang District, Kanchanaburi province collected on 2 July 2009. 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 42667<sup>T</sup>; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107781<sup>T</sup> and CBS-KNAW Fungal Biodiversity Center (CBS), Utrecht, The Netherlands as CBS 12674<sup>T</sup>.



**Fig. 2** Budding cells on YM agar after 3 days at 25 °C. **a** *Ogataea kanchanaburiensis* sp. nov. (KM03<sup>T</sup>) **b** *Ogataea wangdongensis* sp. nov. (KM13<sup>T</sup>). Scale bar = 20 μm

## Etymology

The species epithet *wangdongensis* refers to Wang Dong sub district, Mueang District, Kanchanaburi province, Thailand, where the strain was isolated.

**Acknowledgments** This work was supported by the Kasetsart University Research and Development Institute (KURDI), Kasetsart University, Thailand, the Higher Education Research Promotion and National Research University Project of Thailand and Office of the Higher Education Commission, Thailand. Many thanks go to Mr. Teera Yimyoo and Miss Nampueng Koowadjanakul for their assistance in sample collection and yeasts isolation.

## References

- Altschul SF, Madden TL, Schaffer JZ, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Dlauchy D, Tornai-Lehoczki J, Fulop L, Péter G (2003) *Pichia (Komatagaella) pseudopastoris* sp. nov., a new yeast species from Hungary. *Antonie Van Leeuwenhoek* 83:327–332
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791
- Fonseca A, Inacio J (2006) Phylloplane yeasts. In: Rosa C, Péter G (eds) *Biodiversity and ecophysiology of yeasts*. Springer, Germany, pp 263–301
- Glushakova AM, Yurkov AM, Chernov IY (2007) Massive isolation of anamorphous ascomycete yeasts *Candida oleophila* from plant phyllosphere. *Mikrobiologiya* 76:799–803
- Glushakova AM, Maximova IA, Kachalkin AV, Yurkov AM (2010) *Ogataea cecidiorum* sp. nov., a methanol-assimilating yeast isolated from galls on willow leaves. *Antonie Van Leeuwenhoek* 98:93–101
- Koowadjanakul N, Jindamorakot S, Yongmanitchai W, Limtong S (2011) *Ogataea phyllophila* sp. nov., *Candida chumphonensis* sp. nov. and *Candida matranensis* sp. nov., three methylotrophic yeast species from phylloplane in Thailand. *Antonie Van Leeuwenhoek* 100:207–217
- Kuraishi H, Katayama-Fujimura Y, Sugiyama J, Yokoyama T (1985) Ubiquinone systems in fungi. I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. *Trans Mycol Soc Jpn* 26:383–395
- Kurtzman CP (2005) Description of *Komagataella phaffii* sp. nov. and the transfer of *Pichia pseudopastoris* to the methylotrophic yeast genus *Komagataella*. *Int J Syst Evol Microbiol* 55:973–976
- Kurtzman CP (2011a) A new methanol assimilating yeast, *Ogataea parapolyomorpha*, the ascospore state of *Candida parapolyomorpha*. *Antonie Van Leeuwenhoek* 100:455–462
- Kurtzman CP (2011b) *Ogataea* Y. Yamada, K. Maeda & Mikata. In: Kurtzman CP, Fell JW, Boekhout T (eds) *The yeasts, a taxonomic study*, 5th edn. Elsevier, Amsterdam, pp 645–671
- Kurtzman CP, Robnett CJ (2010) Systematics of methanol assimilating yeasts and neighboring taxa from multigene sequence analysis and the proposal of *Peterozyma* gen. nov., a new member of the Saccharomycetales. *FEMS Yeast Res* 10:353–361
- Lachance M-A, Boekhout T, Scorzetti G, Fell JW, Kurtzman CP (2011) *Candida* Berkhout. In: Kurtzman CP, Fell JW, Boekhout T (eds) *The yeasts, a taxonomic study*, 5th edn. Elsevier, Amsterdam, pp 987–1279

- Landell MF, Billodre R, Ramos JP, Leoncini O, Vainstein MH, Valente P (2010) *Candida aechmeae* sp. nov. and *Candida vrieseae* sp. nov., novel yeast species isolated from the phylloplane of bromeliads in Southern Brazil. *Int J Syst Evol Microbiol* 60:244–248
- Limtong S, Srisuk N, Yongmanitchai W, Kawasaki H, Yurimoto H, Nakase T, Kato N (2004) Three new thermotolerant methylotrophic yeasts, *Candida krabiensis* sp. nov., *Candida sithpensensis* sp. nov., and *Pichia siamensis* sp. nov., isolate in Thailand. *J Gen Appl Microbiol* 50:119–127
- Limtong S, Yongmanitchai W, Tun MM, Kawasaki H, Seki T (2007) *Kazachstania siamensis* sp. nov., an ascomycetous yeast species from forest soil in Thailand. *Int J Syst Evol Microbiol* 57:419–422
- Limtong S, Srisuk N, Yongmanitchai W, Yurimoto H, Nakase T (2008) *Ogataea chonburiensis* sp. nov. and *Ogataea nakhonphanomensis* sp. nov., thermotolerant, methylotrophic yeast species isolated in Thailand, and transfer of *Pichia siamensis* and *Pichia thermomethanolica* to the genus *Ogataea*. *Int J Syst Evol Microbiol* 58:302–307
- Mikata K, Yamada Y (1995) *Ogataea kodamae*, a new combination for a methanol-assimilating yeast species, *Pichia kodamae* van der Walt et Yarrow. *Res Commun Inst Ferment* 17:99–101
- Miller JS, Funk VA, Wagner WL, Barrie F, Hoch PC, Herendeen P (2011) Outcomes of the 2011 botanical nomenclature section at the XVIII International Botanical Congress. *PhytoKeys* 5:1–3
- Morais PB, Teixeira LCRS, Bowles JM, Lachance M-A, Rosa CA (2004) *Ogataea falcaomoraisii* sp. nov., a sporogenous methylotrophic yeast from tree exudates. *FEMS Yeast Res* 5:81–85
- Nagatsuka Y, Saito S, Sugiyama J (2008) *Ogataea neopini* sp. nov. and *O. corticis* sp. nov., with the emendation of the ascomycete yeast genus *Ogataea*, and transfer of *Pichia zsolitii*, *P. dorogensis*, and *P. trehaloabstinens* to it. *J Gen Appl Microbiol* 54:353–365
- Nakase T, Suzuki M (1986) *Bullera megalospora*, a new species of yeast forming large ballistospores isolated from dead leaves of *Oryza sativa*, *Miscanthus sinensis* and *Sasa* sp. in Japan. *J Gen Appl Microbiol* 32:225–240
- Nakase T, Takashima M, Itoh M, Fungsin B, Potacharoen W, Atthasampunna P, Komagata K (2001) Ballistoconidium-forming yeasts found in the phyllosphere of Thailand. *Microbiol Cult Coll* 17:23–33
- Nakase T, Imanishi Y, Ninomiya S, Takashima M (2010) *Candida rishirensis* sp. nov., a novel methylotrophic anamorphic yeast species isolated from soil on Rishiri island in Japan. *J Gen Appl Microbiol* 56:169–173
- Nemecek-Marshall M, MacDonald RC, Franzen JJ, Wojciechowski C, Fall R (1995) Methanol emission from leaves. *Plant Physiol* 108:1359–1368
- Péter G, Dlačhy D, Tornai-Lehoczki J, Kurtzman CP (2005) *Kuraishia molischiana* sp. nov., the teleomorph of *Candida molischiana*. *Antonie Van Leeuwenhoek* 88:241–247
- Péter G, Tornai-Lehoczki J, Dlačhy D (2007) *Ogataea allantospora* sp. nov., an ascomycetous yeast species from phylloplane. *Antonie Van Leeuwenhoek* 92:443–448
- Péter G, Tornai-Lehoczki J, Dlačhy D (2008) *Ogataea nitratroaversa* sp. nov., a methylotrophic yeast species from temperate forest habitats. *Antonie Van Leeuwenhoek* 94:217–222
- Péter G, Tornai-Lehoczki J, Dlačhy D (2009) *Ogataea populiabae* sp. nov., a yeast species from white poplar. *FEMS Yeast Res* 9:936–941
- Péter G, Dlačhy D, Tornai-Lehoczki J, Gouliamova D, Kurtzman CP (2011) *Ogataea saltuana* sp. nov., a novel methanol-assimilating yeast species. *Antonie Van Leeuwenhoek* 100:375–383
- Phaff HJ, Starmer WT (1987) Yeasts associated with plants, insects and soil. In: Rose AH, Harrison JS (eds) *The Yeasts*, 2nd edn. Academic, London, pp 123–180
- Slavikova E, Vadkertiova R, Vranova D (2009) Yeasts colonizing the leaves of fruit trees. *Ann Microbiol* 59:419–424
- Suh S-O, Zhou JJ (2010) Methylotrophic yeasts near *Ogataea (Hansenula) polymorpha*: a proposal of *Ogataea angusta* comb. nov. and *Candida parapolyomorpha* sp. nov. *FEMS Yeast Res* 10:631–638
- Suh SO, Blackwell M, Kurtzman CP, Lachance M-A (2006) Phylogenetics of Saccharomycetales, the ascomycete yeasts. *Mycologia* 98:1006–1017
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28:2731–2739
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
- White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) *PCR protocols: a guide to methods and applications*. Academic, New York, pp 315–322
- Yamada Y, Kondo K (1973) Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus*, and the yeast-like genera *Sporobolomyces* and *Rhodospiridium*. *J Gen Appl Microbiol* 19:59–77
- Yamada Y, Maeda K, Mikata K (1994) The phylogenetic relationships of the hat-shaped ascospore-forming, nitrate-assimilating *Pichia* species, formerly classified in the genus *Hansenula* Sydow et Sydow, based on the partial sequences of 18S and 26S ribosomal RNAs (Saccharomycetaceae): the proposal of three new genera, *Ogataea*, *Kuraishia*, and *Nakazawaea*. *Biosci Biotechnol Biochem* 58:1245–1257
- Yamada Y, Matsuda M, Maeda K, Mikata K (1995) The phylogenetic relationships of methanol-assimilating yeasts based on the partial sequences of 18S and 26S ribosomal RNAs: the proposal of *Komagataella* gen. nov. (Saccharomycetaceae). *Biosci Biotechnol Biochem* 59:439–444
- 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