Antonie van Leeuwenhoek (2011) 100:207–217
DOI 10.1007/s10482-011-9579-9

ORIGINAL PAPER

Ogataea phyllophila sp. nov., *Candida chumphonensis* sp. nov. and *Candida mattranensis* sp. nov., three methylotrophic yeast species from phylloplane in Thailand

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Received: 31 January 2011 / Accepted: 29 March 2011 / Published online: 10 April 2011
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Abstract Five strains (LN12, LN14^T, LN15^T, LN16 and LN17^T) representing three novel methylotrophic yeast species were isolated from the external surface of plant leaves by three-consecutive enrichments. On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics, the sequence analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene and the phylogenetic analysis, the five strains were assigned to be one novel *Ogataea* species and two novel *Candida* species. Three strains (LN12, LN14^T and LN16) represent a single novel species of the genus *Ogataea*, for which the name *Ogataea phyllophila* sp. nov. is proposed. The type strain is LN14^T (= BCC 42666^T = NBRC 107780^T = CBS 12095^T). Strain LN15^T was assigned to be *Candida chumphonensis* sp. nov. (type strain LN15^T = BCC 42667^T = NBRC 107781^T = CBS 12096^T). Strain LN17^T represented another novel species of *Candida* that was named *Candida mattranensis* sp. nov. (type strain LN17^T = BCC 42668^T = NBRC 107782^T = CBS 12097^T).

Keywords *Ogataea phyllophila* sp. nov. · *Candida chumphonensis* sp. nov. · *Candida mattranensis* sp. nov. · Methylotrophic yeast · Phylloplane

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Introduction

Methylotrophic yeasts can utilize methanol as a sole source of carbon and energy. They 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; Peter et al. 2005) and *Candida* (Meyer et al. 1998). In the recent years member of *Ogataea* increased rapidly not only because of transferring many *Pichia* species to this genus after emendation of the genus description for nitrate assimilation, ascospores shaped and ascospores number but also from discovering many novel species in the past few years (Glushakova et al. 2010; Kurtzman and Robnett 2010; Limtong et al. 2008; Nagatsuka

et al. 2008; Peter et al. 2008, 2009; Suh and Zhou 2010). At present more than 30 species are accepted in the genus *Ogataea*.

The external surface of plant leaves usually refer to the phylloplane or phyllosphere (Phaff and Starmer 1987; Fonseca and Inacio 2006). Phylloplane from various regions of the world 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) Peter et al. (2007) reported the occurrence of methylotrophic ascomycetous species on leaves in Hungary. It is suggested that the methylotrophic yeasts on phylloplane utilize methanol that produced by pectin demethylation inside the leaves and emitted to the surface through stomata during transpiration for their growth (Peter et al. 2007).

During investigation of methylotrophic yeasts on phylloplane in Mattra island, Chumphon Islands National Park, Chumphon province, Thailand, five strains of novel species were isolated. Detailed analysis demonstrated that they are belonged to *Ogataea* clade. In this report one novel *Ogataea* species and two novels *Candida* species are described.

Materials and methods

Yeast isolation

Leaves were collected from 21 different plants included banana (*Musa sapientum*), cassava (*Manihot esculenta*), coconut (*Cocos nucifera*), fig (*Ficus racemosa*), sugarcane (*Saccharum officinarum*) and the other 16 unknown plants in a small area of primary rainforest in Mattra island (10°04′N 99°35′E), Chumphon Islands National Park, Chumphon province, Thailand in May, 2009. Methylotrophic yeasts were isolated 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, derived from cutting few leaves to the size that can be put into a 250 ml Erlenmeyer flask, was inoculated into 50 ml enrichment broth in the flask and incubated on a rotary shaker at room temperature 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 YM agar. Purified yeast strains were suspended in YM broth supplemented with 10% glycerol and maintained at −80°C.

DNA sequencing and phylogenetic analysis

Methods for DNA isolation and amplification of the D1/D2 domain of the LSU rRNA gene were as described previously by Limtong et al. (2007). The PCR product was checked by agarose gel electrophoresis and purified by using the QIA quick purification kit (Qiagen, Hilden, Germany). The purified product was sequenced commercially by Macrogen Inc. (Seoul, Korea) for sequencing with primers, NL1 and NL4. The sequences were compared pairwise using a BLASTN 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). 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). The other phylogenetic trees were also constructed with the neighbor-joining and maximum likelihood methods using MEGA 5.0 package (Tamura et al. 2007) and the aligned sequences obtained from MUSCLE version 3.8 alignment program (Edgar 2004). 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. Ascospores formation was investigated on 5% malt extract agar, Gorodkowa 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 in YM broth. Ubiquinones were extracted from cells 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 method 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 and identification

Six methylotrophic yeast strains could be isolated by the enrichment isolation using 0.5% (v/v) methanol-YNB broth from six samples of leaves from cassava and the other five unknown plants out of 21 samples used for isolation. Identification on the basis of similarities of the D1/D2 domain of the LSU rRNA gene sequences revealed that five strains (LN12, LN14^T, LN15^T, LN16 and LN17^T) were found to represent three novel species and one strain was assigned to *Ogataea polymorpha*.

Member of the methylotrophic yeast have been found associated with plant materials including tree bark, tree exudate, flower, leaf, gall on leaf and rotten wood (Dlauchy et al. 2003; Glushakova et al. 2010; Limtong et al. 2004, 2008; Morais et al. 2004; Peter et al. 2003, 2007, 2008, 2009). The result of this investigation showed that methylotrophic yeasts were detected on leaves collected from the small island in Thailand though only a small number of samples were investigated. However, methylotrophic yeast could be detected on only 27% of the leaves which was much lower than 45% that was reported in Hungary (Peter et al. 2007) while 95% of leaves were colonized by the other yeasts.

Novel species delineation

Among the five strains (LN12, LN14^T, LN15^T, LN16 and LN17^T), the sequences of the D1/D2 domain of the LSU rRNA gene of three strains (LN12, LN14^T and LN16) were identical. In terms of pairwise sequence similarity the closest species to the three strains (LN12, LN14^T and LN16) was *Ogataea* sp. NCAIM Y.01896 but with 1.4% nucleotide substitutions (9 nucleotide substitutions out of 568 nt) and the three strains differed from *Ogataea minuta,* their closest described relative, by 1.8% nucleotide substitutions (10 nucleotide substitutions and 1 indel out of 567 nt). Strain LN15^T differed from *Candida* sp. ZIM 2322, it closest phylogenic relative, by 2.8% nucleotide substitutions (18 nucleotide substitutions and 2 gaps out of 570 nt) and from *Ogataea dorogensis*, the known species closest to it, by 3.2% nucleotide substitutions (18 nucleotide substitutions and 3 gaps out of 570 nt). Strain LN17^T was closest to *Candida* sp. NRRL YB-2442 but with 3.5% nucleotide substitutions (20 nucleotide substitutions and 1 indel out of 566 nt) while it differed by 3.7% nucleotide substitutions (21 nucleotide substitutions and 1 indel out of 566 nt) from *Ogataea cecidiorum*, it closest known species. Therefore, the three strains (LN12, LN14^T and LN16) were considered to represent a single novel species while strains LN15^T and LN17^T represented two other novel species (Kurtzman and Robnett 1998).

The phylogenetic trees based on the sequences of the D1/D2 domain of the LSU rRNA gene were constructed by neighbor-joining method using the aligned sequences obtained from CLUSTAL_X alignment program (Fig. 1) or MUSCLE alignment program (data not shown) and by maximum likelihood method using the aligned sequences obtained from either alignment program (data not shown). Though the trees constructed by both methods were not congruent completely, they demonstrated that the five strains were in the *Ogataea* clade. The neighbor-joining tree using aligned sequences obtained from CLUSTAL_X alignment program was selected to show the phylogenetic placement of the new species (Fig. 1). In this neighbor-joining tree the five strains were in the same cluster that separated from the so-called *Pichia methanolica* cluster proposed by Nagatsuka et al. (2008), which latter the *Pichia* species of this cluster were transferred to the genus *Ogataea* (Kurtzman and Robnett 2010). The three strains (LN12, LN14^T and LN16) were located in the same position and formed a subcluster with *Ogataea* sp. NCAIM Y.01896 and *O. minuta*, there closest

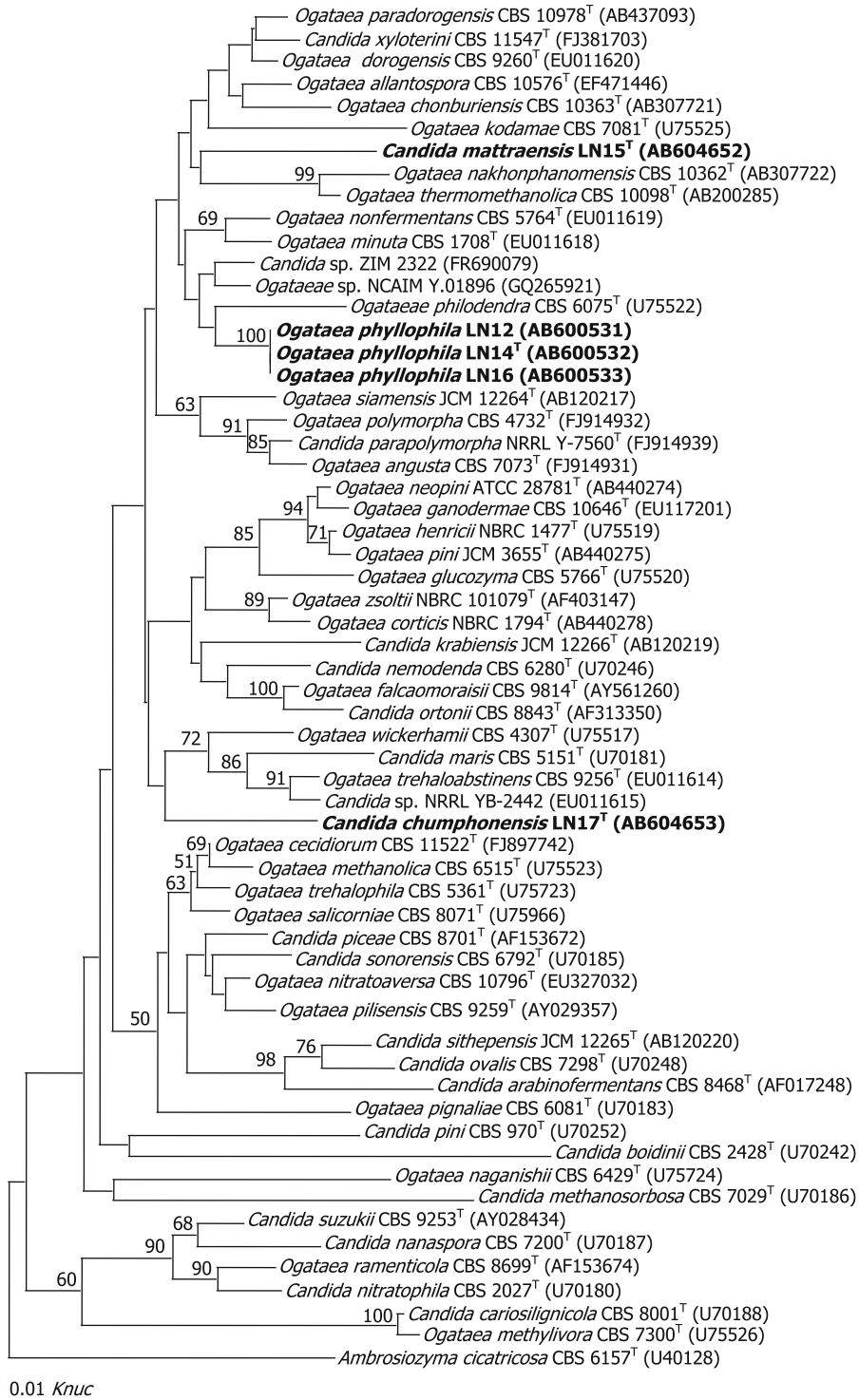

Fig. 1 Phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene, showing positions of *Ogataea phyllophila* sp. nov. (LN12, LN14^T and LN16), *Candida chumphonensis* sp. nov. (LN15^T) and *Candida mattranensis* sp. nov. (LN17^T) with respect to closely relate species. The phylogenetic tree was constructed from evolutionary distance data corrected by two-parameter transformation of Kimura (1980), using the neighbor-joining method. Numbers indicate percentages of bootstrap sampling, derived from 1,000 samples. *Bar* 0.01 *Knuc*

undescribed and described relatives in term of pairwise sequence similarity, but with low bootstrap support. Strain LN15^T form a subcluster with *O. dorogenis* with low bootstrap support its closet described species. Strain LN17^T was related to *Candida* sp. NRRL YB-2442 in a separate subcluster.

Therefore, it is clear that the five strains were separated into three novel species belonging to the *Ogataea* clade but their correct phylogenetic position within the clade remains unclear which was also happened in the case of using single gene sequences for phylogenetic tree construction reported by Nagatsuka et al. (2008); Nakase et al. (2010) and Peter et al. (2010).

On the basis of the evidence of molecular and other taxonomic criteria here we propose three novel species, *O. phyllophila* sp. nov. (MycoBank no.: MB561002) for strains LN12, LN14^T and LN16, *C. chumphonensis* sp. nov. (MycoBank no.: MB561003) for strain LN15^T, and *C. mattranensis* sp. nov. (MycoBank no.: MB561004) for strain LN17^T.

The three novel species *O. phyllophila* sp. nov., *C. chumphonensis* sp. nov. and *C. mattranensis* sp. nov. can be distinguished from each other and from their closest known species *O. minuta*, *O. dorogensis* and *O. cecidiorum*, respectively, not only on the basis of the sequences of the D1/D2 domain of the LSU rRNA gene and ascospores formation but also by several phenotypic characteristics as shown in Table 1.

Latin diagnosis of *Ogataea phyllophila* Koowadjanakul, Jindamorakot, Yongmanitchai et Limtong sp. nov.

In medio liquido 'cum extracto levidins et extracto malti (YM)', post dies 3 ad 25°C cellulae globosae aut ovoideae (2–3 × 2–4 μm), singulae aut binae, per germinationem multipolarem reproducentes. In agaro 'YM', post dies 3 ad 25°C, cultura butyrosa, cremea, glabra et margo glabra. Pseudohyphae et hyphae non formantur in agaro farina Zea mays post dies 7 ad 25°C. Ascosporae galeiformes aut pileiformes, 4 in ascum. Asci inconjugati, conjugati cellularum vel, conjugati cellularum gemmarum que oriumtur. Fermentatio nulla. D-Glucosum, L-sorbosum, D-ribosum, D-xylosum (lente), D-arabinosum (infirme), α-α-trehalosum, cellobiosum, salicinum, glycerolum, erythritolum, ribitolum, D-glucitolum, D-mannitolum, acidum 2-keto-D-gluconicum (infirme), acidum 5-keto-D-gluconicum, acidum DL-lacticum (infirme), acidum succinicum, acidum citricum, methanolum, ethanolum, natrium nitrosum, kalium nitricum, ethylaminum, L-lysinum et cadaverinum assimilantur at non D-galactosum, *N*-acetyl-D-glucosaminum,

Table 1 Phenotypic characteristics of *Ogataea phyllophila* sp. nov., *Candida chumphonensis* sp. nov., *Candida mattranensis* sp. nov., *Ogataea minuta*, *Ogataea dorogensis* and *Ogataea cecidiorum*

Characteristics	1	2	3	4[a]	5[b]	6[c]
Fermentation						
D-Glucose	−	S	W	+/W/−	S	S
D-Galactose	−	−	−	−	−	−
Maltose	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−
α-α-Trehalose	−	−	−	W/S	−/W/S	−
Lactose	−	−	−	−	−	N
Raffinose	−	−	−	−	−	−
Carbon assimilation						
D-Glucose	+	+	+	+	+	+
D-Galactose	−	−	W	−	−	−
L-Sorbose	+	−	S	−	+	+
N-Acethyl-D-glucosamine	−	−	−	−	−	N
D-Ribose	+	W	S	±	+	+
D-Xylose	S	−	W	±	+	V
L-Arabinose	−	−	−	−	L	V
D-Arabinose	W	−	−	V/S	+	V
L-Rhamnose	−	−	−	V/±	−	W
Sucrose	−	−	−	−	−	−
Maltose	−	−	−	−	−	−
α-α-Trehalose	+	−	+	+	+	+
α-Methyl-D-glucoside	−	−	−	−	−	+
Cellobiose	+	W	+	+/S	+	−
Salicin	+	W	+	+	+	N
Melibiose	−	−	−	−	−	−
Lactose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Melezitose	−	−	−	−	−	−
Inulin	−	W	−	−	−	−
Soluble starch	−	−	−	−	−	−
Glycerol	+	W	+	+/S	+	+
Erythritol	+	W	+	−	+	+
Ribitol	+	W	+	+	+	+
D-Glucitol	+	W	+	+	+	N
D-Manitol	+	W	+	+	+	N
Galactitol	−	−	+	−	−	N
Myo-inositol	−	−	−	−	−	−
2-Keto-D-gluconic acid	W	W	−	−	−	−
5-Keto-D-gluconic acid	+	+	−	−	N	−
D-Gluconic acid	−	W	−	V/−	−	N
D-Glucuronic acid	−	−	−	−	−	−
DL-Lactic acid	W	−	W	−	−	−
Succinic acid	+	W	+	V/+/S	+	V
Citric acid	+	−	+	S/+	+	−

Table 1 continued

Characteristics	1	2	3	4[a]	5[b]	6[c]
Methanol	+	+	+	+	+	+
Ethanol	+	W	+	+/W	+	+
Nitrogen assimilation						
Potassium nitrate	+	−	+	V/±	−	+/W
Sodium nitrite	+	−	+	±	−	N
Ethylamine HCl	+	+	+	+	+	N
L-Lysine HCl	+	−	+	+	+	+
Cadaverine HCl	+	+	+	+	+	N
Additional growth tests						
Vitamin-free	W	W	+	−	−	+
Growth at 25°C	+	+	+	+	N	+
Growth at 30°C	+	+	+	+	+	+
Growth at 35°C	+	+	+	+	+	−
Growth at 37°C	−	−	S	±	+	−
Growth at 40°C	−	−	−	±	+/W	−
Growth at 42°C	−	−	−	−	N	−
Growth at 45°C	−	−	−	−	−	−
0.01% Cycloheximide	−	−	−	+	N	+
0.1% Cycloheximide	−	−	−	+	+	+
50% Glucose	+	−	+	−	−	W
60% Glucose	−	−	+	−	N	N
10% NaCl/5% glucose	+	−	+	V	−	−
16% NaCl/5% glucose	−	−	−	−	−	N
Amyloid formation	−	−	−	−	−	−
Urease	−	−	−	−	−	−
Diazonium blue B	−	−	−	−	−	−
Major ubiquinone	Q7	Q7	Q7	Q7	Q7	N

1 O. phyllophila sp. nov., *2 C. chumphonensis* sp. nov., *3 C. mattranensis* sp. nov., *4 O. minuta*, *5 O. dorogensis*, *6 O. cecidiorum*, + positive, *S* slow positive, *W* weak positive, *V* variable, *L* latent, − negative, *N* no data

[a] Data from Kurtzman (1998) and Barnett et al. (2000)

[b] Data from Peter et al. (2003)

[c] Data from Glushakova et al. (2010)

L-arabinosum, L-rhamnosum, sucrosum, maltosum, α-methyl-D-glucosidum, melibiosum, lactosum, raffinosum, melizitosum, inulinum, amylum solubile, galactitolum, inositolum, acidum D-gluconicum nec acidum D-glucuronicum. Vitamina externa ad crescentiam necessaria non sunt (infirme). Crescere potest in temperatura 25, 30 et 35°C at non crescit in temperatura 37, 40, 42 nec 45°C. Crescit in 50% glucosum, et 10% NaCl/5% glucosum. Non crescit in 60% glucosum, 16% NaCl/5% glucosum, 0. 1% cycloheximido et 0.01% cycloheximido. Amylum non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Ubiquinonum majus: Q-7.

Holotypus

Stirps LN14^T isolatus ex folio, in Chumphon provincia, Thailandia. Cultura et conservatus in Collectionie Culturarum in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia ut BCC 42666^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 107780^T et Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands ut CBS 12095^T.

Description of *Ogataea phyllophila* Koowadjanakul, Jindamorakot, Yongmanitchai and Limtong sp. nov.

Growth in yeast extract malt extract (YM) broth: After 3 days at 25°C, cells are spherical to ovoid (2–3 × 2–4 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 produced on 5% malt extract agar and corn meal agar after 2 days at 15 and 25°C (Fig. 2b). Four hat-shaped ascospores are formed in a deliquescent ascus that may be produced unconjugation or conjugation between a parent cell and its bud or between independent cells and ascospores tend to agglutinate after liberation. The other phenotypic characteristics of the species are shown in Table 1. The MycoBank number is MB561002.

Holotype

LN14^T is the holotype of *O. phyllophila*. The strain was isolated from leaves of an unknown tree on Mattra island (10°04′N 99°35′E) in Chumphon Islands National Park, Chumphon province, Thailand, collected in May, 2009. The living culture from type was

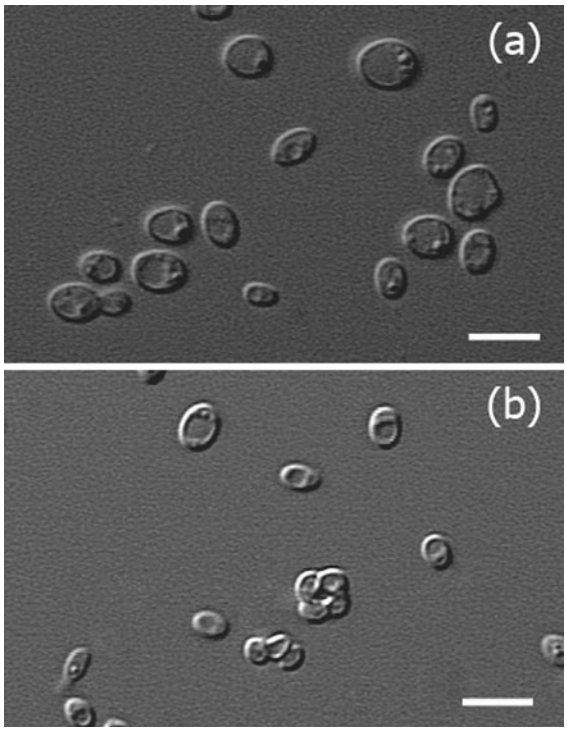

Fig. 2 *Ogataea phyllophila* sp. nov. (LN14^T) **a** Budding cells on YM agar after 3 days at 25°C and **b** hat-shaped ascospores formed on corn meal agar after 3 days at 25°C. *Scale bar* 5 μm

deposited at the BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, as BCC 42666^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107780^T and Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands as CBS 12095^T.

Etymology

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

Latin diagnosis of *Candida chumphonensis* Limtong, Koowadjanakul, Jindamorakot et Yongmanitchai sp. nov.

In medio liquido 'YM', post dies 3 ad 25°C cellulae globosae aut ovoideae (2–4 × 2–4 μm), singulae, binae, aut congregationibus, per germinationem multipolarem reproducentes. In agaro 'YM', post dies 3 ad 25°C, cultura butyrosa, cremea, glabra, umbonatus et margo undulata. Pseudohyphae et hyphae non formantur. Ascosporae non formantur. D-Glucosum (lente) fermentature at non D-galactosum, maltosum, sucrosum, α-α-trehalosum, lactosum nec raffinosum. D-Glucosum, D-ribosum (infirme), cellobiosum (infirme), salicinum (infirme), inulinum (infirme), glycerolum (infirme), erythritolum (infirme), ribitolum (infirme), D-glucitolum (infirme), D-mannitolum (infirme), acidum 2-keto-D-gluconicum (infirme), acidum 5-keto-D-gluconicum, acidum D-gluconicum (infirme), acidum succinicum (infirme), methanolum, ethanolum (infirme), ethylaminum et cadaverinum assimilantur at non D-galactosum, L-sorbosum, *N*-acetyl-D-glucosaminum, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, α-α-trehalosum, α-methyl-D-glucosidum, melibiosum, lactosum, raffinosum, melizitosum, amylum solubile, galactitolum, inositolum, acidum D-glucuronicum, acidum DL-lacticum, acidum citricum, kalium nitricum, natrium nitrosum nec L-lysinum. Vitamina externa ad crescentiam necessaria non sunt (infirme). Crescere potest in temperatura 25, 30 et 35°C at non crescit in temperatura 37, 40, 42 nec 45°C. Non crescit in 50% glucosum, 60% glucosum, 10% NaCl/5% glucosum, 16% NaCl/5% glucosum, 0. 1% cycloheximido et 0.01% cycloheximido. Amylum non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Ubiquinonum majus: Q-7.

Holotypus

Stirps LN15^T isolatus ex folio, in Chumphon provincia, Thailandia. Cultura et conservatus in Collectionie Culturarum in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia ut BCC 42667^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 107781^T et Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands ut CBS 12096^T.

Description of *Candida chumphonensis* Limtong, Koowadjanakul, Jindamorakot and Yongmanitchai sp. nov.

Growth in YM broth: After 3 days at 25°C, cells are spherical to ovoid (2–4 × 2–4 μm) and occur singly or in pairs or in groups (Fig. 3a). Budding is multilateral. Growth on YM agar: After 3 days at 25°C, the streak culture is butyrous, 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 on 5% malt extract agar, Fowell's acetate agar, Gorodkowa agar and corn meal agar after 4 weeks at 15 and 25°C. The other phenotypic characteristics of the species are shown in Table 1. The MycoBank number is MB561003.

Holotype

LN15^T is the holotype of *C. chumphonensis*. The strain was isolated from leaves of an unknown tree on Mattra island (10°04′N 99°35′E) in Chumphon Islands National Park, Chumphon province, Thailand, collected in May, 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^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107781^T and Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands as CBS 12096^T.

Etymology

The species epithet *chumphonensis* refers to Chumphon province, Thailand, where the strain was isolated.

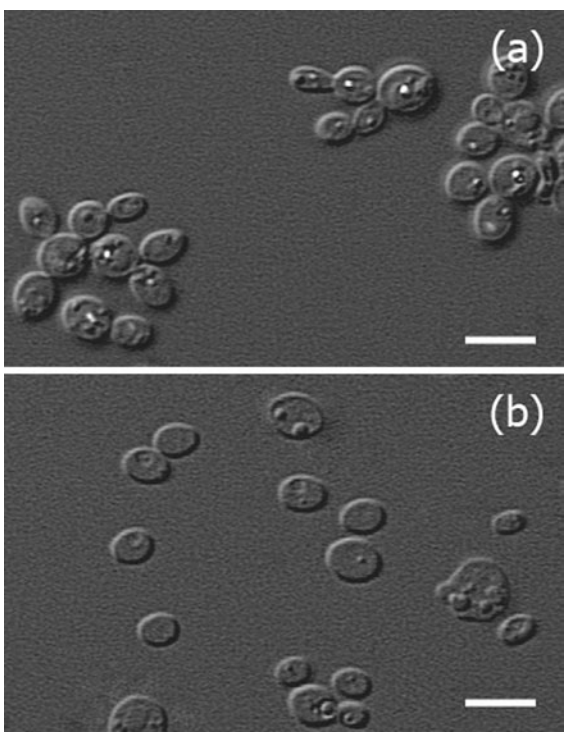

Fig. 3 Budding cells on YM agar after 3 days at 25°C of **a** *Candida chumphonensis* sp. nov. (LN15^T) and **b** *Candida mattranensis* sp. nov. (LN17^T). *Scale bar* 5 μm

Latin diagnosis of *Candida mattranensis* Limtong, Koowadjanakul, Jindamorakot et Yongmanitchai sp. nov.

In medio liquido 'YM', post dies 3 ad 25°C cellulae subglobosae aut ovoideae (1–4 × 2–5 μm), singulae aut binae, per germinationem multipolarem reproducentes. In agaro 'YM', post dies 3 ad 25°C, cultura butyrosa, cremea, glabra et margo glabra. Pseudohyphae et hyphae non formantur. Ascosporae non formantur. D-Glucosum (infirme) fermentature at non D-galactosum, maltosum, sucrosum, α-α-trehalosum, lactosum nec raffinosum. D-Gucosum, D-galactosum (infirme), L-sorbosum (lente), D-ribosum (lente), D-xylosum (infirme), α-α-trehalosum, cellobiosum, salicinum, glycerolum, erythritolum, ribitolum, D-glucitolum, D-mannitolum, galactitolum, acidum DL-lacticum (infirme), acidum succinicum, acidum citricum, methanolum, ethanolum, kalium nitricum, natrium nitrosum, ethylaminum, L-lysinum et cadaverinum at non *N*-acetyl-D-glucosaminum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, α-methyl-D-glucosidum, melibiosum, lactosum, raffinosum, melizitosum, inulinum, amylum solubile, inositolum, acidum 2-keto-D-gluconicum, acidum 5-keto-D-gluconicum, acidum D-gluconicum nec acidum D-glucuronicum. Vitamina externa ad crescentiam necessaria non sunt. Crescere potest in temperatura 25, 30, 35 et 37°C (lente) at non crescit

in temperatura 40, 42 nec 45°C. Crescit in 50% glucosum, 60% glucosum et 10% NaCl/5% glucosum. Non crescit in 16% NaCl/5% glucosum, 0. 1% cycloheximido et 0.01% cycloheximido. Amylum non formatur. Diazonium caeruleum B non respondens. Ureum non hydrolysatur. Ubiquinonum majus: Q-7.

Holotypus

Stirps LN17^T isolatus ex folio, in Chumphon provincia, Thailandia. Cultura et conservatus in Collectionie Culturarum in BIOTEC Culture Collection (BCC), National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailandia ut BCC 42668^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japonia conservatus ut NBRC 107782^T et Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands ut CBS 12097^T.

Description of *Candida mattranensis* Limtong, Koowadjanakul, Jindamorakot and Yongmanitchai sp. nov.

Growth in YM broth: After 3 days at 25°C, cells are subspherical to ovoid (1–4 × 2–5 μm) and occur singly or in pairs (Fig. 3b). 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, Gorodkowa agar and corn meal agar after 4 weeks at 15 and 25°C. The other phenotypic characteristics of the species are shown in Table 1. The MycoBank number is MB561004.

Holotype

LN17^T is the holotype of *C. mattranensis*. The strain was isolated from leaves of an unknown tree on Mattra island (10°04′N 99°35′E) in Chumphon Islands National Park, Chumphon province, Thailand, collected in May, 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 42668^T; NITE Biological Resources Center (NBRC), Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan, as NBRC 107782^T and Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands as CBS 12097^T.

Etymology

The species epithet *mattranensis* refers to Mattra island, Chumphon province, Thailand, where the strain was isolated.

Acknowledgments This work was partially supported by the Thailand Graduate Institute of Science and Technology (TGIST) grant TGIST 01-52-030. The authors would like to thank Assoc Prof Dr Hiroya Yurimoto, Laboratory of Microbial Biotechnology, Graduate School of Agriculture, Kyoto University for allowing to use the microscope for taking yeast micrographs.

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