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ORIGINAL PAPER

Four novel yeasts from decaying organic matter: *Blastobotrys robertii* sp. nov., *Candida cretensis* sp. nov., *Candida scorzettiae* sp. nov. and *Candida vadensis* sp. nov.

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Abstract Four novel yeast species are described, two from decaying mushrooms, viz. *Candida cretensis* and *Candida vadensis*, and two from rotten wood, viz. *Blastobotrys robertii* and *Candida scorzettiae*. Accession numbers for the CBS and ARS Culture Collections, and GenBank accession numbers for the D1/D2 domains of the large subunit of ribosomal DNA are: *B. robertii* CBS 10106^T, NRRL Y-27775, DQ839395; *C. cretensis* CBS 9453^T, NRRL Y-27777, AY4998861 and DQ839393; *C. scorzettiae* CBS 10107^T, NRRL Y-27665, DQ839394; *C. vadensis* CBS 9454^T, NRRL Y-27778, AY498863 and DQ839396. The GenBank accession number for the ITS region of *C. cretensis* is AY498862 and that for *C. vadensis* is AY498864. *C. cretensis* was the only species of the four that displayed fermentative activity. All four type strains grew on *n*-hexadecane. *C. scorzettiae* is the only one of the new species that assimilates some phenolic compounds, viz. 3-hydroxy derivatives of benzoic, phenylacetic and cinnamic acids, but not the corresponding 4-hydroxy acids. This is indicative of an operative gentisate pathway.

Keywords *Blastobotrys robertii* sp. nov. · *Candida cretensis* sp.nov. · *Candida scorzettiae* sp.nov. · *Candida vadensis* sp.nov. · Gentisate pathway · Molecular systematics

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Introduction

Since the pioneering study of Starkey and Henry (1927), many yeast species have been isolated from soil where they play a role in the carbon cycle. Due to vigorous competition of bacteria and filamentous fungi, the concentrations of assimilable organic compounds in soil are low. In view of this, it is interesting to know which yeasts do inhabit decaying organic matter that contains abundant amounts of assimilable carbon compounds. Recently, two studies have been undertaken. Middelhoven (2004, 2006) analysed the yeast flora of two decaying mushrooms as well as that of several samples of rotten wood. In addition to known species, both studies yielded two anamorphic ascomycetous species that could not be identified. In the present study these are described as the following four novel species, viz. *Candida cretensis* and *Candida vadensis* from

decaying mushrooms and *Blastobotrys robertii* and *Candida scorzettiae* from rotten wood.

Materials and methods

Morphological and physiological characteristics

The strains were examined for morphological and physiological properties with standard yeast identification methods (Yarrow 1998; Barnett et al. 2000). Utilization of carbon (10 g per litre) and nitrogen sources (40 mM assimilable N) in liquid Difco Yeast Nitrogen Base and Yeast Carbon Base (2 ml in culture tubes of 18 mm diameter) was tested at 25°C on a rotary shaker at a speed of 100 rpm. Utilization of nitrite was tested by the auxanographic technique. The pH of growth media was adjusted to 5.5 if required, but the pH of media with galacturonic or quinic acid was not adjusted, which is in agreement with the laboratory practice of CBS Utrecht (D.Yarrow, personal communication; cf. Middelhoven 1997). Growth on L-malic, D-galactaric (mucic, 2.5 g per litre), D-glucaric (saccharic) and tartaric acids was tested at pH 4.0. Assimilation of non-traditional carbon compounds was studied according to Middelhoven (2006).

Ribosomal DNA (rDNA) sequencing and sequence analysis

Methods for isolation of nuclear DNA and amplification of LSU rDNA domains 1 and 2 (D1/D2) by the polymerase chain reaction (PCR) were previously given (Kurtzman and Robnett 1998, 2003). The D1/D2 amplicons were sequenced using the ABI TaqDyeDeoxy Terminator Cycle sequencing kit and an ABI Model 3730 automated DNA sequencer (Applied Biosystems, Inc., Foster City, California) following the manufacturer's instructions. GenBank accession numbers for LSU D1/D2 sequences of the new species described here, as well as those of reference species, are given in Figs. 1, 2, 3, 4. Sequences were visually aligned and regions of uncertain alignment were excluded from analysis. Phylogenetic relatedness among species was determined using the maximum parsimony (MP) and neighbor-joining (NJ) algorithms of PAUP* 4.063a (Swofford 1998). The Kimura-2 parameter distance correction was used for NJ analyses.

Results and discussion

On the basis of phylogenetic analysis of nucleotide sequences from D1/D2 LSU rDNA, the four proposed new species, which occur in four separate clades, are seen to differ from all known ascomycetous yeasts (Figs. 1–4).

Latin diagnosis of *Blastobotrys robertii* Middelhoven et Kurtzman sp. nov.

Post 3 dies in medio liquido GPYM dicto cellulae ovales et pseudomycelium praesentes. Pellicula crassa, alba, rugosa et nonnullum sedimentum formatae (praesentes etiam post 30 dies). In agaro maltoso (5%) post 3 dies 25°C colonia alba, pulverulenta, membranacea, margine fimbriata. Cellulae gemmantes et pseudohyphae et hyphae verae praesentes. Cellulae multilateraliter et e denticulis polaribus gemmantes, singulae vel binae cohaerentes, vulgo elongata, utrinque angustatae, raro ellipsoideae, 4–12 × 2.3–3.0 µm. Cellulae hypharum nonnumquam fragmentatae. Ascosporae absentes. Fermentatio nulla. Glucosum, galactosum, sucrosum, maltosum, trehalosum, cellobiosum, salicinum (lente), arbutinum (lente), melibiosum, lactosum, raffinosum, amylum solubile, mannitolum, acidum succinicum, acidum citricum (lente) assimilantur. Sorbosum, glucosaminum, ribosum, xylosum, L-arabinosum, D-arabinosum, rhamnosum, melezitosum, inulinum, glycerolum, erythritolum, ribitolum, xylitolum, arabinitolum, glucitolum, galactitolum, inositolum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum gluconicum, acidum glucuronicum, acidum galacturonicum, acidum lacticum, methanolum et aethanolum non assimilantur. Aethylaminum, lysinum, cadaverinum, D-prolinum, putrescinum assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, glucosaminum, imidazolum, D-tryptophanum. Thiaminum externum crescentiae necessarium. Reactio Diazonii Coeruleaei B negativa. Ureum

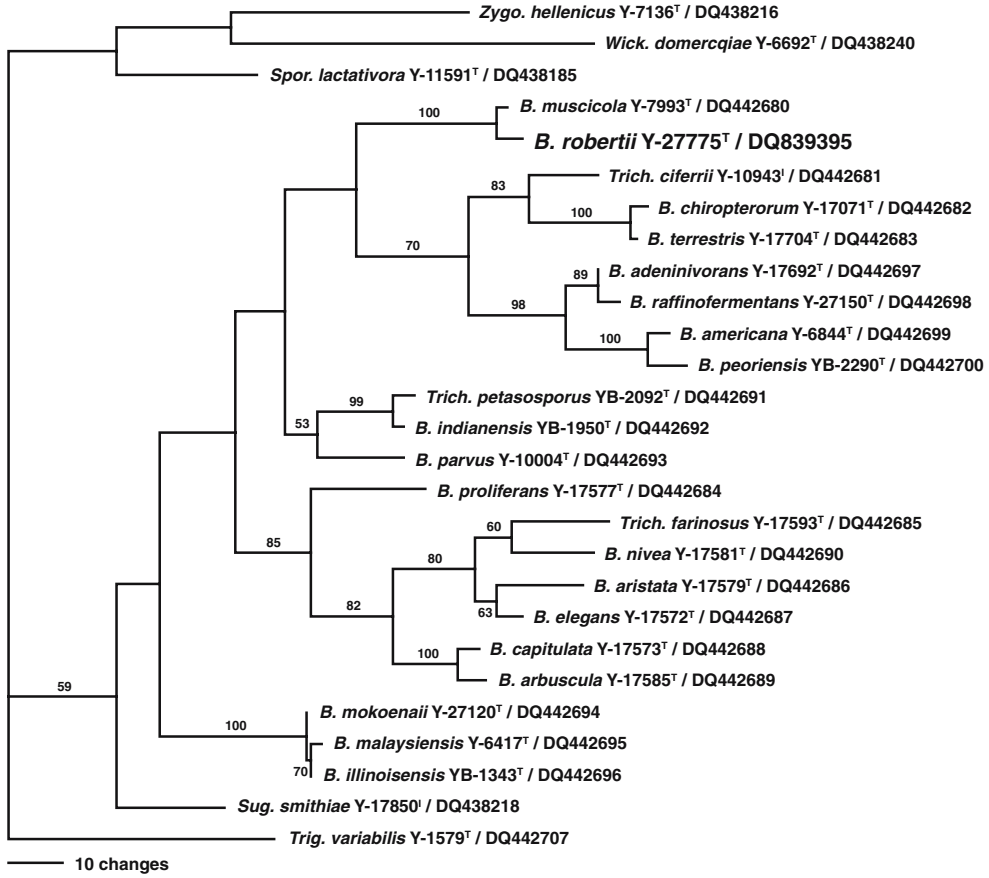


Fig. 1 Placement of *Blastobotrys robertii* in the *Trichomonascus* clade as represented by the single most parsimonious tree from MP analysis of LSU D1/D2 rDNA nucleotide sequences, Consistency index (CI) = 0.517, retention index (RI) = 0.640, rescaled consistencey index (RC) = 0.331, homoplasy index (HI) = 0.483, tree length = 689. Bootstrap values (>50%) are from 1,000 determinations. *Trigonopsis variabilis* was the designated outgroup species. Abbreviations: *B., Blastobotrys; Spor., Sporopachydermia; Sug., Sugiyamaella; Trich., Trichomonascus; Trig., Trigonopsis; Wick., Wickerhamiella; Zygo., Zygoascus*

non finditur. Crescit 32°C neque 35°C. Materia amyloidea non formatur. Typus CBS 10106^T isolatus ex *Pino sylvestri.* Lyophilus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Characteristics of *Blastobotrys robertii* sp. nov.

After 3 days at 25°C in liquid GPYM growth medium, ovate cells and pseudomycelium are present. A thick, white, wrinkled pellicle and some sediment are formed. After one month these are still present. The slant culture on 5% malt extract agar after 3 days at 25°C is white and powdery, membranous and with a mycelial fringe. Budding cells, pseudohyphae and true hyphae are present. Budding is multilateral as well as from short denticles that often form on the tips of elongated cells. Cells are single or in pairs. The cells are usually elongate and tapered, seldom ellipsoidal and measure 4–12 × 2.3–3.0 μm (Fig. 5). After 7 days at 25°C, the Dalmau plate on morphology agar showed aerobic colonies that are flat with a raised centre, white and somewhat powdery in appearance, and the margin is entire. Growth under the cover glass consists primarily of true hyphae with blastoconidia arising on short denticles (Fig. 6). Hyphal cells may detach at the septa. No ascospores were seen in slant cultures on 5% malt extract agar and YM agar incubated

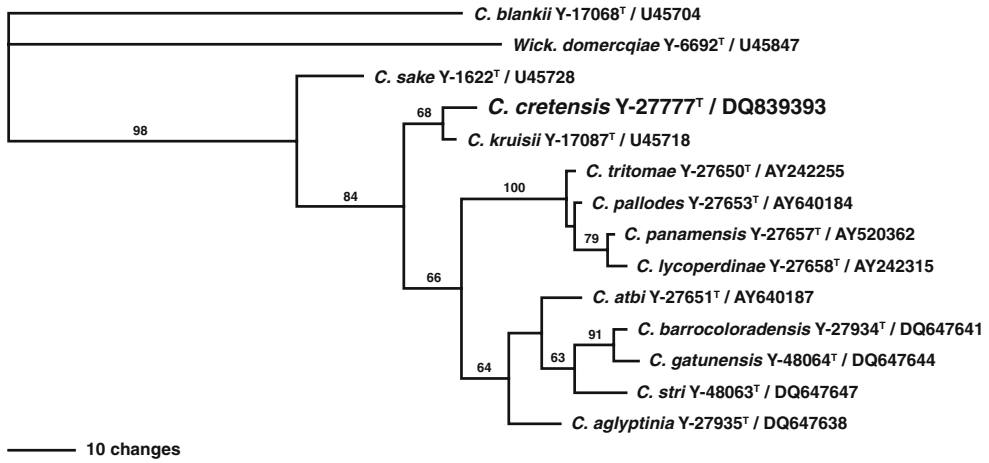


Fig. 2 One of two most parsimonious trees from MP analysis of nucleotide sequences from LSU D1/D2 rDNA showing phylogenetic placement of *Candida cretensis* in the *C. sake*/*C. kruisii* clade. CI = 0.803, RI = 0.690, RC = 0.554, HI = 0.197, tree length = 315. Bootstrap values (>50%) from 1,000 determinations are given at nodes. *Candida blankii* was the designated outgroup species. Abbreviations: *C., Candida; Wick., Wickerhamiella*

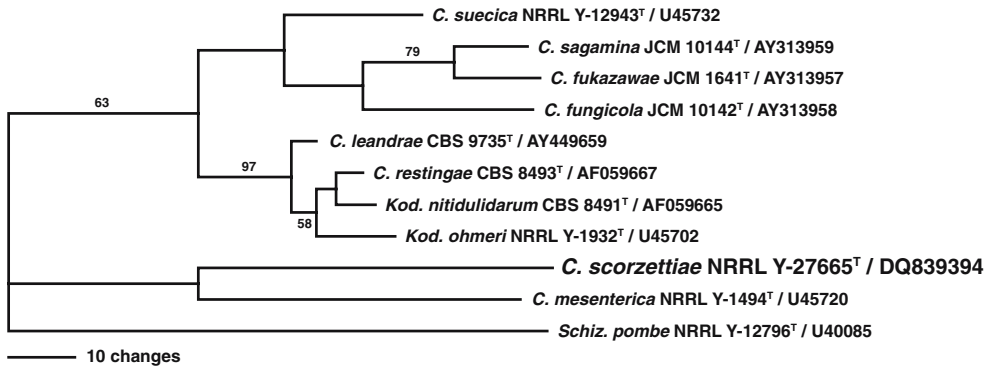


Fig. 3 Placement of *Candida scorzettiae* near *C. mesenterica* as represented by the single most parsimonious tree from MP analysis of LSU D1/D2 rDNA nucleotide sequences. CI = 0.755, RI = 0.500, RC = 0.378, HI = 0.245, tree length = 400. Bootstrap values (>50%) are from 1,000 determinations. *Schizosaccharomyces pombe* was the designated outgroup species. Abbreviations: *C., Candida; Kod., Kodamea; Schiz., Schizosaccharomyces*

at 15°C and 25°C for up to 4 weeks. Growth responses of strain CBS 10106^T to commonly used carbon and nitrogen compounds and other characteristics are shown in Table 1. Sugars were not fermented.

Origin and deposits

The type strain was isolated from rotten pine wood (*Pinus sylvestris* L.) in Wageningen, The Netherlands in August 2002 and originally was assigned strain number PiB-71 (Middelhoven 2006). It was deposited in two culture collections, viz. CBS (Accession number 10106^T) and NRRL (Accession number Y-27775). The ribosomal DNA sequence of the D1/D2 region was deposited at GenBank under the accession number DQ839395. The MycoBank accession number for NRRL Y-27775 (CBS 10106) is MB 510429.

Etymology

The epithet *robertii* is chosen in honour of Dr. Vincent Robert of CBS Utrecht who placed all

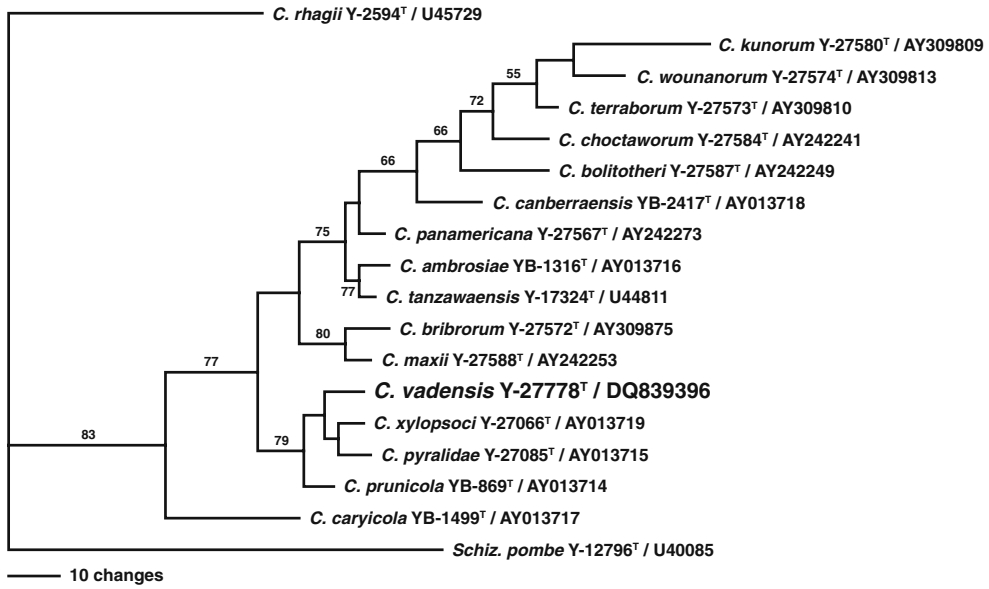


Fig. 4 Placement of *Candida vadensis* among representative species of the *C. tanzawaensis* clade from 1 to 6 most parsimonious trees from MP analysis of LSU D1/D2 rDNA nucleotide sequences. CI = 0.639, RI = 0.667, RC = 0.426, HI = 0.361, tree length = 471. Bootstrap values (>50%) are from 1,000 determinations. *Schizosaccharomyces pombe* was the designated outgroup species. Abbreviations: *C., Candida; Schiz., Schizosaccharomyces*

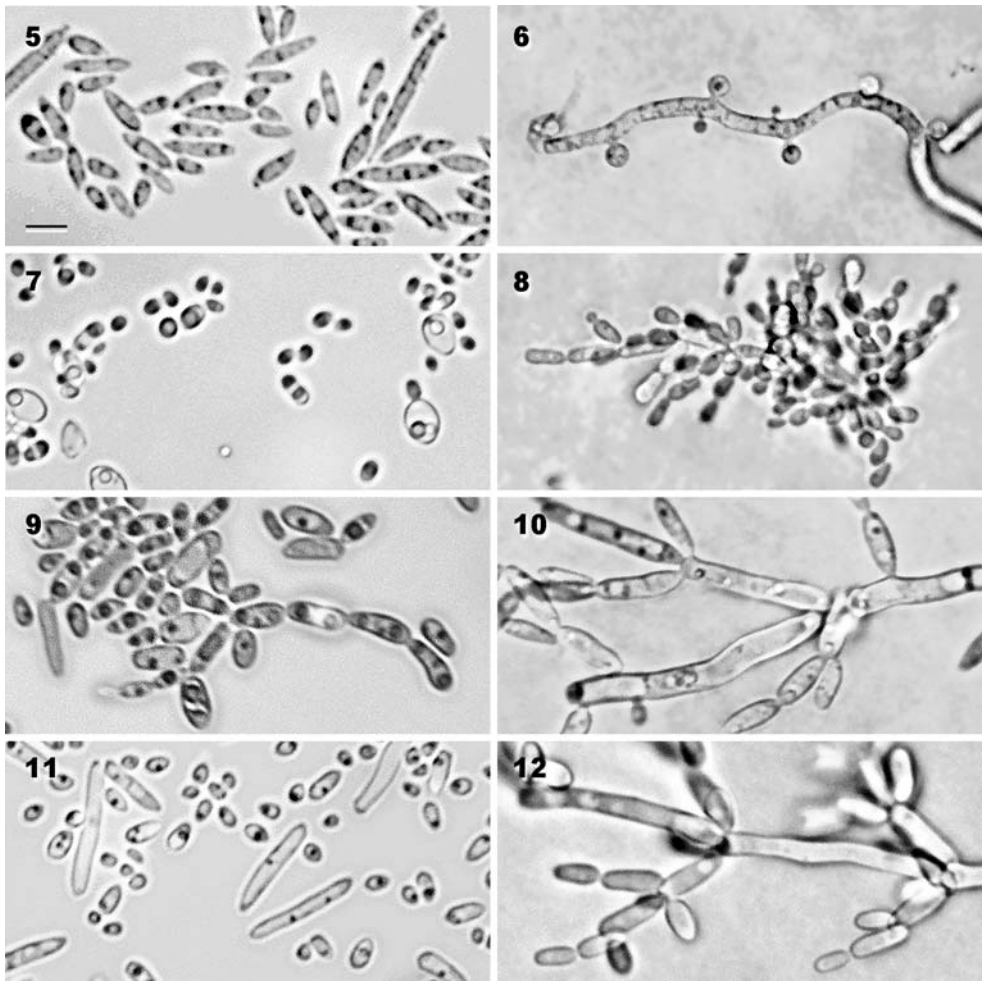
available taxonomic and physiological data for known yeast species on the Internet.

Phylogeny and species recognition

B. robertii is most closely related to *B. muscicola* (Kurtzman 2007; Kurtzman and Robnett 2007), and the two species differ by seven substitutions in the D1/D2 domains of LSU rDNA (Fig. 1). *B. robertii* and *B. muscicola* give similar reactions on standard growth tests, but they can be separated physiologically because *B. robertii* assimilates hexadecane, but does not grow at 37°C, whereas *B. muscicola* does not assimilate hexadecane but does grow at 37°C. Species of *Blastobotrys* have been isolated from air, soil, plant debris, fungi and other substrates, consequently, it is unclear whether there is habitat specificity for any of the species (de Hoog and Smith 1998; Kurtzman 2007).

Latin diagnosis of *Candida cretensis* Middelhoven et Kurtzman sp. nov.

Post 3 dies in medio liquido cellulae globosae vel ovales, multilateraliter gemmantes, singulae vel binae coherentes; hyphae verae absentes. Sedimentum et nonnulae insulae formantur. Post 30 dies annulus et sedimentum floccosum praesentia. In agaro maltoso (5%) post 3 dies 25°C coloniae pallide bubalinae, modice lucidae, planae, butyrosae, margine integra. Cellulae 2.3–6.0 × 1.8–4.0 μm. Ascosporae absentes. Glucosum, galactosum, trehalosum et cellobiosum (lente) fermentur, necque sucrosum, maltosum, melezitosum, methylglucosidum, gluconolactonum et xylosum. Glucosum, galactosum, sorbosum (lente), glucosaminum, D-ribosum (lente), xylosum, D-arabinosum (lente), sucrosum, maltosum, trehalosum, methylglucosidum, cellobiosum, salicinum, arbutinum, melezitosum, glycerolum, ribitolum, xylitolum, glucitolum, mannitolum, gluconolactonum, acidum 2-ketogluconatum, acidum gluconicum (lente), acidum succinicum, acidum citricum et aethanolum assimilantur. L-Arabinosum, rhamnosum, melibiosum, lactosum, raffinosum, inulinum, amylum solubile, erythritolum, arabinitolum, galactitolum, inositolum, acidum 5-ketogluconicum, acidum glucuronicum, acidum galacturonicum, acidum lacticum, methanolum non assimilantur. Aethylaminum, lysinum, cadaverinum, D-prolinum (lente),



Figs. 5-12 Micromorphology of the new *Blastobotrys* and *Candida* species. *B. robertii*: 5. Budding cells. 6. True hyphae with blastoconidia on short denticles. *C. cretensis*: 7. Budding cells. Note the range in cell sizes. 8. Sparingly differentiated pseudohyphae. *C. scorzettiae*: 9. Budding cells. 10. Pseudohyphae. *C. vadensis*: 11. Budding cells. 12. Pseudohyphae. Budding cells were photographed from 3-day 5% malt extract agar growth, 25°C. Pseudohyphae and hyphae were photographed from growth under the cover glass of a Dalmau plate, yeast morphology agar, 7 days, 25°C. Bar = 5 μm for all figures

putrescinum assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, glucosaminum, imidazolum, D-tryptophanum. Biotinum externum crescentiae necessarium. Reactio Diazonii Coeruleaei B negativa. Ureum non finditur. Crescit 30°C neque 32°C. Materia amyloidea non formatur. Typus CBS 9453^{T} (NRRL Y-27777) isolatus ex *Inonoto tamarici* lyophilus praeservatus in collectione zymoticae Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; isotypus in Agricultural Research Service Culture Collection, Peoria. Illinois, USA.

Characteristics of *Candida cretensis* sp. nov.

After 3 days at 25°C in liquid GPYM growth medium containing glucose (1%, wt/vol), peptone (0.5%, wt/vol), yeast extract (0.3%, wt/vol), malt extract (0.3%, wt/vol), the cells are globose and ovate, show multilateral budding and are present singly or in pairs. No hyphae are present. A sediment and some islets are formed. After one month, a ring and a flocculant sediment are present. After 3 days at 25°C the slant culture on 5% malt extract agar is tannish-white, faintly

Table 1 Characteristics of four novel *Candida* spp

	B. robertii	*C. cretensis*	*C. scorzettiae*	*C. vadensis*
CBS Strain Nr.	10106	9453	10107	9454
Assimilation of carbon and nitrogen compounds				
D-Glucose	+	+	+	+
D-Galactose	+	+	+	+
L-Sorbose	–	D	–	–
D-Glucosamine	–	+	–	+D
D-Ribose	–	D	–	–D
D-Xylose	–	+	+	+
L-Arabinose	–	–	–	–
D-Arabinose	–	D	–	–
L-Rhamnose	–	–	–	–
Sucrose	+	+	–	+
Maltose	+	+	+	+
α,α-Trehalose	+	+	+	+
Methyl-α-D-Glucoside	–D	+	–	+
Cellobiose	+	+	+	+
Salicin	D	+	+	+
Arbutin	D	+	+	+
Melibiose	+	–	–	–
Lactose	+	–	W	–
Raffinose	+	–	–	–
Melezitose	–	+	–	+
Inulin	–	–	–	–
Starch	+	–	–	–
Glycerol	–	+	+D	+
Erythritol	–	–	–	–
Ribitol	–	+	+D	+
Xylitol	–	+	–	–
L-Arabinitol	–	–	–	–
D-Glucitol	–	+	+	+
D-Mannitol	+	+	+	+
Galactitol	–	–	–	–
myo-Inositol	–	–	–	–
D-Gluconolactone	–D	+	+D	+
2-Keto-D-gluconate	–	+	+	+
5-Keto-D-gluconate	–	–	–	–
D-Gluconate	–D	D	+	+D
D-Glucuronate	–	–	–	–
D-Galacturonate	–	–	–	–
DL-Lactate	–	–	+	–
Succinate	+	+	+	+
Citrate	D	+	+D	+
Methanol	–	–	–	–
Ethanol	–	+	+	+
Propane-1,2-diol	–	D	D	–
Butane-2,3-diol	–	–	+	–
Quinic acid	–	–	–	–
D-Glucarate	–	–	–	–
D-Galactonate	–	–	–	–
Palatinose	+	+	W	+
Levulinate	–	–	–	–
L-Malic acid	+	+	+	+
L-Tartaric acid	–	–	–	–
D-Tartaric acid	–	–	–	–
meso-Tartaric acid	–	–	–	–
Galactaric acid	–	–	–	–
Uric acid	+	–	D	–

Table 1 continued

	B. robertii	*C. cretensis*	*C. scorzettiae*	*C. vadensis*
Gentobiose	+	+	–	+
Ethylene glycol	–	–	–	–
Tween 60	+	D	D	–
Tween 80	+	D	D	–
N-Acetyl-D-glucosamine	+	+	+	+
n-Hexadecane	+D	+	+	+D
Nitrate	–	–	–	–
Nitrite	–	–	–	–
Ethylamine	+	+	+	+
L-Lysine	+	+	+	+
Cadaverine	+	+	+	+
Creatine	–	–	–	–
Creatinine	–	–	–	–
D-Glucosamine	–W	–	–	D
Imidazole	–	–	–	–
D-Tryptophan	–	–	–	–
D-Proline	+	D	+	D
Putrescine	+	+	+	+
Miscellaneous				
0.01% Cycloheximide	+	+	+	D
0.1% Cycloheximide	+	+	–	–
10% NaCl	–	+	–	+
16% NaCl	–	–	–	–
Growth pH 3	+	+	+	+
Growth pH 9.5	–	–	–	–
Amyloids	–	–	–	–
Urease	–	–	–	–
Diazonium Blue B	–	–	–	–
Growth temperature (°C)	32+,35–	30+,32–	32+,35–	30+,32–
Vitamin required	Biotin	Thiamine	Biot + Thia	Biotin

D means delayed growth, more than 14 days required; W means weak growth

glistening, flat, butyrous, and with an entire margin. Cells show multilateral budding and are single, in pairs and in small clusters. The cells are ellipsoidal and measure 2.3–6.0 × 1.8–4.0 μm (Fig. 7). The appearance does not change over 4 weeks. After 7 days at 25°C, the Dalmau plate culture on morphology agar showed light tannish-white aerobic colonies that are low convex with a central depression, smooth, glistening and butyrous. The margin is usually entire or with infrequent small lobes. Growth under the cover glass is limited and shows only outgrowth of simple pseudohyphae (Fig. 8). No ascospores were seen in slant cultures on 5% malt extract agar and YM agar incubated at 15°C and 25°C for up to 4 weeks. Growth responses of strain CBS 9453^T on commonly used carbon and nitrogen compounds and other characteristics are shown in Table 1. Glucose, galactose, trehalose and cellobiose (delayed) are fermented. Sucrose, maltose, melezitose, methylglucoside, gluconolactone and xylose are not fermented.

Origin and Deposits

The strain was isolated from a rotten polypore *Inonotus tamaricis* (Pat.) Maire growing on a *Tamarix* tree near the sea side in Plakias, Crete, Greece in October 2000 (Middelhoven 2004). The type strain is preserved as a lyophilized preparation in two culture collections, viz. CBS (accession number 9453^T) and NRRL (accession number Y-27777). Ribosomal DNA sequences were deposited by Dr. Vincent Robert in GenBank and received accession numbers AY498861 for D1/D2 and AY498862 for ITS. The D1/D2 domain was resequenced in the present study and received an additional Accession number,

DQ839393. The MycoBank Accession number for NRRL Y-27777 (CBS 9453) is MB 510430.

Etymology

The epithet *cretensis* refers to the Greek island Crete where the strain had been isolated.

Phylogeny and species recognition

Candida cretensis is most closely related to *C. kruisii* (Fig. 2), and differs from this species by eight nucleotides in the D1/D2 domains of LSU rDNA. The *C. kruisii* clade was recently expanded by Suh et al. (2006) with the description of nine new species. These new species were isolated from the digestive tracts of beetles, primarily nitidulids, which had been feeding on agarics growing in the southeastern USA and on Barro Colorado Island, Panama. Consequently, isolation of *C. cretensis* from a rotten polypore reflects a similar habitat, as is the case for the type strain of *C. kruisii*, which was isolated from a fruiting body of *Boletus purpureus* (Meyer et al. 1998). The most reliable method for separation of *C. cretensis* and *C. kruisii* is from sequence analysis, because the two species are essentially identical on standard fermentation and assimilation tests. The only recognized difference is that *C. cretensis* gives a delayed fermentation of cellobiose, whereas *C. kruisii* does not ferment this sugar.

Latin diagnosis of *Candida scorzettiae* Middelhoven et Kurtzman sp. nov.

Post 3 dies in medio GPYM dicto cellulae ovales, singulae vel binae. Hyphae verae absentes. Pellicula tenuis et sedimentum formatae. Post 30 dies annulus et sedimentum praesentes. In agaro maltoso (5%) post 3 dies 25°C colonia alba, quasi pulverulenta, margine pseudohyphis fimbriata. Cellulae multilateraliter gemmantes, ellipsoideae vel elongatae, 4–10 × 2–3 μm, singulae vel binae vel brevis catenis cohaerentes. Hyphae verae et ascosporae absentes. Fermentatio nulla. Glucosum, galactosum, xylosum, maltosum, trehalosum, cellobiosum, salicinum, arbutinum, glycerolum, ribitolum, glucitolum, mannitolum, gluconolactonum, acidum 2-ketogluconicum, acidum gluconicum, acidum lacticum, acidum succinicum, acidum citricum et aethanolum assimilantur. Sorbosum, glucosaminum, ribosum, L-arabinosum, D-arabinosum, rhamnosum, sucrosum, methylglucosidum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile, erythritolum, xylitolum, arabinitolum, galactitolum, inositolum, acidum 5-ketogluconicum, acidum glucuronicum, acidum galacturonicum, methanolum non assimilantur. Aethylaminum, lysinum, cadaverinum, D-prolinum, putrescinum assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, glucosaminum, imidazolum, D-tryptophanum. Biotinum et thiaminum externa crescentiae necessaria. Reactio Diazonii Coeruleaei B negativa. Ureum non finditur. Crescit 32°C neque 35°C. Materia amyloidea non formatur. Typus CBS 10107^{T} isolatus ex *Quercu robore*, lyophilus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Characteristics of *Candida scorzettiae* sp. nov.

After 3 days at 25°C in liquid GPYM growth medium, the cells are ovate, single or in pairs. No hyphae were formed. A thin pellicle and a sediment are formed. After one month a ring and a sediment are present. The slant culture on 5% yeast extract agar after 3 days 25°C is white, almost powdery and with a mycelial fringe consisting of pseudohyphae. Budding is multilateral. Cells are ellipsoidal to elongate, 4–10 × 2–3 μm, single, in budded pairs and in short chains (Fig. 9). After 7 days at 25°C the Dalmau plate on morphology agar shows aerobic colonies that are tannish-white, dull, low convex with numerous cuplike outgrowths, and surrounded by an entire margin composed of pseudohyphae. Growth under the cover glass consists primarily of well-branched pseudohyphae bearing blastoconidia, some of which are spindle-shaped, whereas others are ellipsoidal (Fig. 10). True hyphae were not seen. No ascospores were seen in slant cultures on 5% malt extract agar and YM agar incubated at 15°C and 25°C for up to four weeks. Growth responses of the type strain CBS 10107^{T} (NRRL Y-27665) on commonly used

carbon and nitrogen compounds and other characteristics are shown in Table 1. Sugars were not fermented.

Origin and deposits

The type strain was isolated from rotten oak wood (*Quercus robur* L.) in Wageningen, The Netherlands, in August 2002 and originally was assigned strain number QuB-82 (Middelhoven 2006). The strain was deposited in two culture collections, viz. CBS (Accession number 10107^T) and NRRL (Accession number Y-27665). The D1/D2 LSU rDNA sequence was deposited at GenBank under Accession number DQ839394. The MycoBank Accession number for NRRL Y-27665 (CBS 10107) is MB 510431.

Etymology

The epithet *scorzettiae* is chosen in honour of Dr. Gloria Scorzetti of the University of Miami for her extensive work on basidiomycete taxonomy and phylogeny.

Phylogeny and species recognition

On the basis of phylogenetic analysis of D1/D2 LSU rDNA sequences, *C. scorzettiae* is not closely related to presently described species (Fig. 3). The nearest neighbour is *C. mesenterica*, which differs from *C. scorzettiae* by 102 nucleotide substitutions and 38 indels. The two species can be recognized from standard growth tests because *C. scorzettiae* does not assimilate L-sorbose or sucrose, whereas *C. mesenterica* assimilates both sugars.

Latin diagnosis of *Candida vadensis* Middelhoven et Kurtzman sp. nov.

Post 3 dies in medio liquido GPYM dicto 25°C cellulae globosae vel ovales, singulae vel binae vel breviter catenatae. Nonnullum pseudomycelium formatum. Pellicula et sedimentum formatae. Post 30 dies annulus et sedimentum praesentes. In agaro maltoso (5%) colonia alba, pulverulenta, butyrosa, margine pseudohyphis fimbriata. Cellulae multilateraliter gemmantes, ellipsoideae vel elongatae, 2.1–21 × 2–3 μm, singulae vel binae vel parvis aggregatis cohaerentes. Nonnullae cellulae ex uno vel compluribus denticulis blastoconidia formantes. Neque conjugatio neque hyphae verae nec ascosporae visae. Fermentatio nulla. Glucosum, galactosum, glucosaminum, xylosum, sucrosum, maltosum, trehalosum, methylglucosidum, cellobiosum, salicinum, arbutinum, melezitosum, glycerolum, ribitolum, glucitolum, mannitolum, gluconolactonum, acidum 2-ketogluconicum, acidum gluconicum, acidum succinicum, acidum citricum et aethanolum assimilantur. Sorbosum, L-arabinosum, D-arabinosum, rhamnosum, melibiosum, lactosum, raffinosum, inulinum, amylum solubile, erythritolum, arabinitolum, galactitolum, inositolum, acidum 5-ketogluconicum, acidum glucuronicum, acidum galacturonicum, acidum lacticum, methanolum non assimilantur. Aethylaminum, lysinum, cadaverinum, glucosaminum (lente), D-prolinum (lente), putrescinum assimilantur, neque kalii nitratum, natrii nitritum, creatinum, creatininum, imidazolum, D-tryptophanum. Biotinum externum crescentiae necessarium. Reactio Diazonii Coeruleaei B negativa. Ureum non finditur. Crescit 30°C neque 32°C. Materia amyloidea non formatur. Typus CBS 9454^T isolatus ex *Hericio erinaceo*, lyophilus praeservatus in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Characteristics of *Candida vadensis* sp. nov.

After 3 days at 25°C in liquid GPYM growth medium, the cells are globose and ovate, single or in pairs and short chains. Some pseudomycelium is formed as well. A pellicle and a sediment are formed. After one month a ring and sediment are present. The slant culture on 5% malt extract agar after 3 days at 25°C is white, powdery, butyrous, and fringed with a narrow band of mycelial growth consisting of pseudohyphae. Budding is multilateral. Cells are short ellipsoidal to elongate, 2.5–21 × 2–3 μm, and single, in pairs and in small clusters. Some cells have one or more short denticles that bear blastoconidia (Fig. 11). Infrequent cells produced extensions that resembled conjugation tubes, although conjugation between cells was not observed. After 7 days at 25°C, aerobic colonies on the Dalmau plate on morphol-

ogy agar are flat with a shallow central crater, surrounded by a narrow band of pseudohyphae and have a dull greyish-white, powdery surface. Growth under the cover glass shows extensive well-branched pseudohyphae with blastoconidia (Fig. 12). True hyphae were not observed. No ascospores were seen in slant cultures on 5% malt extract agar and YM agar incubated at 15°C and 25°C for up to 4 weeks. Growth responses of strain CBS 9454^T on commonly used carbon and nitrogen compounds and other characteristics are shown in Table 1. Sugars were not fermented.

Origin and deposits

The strain was isolated from a rotten mushroom, *Hericium erinaceus* (Bull.: Fr.) Pers., growing on a beech tree, *Fagus sylvatica* L., in Wageningen, The Netherlands, in September 2000 (Middelhoven 2004). The type strain was deposited in two culture collections, viz. CBS (Accession number 9454^T) and NRRL (Accession number Y-27778). Ribosomal DNA sequences were deposited at GenBank by Dr. Vincent Robert where they received Accession numbers AY498863 for D1/D2 and AY498864 for ITS. The D1/D2 domain was resequenced in the present study and received an additional Accession number, DQ839396. The MycoBank Accession number for NRRL Y-27778 (CBS 9454) is MB 510432.

Etymology

The epithet *vadensis* refers to the Latin name of Wageningen in the Roman age, Villa Vada, the town where the strain had been isolated.

Phylogeny and species recognition

Phylogenetic analysis of LSU D1/D2 rDNA sequences placed *C. vadensis* in the *C. tanzawaensis* clade most closely related to *C. xylopsoci* and *C. pyralidae*, with *C. vadensis* differing from *C. xylopsoci* by 16 nucleotide substitutions. *C. vadensis* can be separated from the latter two species because it is non-fermentative, whereas *C. xylopsoci* and *C. pyralidae* ferment both glucose and trehalose. *C. tanzawaensis* was described by Nakase et al. (1988) and recognized from LSU D1/D2 rDNA sequence analysis as an isolated lineage (Kurtzman and Robnett 1998). More recently, 22 new species of this clade have been discovered, mostly from insects associated with mushrooms, thus demonstrating members of the clade to be widely distributed in nature and strongly associated with mushroom foraging insects (Kurtzman 2001; Suh et al. 2004).

Physiological characteristics

All four novel species assimilated *n*-hexadecane. Fermentation of sugars was shown only by *C. cretensis*. In contrast to many basidiomycetous yeasts isolated from rotten wood, ascomycetous yeasts did not exhibit much degradative activity towards phenolic compounds and polysaccharides (Middelhoven 2006). *C. cretensis* and *C. vadensis* are notable for assimilation of xylan that supported slow growth of *C. scorzettiae* and *B. robertii*. The latter species grew on soluble starch and slowly on pullulan. *B. robertii* and *C. scorzettiae* (slow) grew on uric acid as sole source of carbon and nitrogen. Moreover, *C. scorzettiae* was the only one of the four novel species that assimilated some phenolic compounds, viz. 3-hydroxybenzoate and 2,5-dihydroxybenzoate (gentisate). This is indicative of an operative gentisate pathway (Middelhoven et al. 1992). *C. scorzettiae* also grew on 3-hydroxyphenylacetate and 3-hydroxycinnamate, showed delayed growth on homoprotocatechuate and homogentisate, but failed to grow on resorcinol, hydroquinone, salicylate, 4-hydroxybenzoate, protocatechuate, 2-hydroxyphenylacetate, 4-hydroxyphenylacetate, and 4-hydroxycinnamate. The other three species did not assimilate any of the phenols, hydroxybenzoates, hydroxyphenylacetates and hydroxycinnamates tested.

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