Cyberlindnera xishuangbannaensis f.a., sp. nov., a yeast isolated from rotting wood

Jun Zheng,† Yun-Feng Lu,† Xiao-Jing Liu and Feng-Li Hui*

Abstract

Three strains representing a novel species of yeast were isolated from samples of rotting wood collected from Xishuangbanna Tropical Rainforest in Yunnan Province, PR China. Phylogenetic analysis based on the concatenated sequences of the internal transcribed spacer (ITS) region and the D1/D2 domains of the large subunit (LSU) rRNA gene revealed that the novel species is a member of the genus Cyberlindnera, although the formation of ascospores was not observed. The novel species was related most closely to the type strain of the species Candida pattaniensis, but they had a 0.5 % sequence divergence (3 substitutions, 0 gap) in the D1/D2 domain and a 5.4 % sequence divergence (21 substitutions, 10 gaps) in the ITS region. The novel species could also be differentiated from the closely related species by some biochemical and physiological characteristics. The species name Cyberlindnera xishuangbannaensis f.a., sp. nov. is proposed to accommodate these strains, with NYNU 16752† (=CICC 33163†=CBS 14692†) designated as the type strain. The MycoBank number is MB 822199.

The genus Lindnera was proposed by Kurtzman et al. to accommodate species of yeast initially classified in the genera Pichia and Williopsis [1, 2]. Because Lindnera is a later homonym of a validly published plant genus, Minter proposed Cyberlindnera as a replacement name [3]. The genus Cyberlindnera belongs to the family Phaffiomycetaceae and it contains 25 teleomorphic species and 19 species, which have an unknown sexual stage [2, 4–9]. The members included in the genus Cyberlindnera possess Q-7 as a predominant ubiquinone, ferment glucose and assimilate a variety of sugars, polyols and other carbon sources, but fail to assimilate methanol and hexadecane [2, 4]. Despite being physiologically similar, species assigned to the genus show noticeable differences in ascospore morphology (spherical, hat-shaped or saturn-shaped). Species of the genus Cyberlindnera have been isolated from a wide variety of habitats, such as plant-related substrates, insect frass, soil and water samples [2, 4–9]. Some species are heterothallic (e.g. Cyberlindnera misumaiensis, Cyberlindnera meyerae and Cyberlindnera xylosilytica), whereas others are homothallic (e.g. Cyberlindnera sargentensis, Cyberlindnera suaveolens, Cyberlindnera saturnus and Cyberlindnera xylebori) [2, 6, 7].

During studies of yeasts associated with rotting wood, we isolated a large number of yeasts from different Chinese regions [10–12]. The majority of the yeasts belonged to several major clades in the subphylum Saccharomycotina; some of these species have been identified as novel species in earlier papers [10–12]. Amongst these associates, we focus here on three strains of an asexual ascomycetous yeast species from Xishuangbanna Tropical Rainforest. Analysis of the sequences of the internal transcribed spacer (ITS) region and the D1/D2 domains of the large subunit (LSU) rRNA gene revealed that the strains represent a novel species of the genus Cyberlindnera. However, asc and ascospores were not observed. In the present study, we describe this species as Cyberlindnera xishuangbannaensis f.a., sp. nov.

Rotting wood samples were collected from different locations in the Xishuangbanna Tropical Rainforest, Yunnan Province, China in 2016 [10]. The two strains, NYNU 16752† and NYNU 16785, were isolated from two samples of rotting wood collected in Menglun, Mengla. The other strain NYNU 16739 was recovered from rotting wood collected in Galan, Jinghong. The yeast strains were isolated from rotting wood samples in accordance with the methods described by Zheng et al. [10]. Each sample (1 g) was added to 20 ml sterile yeast extract-malt extract (YM) broth [1 % glucose (w/v), 0.5 % peptone (w/v), 0.3 % yeast extract (w/v) and 0.3 % malt extract (w/v); pH 5.4] supplemented with 0.02 % chloramphenicol (w/v) in a 150 ml Erlenmeyer flask and then incubated at 25 °C for 3 days on a rotary shaker. Subsequently, 0.1 ml enrichment culture and appropriate decimal dilutions were spread on YM agar plates.

Author affiliation: School of Life Science and Technology, Nanyang Normal University, Nanyang 473061, PR China.
*Correspondence: Feng-Li Hui, fenglihui@yeah.net
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Abbreviations: ITS, internal transcribed spacer; LSU, large subunit.
†These authors contributed equally to this work.
The GenBank/EMBL/DDBJ accession numbers for the sequences of the D1/D2 domains of the LSU rRNA gene and ITS region of Cyberlindnera xishuangbannaensis sp. nov. NYNU 16752† are KY213813 and KY213821, respectively.
supplemented with 0.02 % chloramphenicol (w/v) and then incubated at 25 °C for 3–4 days. Different yeast morphotypes were purified at least twice and then stored on YM agar slants at 4 °C or in 15 % (v/v) glycerol at −80 °C.

The yeast strains were characterized according to the standard procedures described by Kurtzman et al. [13]. Assimilation tests for carbon and nitrogen sources were performed in liquid media. Starved inocula were used in nitrogen assimilation tests. Ascosporulation was investigated on McClary’s acetate agar, corn meal agar (CMA), malt extract agar, V8 agar and yeast carbon base supplemented with 0.01 % ammonium sulphate (w/v; YCBAS) agar (1.1 % yeast carbon base, 0.01 % ammonium sulphate and 1.8 % agar; all w/v) in pure and mixed cultures at 15 and 25 °C [6, 7]. The cultures were examined weekly for up to 4 weeks.

Genomic DNA was extracted using an Ezup Column Yeast Genomic DNA Purification Kit according to the manufacturer’s protocol (Sangon Biotech). The D1/D2 domains of the LSU rRNA gene and ITS region were amplified by PCR and sequenced using primers NL1 and NL4 [14] and ITS1 and ITS4 [15], respectively. Each 50 µl PCR mixture included 21 µl of PCR-grade water, 1 µl DNA template, 1.5 µM of each primer and 1 µl PCR Master Mix (2x) (0.05 µl µl−1 de Taq DNA polymerase, reaction buffer, 4 mM MgCl₂, 0.4 mM of each dNTP; Sangon Biotech). PCR reactions were carried out in a S1000 thermal cycler (Bio-Rad Laboratories). The amplified products were purified with a QIAquick purification kit (Sangon Biotech), according to the manufacturer’s instructions. Sangon Biotech (Shanghai, China) performed direct sequencing of the purified LSU rRNA gene and ITS PCR products using primers NL1 and NL4, and ITS1 and ITS4 with a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems), according to the manufacturer’s protocol. Purified sequencing reaction mixtures were separated with a 3730XL automated DNA analyser (Applied Biosystems).

Pairwise sequence comparisons were made using a Basic Local Alignment Search Tool (BLAST) search [16] and aligned with the sequences of related species retrieved from GenBank by using the multiple alignment program, MUSCLE 3.8.31 [17]. A phylogenetic tree was reconstructed based on the concatenated sequences of the ITS and D1/D2 domains of the LSU rRNA gene with MEGA software version 7.0 [18]. The evolutionary distance data were calculated using the maximum composite likelihood method [19] in the neighbour-joining analyses. Pichia membranifaciens NRRL Y-2026T was used as an outgroup. The confidence levels of the clades were estimated through bootstrap analysis (1000 replicates) [20]. Only values greater than 50 % were recorded on the resulting tree. Reference sequences were retrieved from GenBank under the accession numbers indicated in the tree.

SPECIES DELINEATION, CLASSIFICATION AND ECOLOGY

We have isolated three strains (NYNU 16752T, NYNU 16785 and NYNU 16793), from independent rotten wood samples collected from two Xishuangbanna Tropical Rainforest sites. A sequence similarity search using MEGABLAST against the GenBank database and the D1/D2 LSU as query revealed that these three strains had identical sequences and belong to the genus Cyberlindnera (Fig. 1). In terms of pairwise sequence similarities, the three strains displayed 0.5 % sequence divergence (3 substitutions, 0 gap) with Candida pattaniensis CBS 11707T, 1.1 % sequence divergence (6 substitutions, 0 gap) with Candida stauntonica CBS 12241T and 1.4 % sequence divergence (8 substitutions, 0 gap) with Cyberlindnera fabianii CBS 5640T. Despite the close phylogenetic relationship based on the D1/D2 LSU sequences, a novel species of the genus Cyberlindnera could be readily distinguished from its closely related species by ITS sequencing. In the ITS region, strains NYNU 16752T, NYNU 16785 and NYNU 16793 showed identical nucleotide sequences. They had 5.4 % sequence divergence (21 substitutions, 10 gaps) compared with Candida pattaniensis CBS 11707T, 11.3 % sequence divergence (41 substitutions, 23 gaps) compared with Candida stauntonica CBS 12241T and 11 % sequence divergence (41 substitutions, 22 gaps) compared with Cyberlindnera fabianii CBS 5640T. Although D1/D2 LSU is still an appropriate region to use for higher level taxon delimitations, it is clear from the present study that the use of the D1/D2 LSU marker solely as a barcode is insufficient for the differentiation of all the species in the Cyberlindnera clade. The newly recommended fungal barcode, the ITS region [21], is a good alternative marker for increasing the accuracy of species discrimination among the Cyberlindnera clade.

A phylogenetic analysis, based on the concatenated sequences of the ITS and D1/D2 domains of the LSU rRNA gene, indicated that the novel taxon (as represented by strains NYNU 16752T, NYNU 16785 and NYNU 16793) belongs to the Cyberlindnera clade, and forms a distinct (bootstrap value, 99 %) species pair with Candida pattaniensis (Fig. 1). However, sequence comparisons of the ITS and D1/D2 domains of the LSU rRNA gene demonstrated greater diversity and supports the separation of the two species. All isolates and mixtures of these isolates were tested for ascospore formation on McClary’s acetate agar, corn meal agar (CMA), malt extract agar, V8 agar and yeast carbon base agar supplemented with 0.01 % ammonium sulphate (w/v) [6, 7]. No ascospores were observed at 15 or 25 °C after 4 weeks of incubation. Considering all the data described above, we propose a novel species Cyberlindnera xishuangbannaensis f.a., sp. nov.

Cyberlindnera xishuangbannaensis sp. nov. is physiologically similar to its closest phylogenetically recognized species Candida pattaniensis [4], but can be distinguished from the latter species based on growth in D-ribose, inulin, raffinose, ribitol and 2-keto-D-gluconate, which are positive for the novel species and negative for Candida pattaniensis. Given that these two species exhibit nearly identical growth
profiles, separating them is difficult on the basis of their phenotypic characteristics alone. Therefore, sequencing is recommended to differentiate these species.

The three strains of *Cyberlindnera xishuangbannaensis* sp. nov. recovered from independently rotting wood samples collected in Xishuangbanna Tropical Rainforest suggest that this substrate could represent its ecological niche. *Cyberlindnera xishuangbannaensis* sp. nov., *Candida hungchunana*, *Candida pattaniensis*, *Cyberlindnera xylosilytica*, *Cyberlindnera japonica* and many other species of the genus *Cyberlindnera* have been isolated from decaying wood, wood-ingesting insects and insect frass, and are able to assimilate cellobiose and xylose [2, 4–7], the building blocks of cellulose and hemicellulose, two major constituents of wood. It is probable that these yeasts are beneficiaries of the degradation of wood.

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![Phylogenetic tree](image-url)
Two yeast strains, NYNU 16752\textsuperscript{T} and NYNU 16785, were isolated from rotting wood collected in Menglun, Mengla, Yunnan Province, China. The strain NYNU 16793 was isolated from rotting wood collected in Galan, Jinghong, Yunnan Province, China. The type strain is strain NYNU 16752\textsuperscript{T}, permanently preserved in a metabolically inactive state in the China Centre of Industrial Culture Collection (CICC), Beijing, China, as strain CICC 33163\textsuperscript{T}. Ex-type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 14692\textsuperscript{T}. The MycoBank number is MB 822199.

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### Conflicts of interest
The authors declare that there are no conflicts of interest.

### References


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