Candida saraburiensis sp. nov. and *Candida prachuapensis* sp. nov., xylose-utilizing yeast species isolated in Thailand

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Four strains of two novel xylose-utilizing yeast species were obtained from samples collected in Thailand from decaying corn cobs (strains KU-Xs13^T and KU-Xs18), a decaying grass (KU-Xs20) and estuarine water from a mangrove forest (WB15^T). On the basis of morphological, biochemical, physiological and chemotaxonomic characteristics and sequence analysis of the D1/D2 domain of the large subunit rRNA gene, the four strains were found to represent two novel species of the genus *Candida* in the *Candida albicans*/*Lodderomyces elongisporus* clade. Three strains (KU-Xs13^T, KU-Xs18 and KU-Xs20) were assigned as a single novel species, which was named *Candida saraburiensis* sp. nov. The type strain is KU-Xs13^T (=CBS 11696^T=NBRC 106721^T=BCC 39601^T). Strain WB15^T represented another novel species of the genus *Candida* that was named *Candida prachuapensis* sp. nov. The type strain is WB15^T (=CBS 11024^T=NBRC 104881^T=BCC 29904^T).

INTRODUCTION

Lignocellulose in plant biomass is currently of interest as a substrate for the production of many microbial products such as xylitol, acetic acid and ethanol (Carvalho *et al.*, 2002; Doran-Peterson *et al.*, 2008; Hahn-Hägerdal *et al.*, 2006). D-Xylose is the second most abundant sugar in the lignocellulose of plant cell walls. It is a major sugar component of xylan, which is one of the principal polysaccharide constituents of lignocellulose. Many yeast species can assimilate xylose as a sole carbon source. In addition, many species have been tested for D-xylose fermentation (Barnett *et al.*, 2000; Kurtzman & Fell, 1998; Toivola *et al.*, 1984). Only a few yeast species have been reported to ferment D-xylose to ethanol. These include *Brettanomyces naardenensis*, *Candida lyxosophila*, *Candida intermedia*, *Candida shehatae*, *Candida tenuis*, *Pachysolen tannophilus*, *Pichia segobiensis*, *Scheffersomyces* (*Pichia*) *stipitis* (Barnett *et al.*, 2000; Kurtzman & Fell, 1998),

Candida jeffriesii, *Spathaspora passalidarum* (Nguyen *et al.*, 2006) and *Spathaspora arborariae* (Cadete *et al.*, 2009).

During investigations of xylose-utilizing yeasts and yeasts in mangrove forest ecosystems in Thailand, four xylose-utilizing yeast strains were found to represent two novel species of the genus *Candida* in the *Candida albicans*/*Lodderomyces elongisporus* clade based on the analysis of the D1/D2 domain of the large subunit (LSU) rRNA gene sequence. In this paper, three D-xylose-fermenting strains (KU-Xs13^T, KU-Xs18 and KU-Xs20) are described as *Candida saraburiensis* sp. nov. and one D-xylose-assimilating strain (WB15^T), is described as *Candida prachuapensis* sp. nov.

METHODS

Yeast isolation and characterization. Xylose-utilizing yeasts were isolated from decaying agricultural residues and soil using an enrichment technique. Each sample (1 g) was added to 50 ml D-xylose-yeast nitrogen base (YNB) broth (2 % D-xylose, 0.67 % Difco yeast nitrogen base; adjusted to pH 5.5 with 1 M HCl) supplemented with 0.025 % sodium propionate and 200 mg chloramphenicol l⁻¹ in a 250 ml Erlenmeyer flask and incubated on a rotary shaker at 25 °C for 48 h. The enrichment culture was spread on D-xylose-YNB agar and incubated at 25 °C. After incubation, yeast colonies were picked

Abbreviation: LSU, large subunit.

The GenBank/EMBL/DBJ accession numbers for the sequence of the D1/D2 domain of the LSU rRNA gene of strains KU-Xs13^T and WB15^T are AB534915 and AB439258, respectively.

A supplementary figure is available with the online version of this paper.

and purified by streaking on yeast extract malt extract (YM) agar (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1 % glucose and 1.5 % agar). Yeasts in estuarine water were isolated by a membrane filtration technique according to the method of Limtong *et al.* (2007a). 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 domain of the LSU rRNA gene were determined from PCR products amplified from genomic DNA that was extracted from yeast cells by using a slightly modified version of the method described by Lachance *et al.* (1999). The D1/D2 domain of the LSU rRNA gene was amplified by PCR with the forward primer NL-1 and the reverse primer NL-4 (O'Donnell, 1993). The PCR product was checked by agarose gel electrophoresis and purified by using the QIAquick purification kit (Qiagen). The purified PCR product was submitted to Macrogen Inc. (Korea) for sequencing with the external primers NL-1 and NL-4. 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 CLUSTAL_X version 1.81 multiple alignment program (Thompson *et al.*, 1997). A phylogenetic tree was constructed from the evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980) using the neighbour-joining method (Saitou & Nei, 1987). Confidence levels of the clades were estimated from bootstrap analysis (1000 replicates) (Felsenstein, 1985).

Phenotypic characterization and xylose fermentation tests. The novel strains were characterized morphologically, biochemically and physiologically according to the standard methods described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula following the method of Nakase & 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 200 ml yeast extract peptone glucose (YPG) broth (1 % yeast extract, 2 % peptone and 2 % glucose) on a rotary shaker at 28°C for 72 h and purified according to the methods described by Yamada & Kondo (1973) and Kuraishi *et al.* (1985). The isoprenologues were identified by HPLC as described previously (Limtong *et al.*, 2007b).

The fermentation of D-xylose was tested by cultivation in 50 ml 4 % D-xylose yeast extract peptone (YP) broth (4 % D-xylose, 2 % peptone and 1 % yeast extract) in a 250 ml Erlenmeyer flask on a rotary shaker at 150 r.p.m. and 30°C . Ethanol production and growth were measured at every 24 h for 120 h. Ethanol was analysed by GC (GC-9A; Shimadzu) with n-propanol as the internal standard and the cell concentration was determined at OD₆₆₀. *Scheffersomyces stipitis* CBS 5773^T was used as a positive control and *Saccharomyces cerevisiae* M30 was used as a negative control.

RESULTS AND DISCUSSION

Species delineation, classification and ecology

A total of 133 xylose-utilizing yeast strains were isolated from 84 samples of decaying agricultural residues and soils collected in various areas of Thailand. Seventy-eight strains were assigned to 18 recognized species in nine genera of the phylum Ascomycota (*Barnettozyma californica*, *Candida blankii*, *Candida coipomoensis*, *Candida maltosa*, *Candida membranifaciens*, *Candida pseudointermedia*, *Candida pyralidae*, *Candida solani*, *Candida tropicalis*, *Debaryomyces hansenii* var. *fabryi*, *D. nepalensis*, *Geotrichum silvicola*,

Lindnera rhodanensis, *Pichia caribbica*, *Pichia kudriavzevii*, *Saturnispora saitoi*, *Sporopachydermia lactativora* and *Zygoascus hellenicus*). Forty-two strains were assigned to nine recognized species in two genera of the phylum Basidiomycota (*Cryptococcus* cf. *podzolicus*, *Cryptococcus heveanensis*, *Cryptococcus humicola*, *Cryptococcus laurentii*, *Cryptococcus terrestris*, *Trichosporon asahii*, *T. moniliiforme*, *T. mycotoxinivorans* and *T. terricola*). Four strains were similar to three undescribed species and nine strains were found to represent seven novel species.

All 133 strains were tested for D-xylose fermentation using shake-flask cultivation. The results revealed that only eight strains could ferment D-xylose to ethanol. These were two strains of *Zygoascus hellenicus* (SN1-1 and SN1-4), three strains of *Candida blankii* (A6-1, A8-1 and A8-2) and three strains of a novel species (KU-Xs13^T, KU-Xs18 and KU-Xs20). Among the xylose-fermenting strains of the novel species, two strains (KU-Xs13^T and KU-Xs18) were isolated from decaying corncobs and one strain (KU-Xs20) was obtained from decaying grass collected in Saraburi province, Thailand. The sequences of the D1/D2 domain of the LSU rRNA gene of strains KU-Xs13^T and KU-Xs20 were identical and differed from the sequence of strain KU-Xs18 by only one gap out of 571 nt. With respect to their closest species in term of pairwise sequence similarity, the three new strains differed by 2.3 % nucleotide substitutions (13 nt substitutions and one to two gaps out of 571 nt) from the type strain of *Candida viswanathii* and by 1.9 % nucleotide substitutions (11 nt substitutions and one to two gaps out of 571 nt) from the type strain of *Candida lodderae*. *Candida viswanathii* and *Candida lodderae* were reported to be synonymous since they differ from each other by only two nucleotides in the D1/D2 domain of their LSU rRNA gene sequences (Kurtzman & Robnett, 1997) and belong to the *Candida albicans/Lodderomyces elongisporus* clade. According to Kurtzman & Robnett (1998), yeast strains showing nucleotide substitutions of greater than 1 % in the D1/D2 domain of the LSU rRNA gene are usually representative of different species. The phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene further demonstrated that the three new strains (KU-Xs13^T, KU-Xs18 and KU-Xs20) formed a clade that was distinct from *Candida viswanathii*, *Candida lodderae* and other related species in the *Candida albicans/Lodderomyces elongisporus* clade (Fig. 1).

Strain WB15^T was isolated from estuarine water collected from a mangrove forest in Prachuap Khiri Khan province, Thailand, during another investigation of yeasts in mangrove forest ecosystems. Strain WB15^T showed the ability to assimilate but not ferment D-xylose, and represented another novel species. The closest species in terms of pairwise sequence similarity of the D1/D2 domain of the LSU rRNA gene were *Candida viswanathii* and its synonymous species, *Candida lodderae*, but with 2.3 % nucleotide substitutions (13 nt substitutions out of 570 nt) and 1.9 % nucleotide substitutions (11 nt substitutions out

Fig. 1. Phylogenetic tree based on the sequences of the D1/D2 domain of the LSU rRNA gene showing the positions of *Candida saraburiensis* sp. nov. (KU-Xs13^T, KU-Xs18 and KU-Xs20) and *Candida prachuapensis* sp. nov. (WB15^T) with respect to closely related species. The phylogenetic tree was constructed from evolutionary distance data with Kimura's two-parameter correction (Kimura, 1980), using the neighbour-joining method. Numbers indicate the percentages of bootstrap sampling, derived from 1000 samples. Bar, 0.02 K_{nuc} .

of 570 nt), respectively. The sequence of strain WB15^T differed from those of strains KU-Xs13^T, KU-Xs18 and KU-Xs20 by 2.1% nucleotide substitutions (12 nt substitutions and one to two gaps out of 571 nt). In the phylogenetic tree (Fig. 1), strain WB15^T was placed in the *Candida albicans*/*Lodderomyces elongisporus* clade at a separate branch from *Candida viswanathii*, *Candida lodderae* and strains KU-Xs13^T, KU-Xs18 and KU-Xs20.

Cells of the novel strains proliferated by multilateral budding and were negative for Diazonium blue B and urease reactions. Starch-like compounds were not produced in common with the other members of *Candida albicans*/*Lodderomyces elongisporus* clade. Ascospores were not produced from individual strains or strains paired on 5% malt extract agar, Fowell's acetate agar, Gorodkova agar or YM agar after 5 weeks at 25 °C. These characteristics matched well with those of other members the genus *Candida*. Therefore, it was concluded that the four new strains represented two novel species. Strains KU-Xs13^T, KU-Xs18 and KU-Xs20 were assigned to *Candida saraburiensis* sp. nov. with strain KU-Xs13^T as the type strain. Strain WB15^T was proposed as the type strain of *Candida prachuapensis* sp. nov.

Candida saraburiensis sp. nov. and *Candida prachuapensis* sp. nov. could be distinguished from each other and from *Candida viswanathii* and *Candida lodderae*, their closest recognized species, by many characteristics (as shown in Table 1).

The *Candida albicans*/*Lodderomyces elongisporus* clade consists of more than 20 species and includes some medically important species such as *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis* and *Candida viswanathii*. The members of this clade have been isolated not only from humans, but also from soil, water, plants, insects and from industrial processes (Ji *et al.*, 2009; Kurtzman *et al.*, 2001; Meyer *et al.*, 1998; Nguyen *et al.*, 2007; Rao *et al.*, 2007; Suh *et al.*, 2008; Vallini *et al.*, 1997). The two novel species of the genus *Candida* described in this study were obtained from different habitats. Strains of *Candida saraburiensis* sp. nov. were isolated from decaying corn cobs and a decaying grass, while *C. prachuapensis* sp. nov. was isolated from estuarine water from a mangrove forest. The two novel species were closely related to *Candida viswanathii*, but it is not known whether they are pathogenic.

Xylose fermentation

Ethanol fermentation from xylose was determined for the four strains of the two novel species by shaking-flask cultivation using 4% D-xylose-YP broth. The results revealed that all three strains of *Candida saraburiensis* sp. nov. produced ethanol at 3.1–3.6 g l⁻¹ at 72 h, but all ethanol was either consumed or evaporated after 96 h (see Supplementary Fig. S1 in IJSEM Online). *Candida prachuapensis* sp. nov. WB15^T did not produce ethanol from xylose and displayed similar properties to the non-D-

Table 1. Differential phenotypic characteristics of *Candida saraburiensis* sp. nov. and *Candida prachuapensis* sp. nov., *Candida viswanathii* and *Candida lodderae*

Taxa: 1, *C. saraburiensis*; 2, *C. prachuapensis*; 3, *C. viswanathii* and *C. lodderae*. +, Positive; w, weak; v, variable; –, negative; ND, not determined. Data for *C. viswanathii* and *C. lodderae* were taken from Meyer *et al.* (1998) and Barnett *et al.* (2000).

Characteristic	1	2	3
Fermentation of:			
D-Galactose	w	–	v
Maltose	w	+	+
Sucrose	+	+	v
Trehalose	+	–	v
Assimilation of:			
D-Galactose	+	–	+
L-Sorbose	+	+	v
D-Ribose	–	–	v
L-Arabinose	–	–	v
Cellobiose	w	+	+
Salicin	w	+	v
Melibiose	–	w	–
Raffinose	–	+	–
Inulin	–	+	–
Soluble starch	–	+	+
5-Ketogluconic acid	–	+	+
DL-Lactic acid	+	+	–
Growth at/with			
Vitamin-free medium	+	+	–
0.01 % Cycloheximide	–	–	+
0.1 % Cycloheximide	–	–	+
60 % Glucose	+	+	–
16 % NaCl/5 % glucose	+	ND	–
Acid formation from glucose	+	ND	–
40 °C	+	+	v
42 °C	–	w	–

xylose fermenting reference yeast, *Saccharomyces cerevisiae* M30. In the same experiment *Scheffersomyces stipitis* CBS 5773^T, the D-xylose-fermenting control yeast strain, produced the highest ethanol concentration at 8.4 g l^{–1} after 72 h, although ethanol was also reduced to 6.3 g l^{–1} and 2.2 g l^{–1} after 96 h and 120 h, respectively. In contrast, when D-xylose fermentation was tested by the standard methods described by Yarrow (1998), i.e. the Durham tube fermentation test, all four strains gave a negative result.

Latin diagnosis of *Candida saraburiensis* Nitiyon, Boonmak, Am-In, Jindamorakot, Kawasaki, Yongmanitchai et Limtong sp. nov.

In agaro YM post dies 3 ad 25 °C cellulae ovoidae aut cylindricae, (2–6 × 4–10 µm), singulae, binae, aut pseudohyphae fiunt, per germinationem multipolarem reproducentes. Cultura butyrosa, cremea, glabra, seminitidae, centrum colonia altum, margine glabra. In agaro YM post dies 5 ad 25 °C

pseudohyphae formantur, hyphae non formantur. Ascosporae non formantur. D-Glucosum, D-galactosum (infirmum), maltosum (infirmum), sucrosus et α,α-trehalosus fermentantur at non melibiosum, lactosum nec raffinolum. D-Glucosum, D-galactosum, L-sorbosum, N-acetyl-D-glucosaminum, D-xylosum, sucrosus, maltosum, α,α-trehalosus, methyl α-D-glucosidum, cellobiosum (infirmum), salicinum (infirmum), melezitolum, glycerolum, ribitolum, D-glucitolum, D-mannitolum, D-glucono-1,5-lactonum, acidum 2-ketogluconicum, acidum D-gluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, ethanolum, ethylaminum, L-lysinum et cadaverinum assimulantur. D-Ribosum, L-arabinosum, D-arabinosum, L-rhamnosum, melibiosum, lactosum, raffinolum, inulinum, amylum solubile, erythritolum, galactitolum, inositolum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, methanolum, kalium nitricum nec natrum nitrosus non assimulantur. Vitamina externa crescentiae non necessaria sunt. Crescit postest in temperatura 40 °C at non in 42 °C. Non crescit in 0.01 % cycloheximido nec 0.1 % cycloheximido. Crescit in 50 % glucosum, 60 % glucosum, 10 % natrii chloridum/5 % glucosum et 16 % natrii chloridum/5 % glucosum. Amylum non formatur. Ureum non hydrolysatur. Diazonium caerulum B non respondens. Ubiquinonum majus: Q-9.

Typus stirpis, KU-Xs13^T(=CBS 11696^T=NBRC 106721^T=BCC 39601^T) isolatus digesta granum core, Saraburi provincia, Thailandica, conservatur in collectionibus utrarum quas Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands), NITE Biological Resource Center (Chiba, Japan) et BIOTEC Culture Collection, National Centre for Genetic Engineering and Biotechnology, Thailand (Pathumthani, Thailand) deposita est.

Description of *Candida saraburiensis* Nitiyon, Boonmak, Am-In, Jindamorakot, Kawasaki, Yongmanitchai & Limtong sp. nov.

Candida saraburiensis (sa.ra.bu.ri.en'sis. N.L. fem. adj. *saraburiensis* of or belonging to Saraburi, Thailand).

After growth on YM agar for 3 days at 25 °C, cells are ovoid to cylindrical (2–6 × 4–10 µm) and occur singly, in pairs or in short chains (Fig. 2). Budding is multilateral. Colonies are butyrous, cream-coloured, smooth, semiglistening, umbonate and have an entire margin. Pseudohyphae are formed, but true hyphae are not formed in slide culture on cornmeal agar after 5 days at 25 °C. Ascospores are not produced from individual strain or strains paired on 5 % malt extract agar, Fowell's acetate agar, Gorodkova agar or YM agar after 5 weeks at 25 °C. The major ubiquinone is Q-9. Tests for fermentation of D-glucose, D-galactose (weak), maltose (weak), sucrose and α,α-trehalose are positive, but negative results are obtained for melibiose, lactose and raffinose. D-Glucose, D-galactose, L-sorbose, N-acetyl-D-glucosamine, D-xylose, sucrose, maltose, α,α-trehalose, methyl α-D-glucoside, cellobiose (weak), salicin (weak), melezitose, glycerol, ribitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, 2-ketogluconic acid, D-gluconic acid, DL-lactic acid, succinic acid, citric

Fig. 2. Images of cells of *Candida saraburiensis* sp. nov. KU-Xs13^T. (a) Cells grown on YM agar after 3 days at 25 °C. (b) Pseudohyphae produced on cornmeal agar after 5 days at 25 °C. Bars, 10 µm.

acid, ethanol, ethylamine-HCl, L-lysine-HCl and cadaverine are assimilated, but D-ribose, L-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, raffinose, inulin, soluble starch, erythritol, galactitol, inositol, 5-ketogluconic acid, D-glucuronic acid, D-galacturonic acid, methanol, potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Growth at 40 °C is positive, but no growth at 42 °C. No growth with 0.01 % or 0.1 % cycloheximide. Growth is positive on media containing 50 % (w/v) glucose, 60 % (w/v) glucose, 10 % (w/v) sodium chloride/5 % (w/v) glucose or 16 % (w/v) sodium chloride/5 % (w/v) glucose. Starch-like compounds are not produced. Diazonium blue B and urease reactions are negative.

The type strain KU-Xs13^T (=CBS 11696^T=NBRC 106721^T=BCC 39601^T) was isolated from decaying corn-cobs collected in Saraburi province, Thailand.

Latin diagnosis of *Candida prachuapensis* Boonmak, Nitiyon, Am-In, Jindamorakot, Kawasaki, Yongmanitchai et Limtong sp. nov.

*In agar*o YM post dies 3 ad 25 °C cellulae ovoidae aut cylindricae, (3–6 × 3–13 µm), singulae aut binae, per germinationem multipolarem reproducentes. Cultura butyrosa, crenea, centrum colonia altum et margine filamentum. *In agar*o farina Zea mays post dies 7 ad 25 °C pseudohyphae

formantur, hyphae non formantur. Ascospores non formantur. D-glucosum, sucrosum et maltosum fermentantur at non D-galactosum, lactosum, raffinolum nec α,α-trehalosum. D-Glucosum, L-sorbozum, sucrosum, maltosum, cellobiosum, melibiosum (infirme), raffinolum, melezitosum, inulinum, amyllum solubile, D-xylosum, ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, methyl α-D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, N-acetyl-D-glucosaminum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-gluconicum, D-glucono-1,5-lactonum, xylitolum, ethylaminum, L-lysinum et cadaverinum assimilantur. D-Galactosum, lactosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, erythritolum, galactitolum, inositolum, methanolum, acidum D-glucuronicum, acidum D-galacturonicum, kalium nitricum nec natrum nitrosum non assimilantur. Vitamina externa crescentiae non necessar sunt. Crescit in temperatura 42 °C (infirme) at non in 45 °C. Non crescit in 0.01 % cycloheximido nec 0.1 % cycloheximido. Crescit in 50 % glucosum, 60 % glucosum et 10 % natrii chloridum/5 % glucosum. Amyllum non formatur. Ureum non hydrolysat. Diazonium caeruluum B non respondens. Ubiquinonum majus: Q-8.

Typus stirpis WB15^T (=CBS 11024^T=NBRC 104881^T=BCC 29904^T) isolatus aqua, Prachuap Khiri Khan provincia, Thailandica, conservatur in collectionibus culturarum quas Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands), NITE Biological Resource Center (Chiba, Japan) et BIOTEC Culture Collection, National Centre for Genetic Engineering and Biotechnology, Thailand (Pathumthani, Thailand) deposita est.

Description of *Candida prachuapensis* Boonmak, Nitiyon, Am-In, Jindamorakot, Kawasaki, Yongmanitchai & Limtong sp. nov.

Candida prachuapensis (pra.chuap.en'sis. N.L. fem. adj. *prachuapensis* of or belonging to Prachuap Khiri Khan, Thailand).

After growth on YM agar for 3 days at 25 °C, cells are ovoid to cylindrical (3–6 × 3–13 µm) and occur singly, in pairs or in short chains (Fig. 3). Budding is multilateral. Colonies are butyrous, cream-coloured and umbonate with a mycelial margin. Pseudohyphae are formed, but true hyphae are not formed in slide culture on cornmeal agar after 7 days at 25 °C. Ascospores are not produced on 5 % malt extract agar, Fowell's acetate agar, Gorodkova agar or YM agar after 6 weeks at 15 °C. The major ubiquinone is Q-8. Tests are positive for fermentation of D-glucose, sucrose and maltose, but negative results for D-galactose, lactose, raffinose and α, α-trehalose. D-Glucose, L-sorbose, sucrose, maltose, cellobiose, melibiose (weak), raffinose, melezitose, inulin, soluble starch, D-xylose, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, N-acetyl-D-glucosamine, 2-ketogluconic acid, 5-ketogluconic acid, D-gluconic acid, D-glucono-1,5-lactone, xylitol, ethylamine-HCl, L-lysine-HCl and cadaverine are assimilated,

Fig. 3. Images of cells of *Candida prachuapensis* sp. nov. WB15^T. (a) Cells grown on YM agar after 3 days at 25 °C. (b) Pseudohyphae produced on cornmeal agar after 5 days at 25 °C. Bars, 10 µm.

but D-galactose, lactose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, galactitol, inositol, methanol, D-glucuronic acid, D-galacturonic acid, potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Weak growth at 42 °C, but no growth at 45 °C. Growth with 0.01 % and 0.1 % cycloheximide is negative. Growth on medium containing 50 % (w/v) glucose, 60 % (w/v) glucose or 10 % (w/v) sodium chloride/5 % (w/v) glucose is positive. Starch-like compounds are not produced. Diazonium blue B and urease reactions are negative.

The type strain, WB15^T (=CBS 11024^T=NBRC 104881^T=BCC 29904^T), was isolated from water collected from a mangrove forest in Prachuap Khiri Khan province, Thailand.

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