Short Communication

***Candida baotianensis* sp. nov., an ascomycetous yeast species from forest soil in China**

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Forest soil is a rich source of new yeast species. In the past few years, many yeast species have been isolated from soil samples collected in different forests worldwide. Several yeast strains have been recognized as new species, such as *Candida dajiaensis*, *Candida yuanshanicus*, *Candida jianshihensis* and *Candida sanyiensis* (Liu et al., 2008), and *Millerozyma phetchabunensis* (Tammawong et al., 2010). Yeasts from these habitats may hold keys to nutrient cycling, metal detoxification, and food-webs in the natural environment, as well as potentially provide unique biomolecules for industry and medicine.

Baotianman Nature Reserve, which is located in Henan Province of Central China, has a transitional climate between the northern subtropical zone and warm temperate zone. Due to its unique geographical position and very rich diversity of wildlife resources, a survey of the yeast community associated with forest soil was conducted in Baotianman Nature Reserve. During this investigation, a number of yeast strains belong to

known species in the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Hanseniaspora*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Scheffersomyces*, *Trichosporon*, *Williopsis* and *Zygosaccharomyces* were isolated. However, two novel strains, BY-29^T and BY-30, were obtained and we propose the name *Candida baotianensis* sp. nov. for these isolates based on morphological, physiological and molecular characteristics.

The two strains were isolated from soil collected in a mixed deciduous forest (BY-29^T) and a *Quercus variabilis* forest (BY-30) in Baotianman Nature Reserve, by using an enrichment technique. Each soil sample (5 g) was added to 50 ml acidified YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose; adjusted to pH 4.0–4.5 with 1 M HCl) supplemented with 0.025% sodium propionate and 200 mg/L chloramphenicol in a 250 ml Erlenmeyer flask and incubated at 25°C for 3 days on a rotary shaker (Limtong et al., 2007). The enrichment culture was then spread out on YM agar supplemented with 0.025% sodium propionate and 200 mg/L chloramphenicol. Representative colonies were purified by the conventional streaking technique on YM agar plates. Purified yeast strains were suspended in YM broth supplemented with 10% glycerol and maintained at –80°C.

The morphological, physiological and biochemical characteristics were examined according to standard methods that are employed in yeast taxonomy (Yarrow, 1998). All assimilation tests were performed three times, and the results were read after 5 and 21 days of

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The GenBank/EMBL/DDBJ accession numbers for the gene sequences of the D1/D2 domains of the LSU rDNA and ITS region are HM588005 and HQ117932 for strain BY-29^T, respectively, and HM588006 and HQ117933, respectively, for strain BY-30.

incubation. Assimilation of nitrogen compounds was investigated on liquid media with starved inocula (Nakase and Suzuki, 1986). Growth at various temperatures was determined by cultivation in YM broth. Mating and ascus formation were assessed individually or by mixing strains in pairs on 5% malt extract agar, cornmeal agar or yeast carbon base agar (Difco) supplemented with 0.01% ammonium sulfate (Rosa et al., 2007).

Total genomic DNA was isolated from stationary-phase cultures according to the method of Makimura et al. (1994). The sequences of the D1/D2 domain of the LSU rRNA gene and the internal transcribed spacer (ITS) region were determined by the methods of Kurtzman and Robnett (1998) and White et al. (1990), respectively. The PCR product was checked by agarose gel electrophoresis, purified using the QIA quick purification kit (Qiagen) and cycle-sequenced using the ABI BigDye terminator cycle sequencing kit, version 3.1 (Applied Biosystems). The sequences were determined with an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) according to the instructions of the manufacturer. The generated sequences were aligned with related species using

CLUSTAL_X version 1.8 software (Thompson et al., 1997). The phylogenetic trees were constructed from the evolutionary distance data calculated from Kimura's two-parameter model (Kimura, 1980) using the neighbor-joining method (Saitou and Nei, 1987). Bootstrap analyses were performed based on 1,000 random resamplings (Felsenstein, 1985). Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

Strains BY-29^T and BY-30 have identical sequences in the D1/D2 domain and ITS region, suggesting that they are strains of the same species. Sequences of related species were retrieved from the nucleic acid databases and phylogenetic tree was constructed for the D1/D2 domain of the LSU rDNA (Fig. 1). Phylogenetic analysis showed that strain BY-29^T belongs to the *Metschnikowia* clade, clustering in a group most closely related to *Candida melibiosica* with high bootstrap value (Fig. 1). Thus, strain BY-29^T is phylogenetically distinct. The D1/D2 domain phylogenetic tree also makes it clear that strain KBP3959, identified as *C. melibiosica*, constitutes a cluster with strain BY-29^T, and differs from the latter strain by only one substitution in the compared region (490 nt), indicating their con-

Fig. 1. Neighbor-joining phylogenetic tree based on the D1/D2 domain of the LSU rRNA gene, depicting the relationship of *Candida baotianensis* sp. nov. with closely related species.

Saccharomyces cerevisiae was used as an outgroup. Bootstrap values of 50% or above are shown. Bar, 0.02 substitutions per nucleotide position.

specificity. The strain KBP3959 was isolated from soil in Bronnitsy, Moscow, Russia. The existence of strains of the new species in two such widely separated locations also suggests a broad range of occurrence. Strain BY-29^T is related more distantly to *Clavispora reshetovae*, *Clavispora opuntiae* and *Clavispora lusitanae*. The isolate is therefore assigned to the genus *Candida*, as it failed to produce the conical or clavate shaped ascospores characteristic of *Clavispora*.

Sequence analysis showed that strain BY-29^T differs from the type strain of *C. melibiosica* by 2.5% divergence (6 nucleotide substitutions and 7 gaps out of 521 nt) in the D1/D2 domain of the LSU rDNA, and by 8.2% mismatches (12 nucleotide substitutions and 13 indels out of 304 nt) in the ITS sequence. Previous studies have shown that conspecific yeast strains usually have fewer than 1% nucleotide differences in the D1/D2 domain sequence (Kurtzman and Robnett, 1998; Lu et al., 2004) and 1–2% nucleotide differences in the ITS region (Bai et al., 2001; Nagahama et al., 1999). Hence, results acquired from D1/D2 and ITS sequence analyses clearly indicate that strain BY-29^T

is a different species from *C. melibiosica*.

Strain BY-29^T also resembles *C. melibiosica* in the phenotypic characteristics but is distinguished from the latter species by its inability to ferment trehalose, melibiose, cellobiose, melezitose and raffinose, ability to assimilate L-rhamnose, methyl-D-glucoside, glycerol, soluble starch and D-galacturonate, inability to assimilate melibiose and raffinose, and ability to grow in a vitamin-free medium (Table 1).

The molecular and phenotypic comparison showed that the strain BY-29^T represents a novel species of the genus *Candida*. The name *Candida baotianensis* sp. nov. is proposed for it.

Latin diagnosis of *Candida baotianensis* F. -L. Hui et T. Ke sp. nov.

In medio liquido YM, post dies 3 ad 25°C cellulae ovoideae aut ellipsoideae (2–4 × 2–6 µm), singulae aut binae, per germinationem multipolarem reproducentes. In agarō YM, post dies 3 ad 25°C, cultura butyrosa, albida, sublatum, glabra et margine glabra. In agarō farinae *Zea mays* post dies 7, pseudohyphae formantur nec hyphae non formantur. Asci non formantur. D-glucosum, D-galactosum, maltosum (lente, infirme) et sucrosus (lente, infirme) fermentatur at non trehalosum, melibiosum, cellulobiosum, melezitosum nec raffinolum. D-glucosum, D-galactosum, L-sorbose, D-glucosaminum, D-ribosum (variabile), D-xylosum, L-rhamnosum (infirme), sucrosus, maltosum, trehalosum, methyl-D-glucosidum (infirme), cellulobiosum, salicinum, arbutinum, melezitosum, amyllum solubile, glycerolum (infirme), ribitolum, xylitolum, D-glucitolum, D-mannitolum, D-glucono-1,5-lactonum, 2-keto-D-glucuronatum, D-gluconatum (infirme), D-galacturonatum, saccinatum, citratum, ethanolum et acidum quinicum assimilantur at non L-arabiosum, D-arabiosum, melibiosum, lactosum, raffinolum, inulinum, erythritolum, L-arabinitolum, galactitolum, inositolum, 5-keto-D-glucuronatum, D-glucuronatum, DL-lactatum, methanolum, propanum-1,2-diolum nec D-glucaratum. Ethylaminum, L-lysinum, cadaverinum, glucosaminum (infirme) et D-tryptophanum assimilantur at non nitricum, nitrosus, creatinum, creatininum nec imodazolium. Vitamina externa ad crescentiam necessaria non sunt. Crescit in 10% natrium chloridum/5% glucosum et 50% glucosum. Non crescit in 16% natrium chloridum/5% glucosum, 60% glucosum, 0.01% cycloheximido nec 0.1% cycloheximido. Crescere potest in temperatura 25, 30, 35 et 37°C, at non crescit in temperatura 40, 42 nec

Table 1. Physiological characteristics that differentiate *Candida baotianensis* sp. nov. from related species.

Characteristic	1	2	3	4	5
Fermentation of					
D-Galactose	+	+	ND	+	+
Trehalose	–	D	ND	+	–
Melibiose	–	+	ND	V	–
Cellobiose	–	D	ND	+	–
Melezitose	–	D	ND	V	D
Raffinose	–	+	ND	–	–
Assimilation of					
L-Sorbose	+	V	+	V	–
D-Glucosamine	+	V	–	V	D
L-Rhamnose	W	–	+	V	+
Methyl-D-glucoside	W	–	ND	V	+
Melibiose	–	+	ND	–	–
Raffinose	–	+	–	–	–
Starch	+	–	–	–	+
Glycerol	W	V	+	+	–
D-Galacturonic acid	+	–	ND	–	–
Vitamin-free medium	+	–	–	–	–

Strains: 1, *Candida baotianensis* sp. nov. BY-29^T; 2, *Candida melibiosica*; 3, *Clavispora reshetovae*; 4, *Clavispora lusitanae*; 5, *Candida oregonensis*. +, Positive; –, negative; D, delayed positive; W, weakly positive; V, variable; ND, not determined.

Data for reference species were taken from Barnett et al. (2000) and Yurkov et al. (2009).

45°C. Amylum et acidum non formantur. Diazonium caeruleum B non respondens. Ureum non hydrolysat-ur.

Typus stirpis BY-29^T (= CICC 1968^T = CGMCC 2.4378^T = CBS 11898^T) isolatus ex terra, Baotianman, Henan *Provincia*, China in 2009. China Center of Industrial Culture Collection (Beijing, China), China General Microbiological Culture Collection Center (Beijing, China) et Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) deposita est.

Description of *Candida baotianensis* F. -L. Hui & T. Ke sp. nov.

Candida baotianensis (bao.tian.en'sis. N.L. fem. adj. *baotianensis* of Baotianman, referring to the geographical origin of the type strain).

In YM broth, after 3 days at 25°C, cells are ovoid to ellipsoid (2–4 × 2–6 µm) and occur singly or in pairs (Fig. 2a). Budding is multilateral. In YM agar, after 3 days at 25°C, the streak culture is butyrous, white, raised, with a smooth surface and has an entire margin. In Dalmau plates after 7 days on cornmeal agar at 25°C, pseudohyphae are formed (Fig. 2b), but true hyphae are not formed. Asci are not formed on common sporulation media. D-Glucose, D-galactose, maltose (weak, delayed) and sucrose (weak, delayed) are fermented, but not trehalose, melibiose, cellobiose, melezitose or raffinose. The following carbon compounds are assimilated: D-glucose, D-galactose, L-sorbose, D-glucosamine, D-ribose (variable), D-xylose, L-rhamnose (weak), sucrose, maltose, trehalose, methyl-D-glucoside (weak), cellobiose, salicin, arbutin, melezitose, soluble starch, glycerol (weak), ribitol, xylitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone, 2-keto-D-glu-

conate, D-gluconate (weak), D-galacturonate, succinate, citrate, ethanol and quinic acid. Other carbon compounds tested in this study, including L-arabinose, D-arabinose, melibiose, lactose, raffinose, inulin, erythritol, L-arabinitol, galactitol, myo-inositol, 5-keto-D-gluconate, D-glucuronate, DL-lactate, methanol, propane 1, 2 diol and D-glucarate are not assimilated. Ethylamine, L-lysine, cadaverine, glucosamine (weak) and D-tryptophan are assimilated, but nitrate, nitrite, creatine, creatinine and imidazole are not assimilated. Growth in vitamin-free medium is positive. Growth occurs in 10% sodium chloride plus 5% glucose and 50% glucose, but does not occur in 16% sodium chloride plus 5% glucose, 60% glucose, 0.01% cycloheximide or 0.1% cycloheximide. Growth occurs at 25, 30, 35 and 37°C, but does not occur at 40, 42 or 45°C. No starch-like substance is produced. Acid production on chalk agar is negative. Urease hydrolysis and Diazonium blue B reactions are negative.

The type strain of *Candida baotianensis*, strain BY-29^T (= CICC 1968^T = CGMCC 2.4378^T = CBS 11898^T), was isolated from forest soil collected from Baotianman Nature Reserve, Henan Province, China in 2009.

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Fig. 2. *Candida baotianensis* sp. nov. BY-29^T.

(a) Vegetative cells grown on YM broth for 3 days at 25°C. (b) Pseudohyphae formed on cornmeal agar after 7 days at 25°C. Bar, 10 µm.

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