Short Communication

Lindnera wuzhiensis sp. nov., a novel ascomycetous yeast species

Shi-An Wang^{1,2} and Feng-Yan Bai^{1,*}

¹ Key Laboratory of Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China

² Key Laboratory of Biofuels, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, 266101, China

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Recently, phylogenetic relationships among species assigned to the genera *Pichia*, *Issatchenkia* and *Williopsis* were re-analyzed by using multigene sequence analysis. The concepts of related genera were consequently revised and three new genera *Barnettozyma*, *Lindnera* and *Wickerhamomyces* were proposed (Kurtzman et al., 2008). The genus *Lindnera* was established to accommodate the *Pichia* species in the *Pichia anomala* clade (Kurtzman, 2000; Kurtzman et al., 2008). During our investigation of ascomycetous yeast diversity distribution in plant materials from Hainan Province in China, one strain, YSHM21C^T, isolated from a leaf of herbaceous plant was revealed to represent a novel *Lindnera* species by phenotypic characterization and sequence analyses of the 26S rDNA D1/D2 domain and internal transcribed spacer (ITS) region. A complete description of the species is given in the present study.

Samples of leaves were placed into acidified YPD broth containing 1% yeast extract, 2% peptone, 2% dextrose and 200 µg/ml chloramphenicol, adjusted to pH 4.0–4.5 with HCl. After incubation at room temperature (about 25°C) for 12–24 h, aliquots (100 µl) of the

10⁻¹ or 10⁻³ diluted cultures were spread on malt extract plates. After 2 days' incubation at room temperature or 25°C, colonies with different morphological characters were transferred onto malt extract agar slants for further purification and examination.

The morphological, physiological and biochemical characteristics were examined according to standard methods commonly used in yeast taxonomy (Yarrow, 1998). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase and Suzuki, 1986).

Nuclear DNA was extracted by the method of Makimura et al. (1994). The DNA fragment covering the ITS region (including 5.8S rDNA) and the large-subunit rDNA D1/D2 domain was amplified and sequenced as described previously (Lu et al., 2004). Molecular phylogenetic analysis was performed by the methods described by Bai et al. (2002). Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree.

Phylogenetic analysis from the large subunit (26S) rRNA gene D1/D2 domain sequence showed that strain YSHM21C^T was most closely related to *Lindnera veronae* and *Lindnera fabianii* (Fig. 1), two species formerly assigned to *Pichia* (Kurtzman, 2000; Kurtzman et al., 2008). The species in *Lindnera* were clustered together by analysis of either the individual gene section of the 26S rDNA D1/D2 domain or three concatenated gene sequences from large and small subunit

* Address reprint requests to: Dr. Feng-Yan Bai, Key Laboratory of Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.

E-mail: baify@im.ac.cn

Fig. 1. Phylogenetic tree drawn from neighbor-joining analysis of the 26S rDNA D1/D2 domain, depicting the relationships of *Lindnera wuzhiensis* sp. nov. with closely related taxa.

Bootstrap percentages over 50% from 1,000 bootstrap replicates are shown. Reference sequences were retrieved from GenBank under the accession numbers indicated.

rRNAs and translation elongation factor-1 α (Kurtzman, 2000; Kurtzman et al., 2008). These supported that strain YSHM21C^T should be a *Lindnera* species. The two described species, *L. veronae* and *L. fabianii*, have identical D1/D2 sequences. Strain YSHM21C^T differed from them by 8 substitutions (~1.3%) in this domain. According to Kurtzman and Robnett (1998), yeast strains that show nucleotide substitution greater than 1% in the D1/D2 domain usually represent different species.

The distinction of YSHM21C^T was further supported by ITS sequence comparison. In this region, strain YSHM21C^T (GenBank accession no. FJ606824) differed from *Lindnera veronae* (AF335966) and *Lindnera fabianii* (AF335967) by ~14% mismatches (65 substitutions and 10 gaps).

Strain YSHM21C^T typically formed two hat-shaped ascospores in a deliquescent ascus. Asci were unconjugated or conjugated between independent cells (Fig. 2b). Its nearest neighbors, *L. veronae* and *L. fabianii*, formed up to four hat-shaped ascospores in an ascus (Barnett et al., 2000). In the genus *Lindnera*, closely related species may form ascospores with different shapes (Kurtzman, 2000; Kurtzman et al., 2008). The discovery of strain YSHM21C^T showed that as-

Fig. 2. *Lindnera wuzhiensis* sp. nov. YSHM21C^T. (a) Vegetative cells grown in YM broth for 2 days at 25°C. (b) Asci formed on acetate agar after 2 days at 25°C. Bars, 10 μm.

Table 1. Physiological characteristics that differentiate *Lindnera wuzhiensis* sp. nov. from *Lindnera veronae* and *Lindnera fabianii*.

Characteristic	<i>Lindnera wuzhiensis</i>	<i>L. veronae</i>	<i>L. fabianii</i>
Fermentation of:			
Maltose	D	–	+ / D
Raffinose	W	–	+
Assimilation of:			
Raffinose	D	–	+
Soluble starch	+	–	+ / D
L-Arabinose	W	+ / D	–
L-Rhamnose	–	+	–
Citric acid	W	+	+
Potassium nitrate	–	–	+
Sodium nitrite	–	–	+
Vitamin-free medium	+	–	–

Symbols: +, positive; –, negative; D, delayed positive; and W, weak.

cospore numbers in an ascus formed by close relatives in the genus *Lindnera* may also be different.

Physiological characteristics that differentiate strain YSHM21C^T from *L. veronae* and *L. fabianii* are shown in Table 1. Strain YSHM21C^T cannot utilize nitrate as a sole nitrogen source, being similar to *L. veronae* but different from *L. fabianii*. Actually, nitrate utilization is not of phylogenetic importance in *Lindnera* (Kurtzman, 1984; Kurtzman and Robnett, 1998; Kurtzman et al., 2008). In other physiological characteristics, strain YSHM21C^T differs from *L. veronae* more significantly than from *L. fabianii* (Table 1). Strain YSHM21C^T can be phenotypically differentiated from both species by its ability to grow in vitamin-free medium.

The molecular and phenotypic comparison showed that strain YSHM21C^T represents a novel species of the genus *Lindnera*. The name *Lindnera wuzhiensis* sp. nov. is proposed for it.

Latin diagnosis of *Lindnera wuzhiensis* F.-Y. Bai et S.-A. Wang sp. nov.

In media liquido YM post dies 3 ad 25°C, cellulae ovoideae vel elongatae, 1.0–5.0 × 3.0–10.0 µm, singulae, binae et adhaerentes. Per gemmationem multipolarem reproducentes. Post 1 mensem sedimentum formatur. In agarō YM post 1 mensem ad 25°C, candida vel crenea, glabra, margo glabro. In agarō farinae Zea mays post dies 7, pseudohyphae eumorphum. Ascospores pileiformes, 2 in ascum. Asci liberi. Glucosum, sucrosum, maltosum (lente) et raffinose (in-

firme) fermentantur at non galactosum nec lactosum. Glucosum, sucrosum, maltosum, cellobiosum, trehalosum (lente), raffinose (lente), melezitose, amy-lum solubile, D-xylosum, L-arabinosum (infirme), ethanolum, glycerolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, DL-lacticum, acidum succinicum et acidum citricum (infirme) assimilantur at non galactosum, L-sorbose, lactosum, melibiosum, inulinum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, erythritolum, ribitolum, galactitolum, inositolum nec hexadecanum. Ammonium sulfatum, ethylaminum, cadaverinum et L-lysinum assimilantur at non kalium nitricum nec natrum nitrosum. Ad crescentiam vitaminum non necessarium est. Maxima temperatura crescentiae: 33°C. Materia amyloidea iodophila non formantur. Diazonium caeruleum B non respondens. Ureum non hydrolysat.

Description of *Lindnera wuzhiensis* F.-Y. Bai & S.-A. Wang sp. nov.

Lindnera wuzhiensis (wu.zhi.en'sis. N.L. fem. adj. *wuzhiensis* of Wuzhi, referring to the geographical origin of the type strain).

In YM broth, after 3 days at 25°C, cells are ovoid to elongate, 1.0–5.0 × 3.0–10.0 µm and occur singly, in pairs or in groups (Fig. 2a). Budding is multilateral. After a month at 25°C, sediment is present. Growth on YM agar, after a month at 25°C, the streak culture is white to cream, smooth and somewhat dull, with an entire margin. Dalmau plate culture on corn-meal agar: after 7 days at 25°C, pseudohyphae are present. On acetate agar after 2 days at 25°C, two hat-shaped spores are formed which are rapidly liberated from the asci upon maturity. Glucose, sucrose, maltose (delayed) and raffinose (weak) are fermented; galactose and lactose are not fermented. Glucose, sucrose, maltose, cellobiose, trehalose (delayed), raffinose (delayed), melezitose, soluble starch, D-xylose, L-arabinose (weak), ethanol, glycerol, D-mannitol, D-glucitol, α-methyl-D-glucoside, salicin, DL-lactic acid, succinic acid and citric acid (weak) are assimilated; galactose, L-sorbose, lactose, melibiose, inulin, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, erythritol, ribitol, galactitol, inositol and hexadecane are not assimilated. Ammonium sulfate, ethylamine hydrochloride, cadaverine hydrochloride and L-lysine are assimilated; potassium nitrate and sodium nitrite are not assimilated. Growth in vitamin-free medium is positive. Maximum growth temperature is 33°C. Starch-

like compounds are not produced. Diazonium blue B reaction is negative. Urease activity is negative. The type strain YSHM21C^T was isolated from a leaf of herbaceous plant collected in Wuzhi Mountain, Hainan Province in the south of China in November 2006. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.3480^T (= CBS 10698^T).

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