Kodamaea neixiangensis f.a., sp. nov. and Kodamaea jinghongensis f.a., sp. nov., two yeast species isolated from rotting wood

Wan-Li Gao, Tian-tian Liu, Jun Zheng and Feng-Li Hui

Abstract

Seven strains representing two novel yeast species were isolated from rotting wood in Henan and Yunnan Provinces, PR China. The results of phylogenetic analysis based on the D1/D2 domains of the large subunit (LSU) rRNA gene revealed that these two species are members of the genus Kodamaea, although the formation of ascospores was not observed. Kodamaea neixiangensis f.a., sp. nov. (type strain NYNU 167139T=CICC 33170T=CBS 14699T) formed a clade with Candida kaohsiungensis and Candida hsintzibensis, from which it differed by 10–16 substitutions in the D1/D2 domain. The ITS sequences of K. neixiangensis sp. nov. differed by 27 substitutions from those of the type strain of C. kaohsiungensis. The most closely related species with a validly published name to Kodamaea jinghongensis f.a., sp. nov. (type strain NYNU 167162T=CICC 33171T=CBS 14700T) was Candida fukazawae, but this differed by 14 substitutions in the D1/D2 domain and by 15 substitutions in the ITS region.

The Kodamaea clade, which is part of the family Metschnikowiaeae, is recognizable from combined gene sequences for the nearly complete small subunit (SSU) rRNA and the large subunit (LSU) rRNA [1]. On the basis of the results of multigene phylogenetic analysis of the nearly entire LSU rRNA, SSU rRNA, translation elongation factor-1a (EF-1a), and RNA polymerase II subunits 1 (RPB1) and 2 (RPB2), this clade has been found to be closely related to the Aciculoc withholdum clade [2]. The Kodamaea clade currently consists of 22 species, among them five species with a sexual state, Kodamaea anthropila, Kodamaea kakaduensis, Kodamaea laetipori, Kodamaea nitidulidarum and Kodamaea ohmeri, and 18 species that are not known to form ascospores [1, 3–6]. In addition, several unpublished sequences of potential novel species deposited in the NCBI GenBank database have been clustered to the Kodamaea clade in phylogenetic analyses [4, 6]. K. ohmeri, the type species of the genus, appears to be a generalist, but the other species in the clade are associated with plants, mushrooms or nitidulid beetles [6]. K. ohmeri and Candida (iter. nom. Kodamaea) mesenterica are also found in clinical specimens [4, 7].

During a study on yeasts associated with rotting wood in PR China, seven unidentified yeast strains were obtained. The results of sequence analysis of the D1/D2 domains of the LSU rRNA gene indicated that these yeasts represent two novel species belonging to the Kodamaea clade. All the yeast strains exhibited affinity to the Kodamaea clade, but ascospores from the species were not found. In this study, we describe these two species as Kodamaea neixiangensis f.a., sp. nov. and Kodamaea jinghongensis f.a., sp. nov.

The yeast strains were isolated from rotting wood samples collected from two locations in PR China in 2016 in accordance with the method described by Hui et al. [8]. Each sample (1 g) was added to 20 ml sterile yeast extract–malt extract (YM) broth (1 % glucose, 0.5 % peptone, 0.3 % yeast extract and 0.3 % malt extract; pH 5.4) supplemented with 0.02 % chloramphenicol 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 supplemented with 0.02 % chloramphenicol 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 % (w/v) glycerol at −80 °C.

The yeast strains were characterized by standard procedures described by Kurtzman et al. [9]. Assimilation tests for

Author affiliations: 1 School of Agricultural Engineering, Nanyang Normal University, Nanyang 473061, PR China; 2 School of Life Science and Technology, Nanyang Normal University, Nanyang 473061, PR China.

*Correspondence: Feng-Li Hui, fenglihui@yeah.net

Keywords: Kodamaea neixiangensis; Kodamaea jinghongensis; rotting wood.

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DBJ accession numbers for the sequences of the ITS region and the D1/D2 domains of the LSU rRNA gene of Kodamaea neixiangensis sp. nov. NYNU 167139T are KY213808 and KY213820, respectively; those of Kodamaea jinghongensis sp. nov. NYNU 167162T are KY213814 and KY213807, respectively.

002117 © 2017 IUMS

Downloaded from www.microbiologyresearch.org by
IP: 53358.10.11
On: Thu, 03 Jan 2019 11:39:21
carbon and nitrogen sources were performed in liquid media. Starved inocula were used in nitrogen assimilation tests. Ascosporulation was investigated on YM agar, 5% malt extract agar (MEA), corn meal agar (CMA), V8 agar, Gorodkowa agar, McClary’s acetate agar and yeast carbon base supplemented with 0.01% ammonium sulphate (YCBS) agar (1.1% yeast carbon base, 0.01% ammonium sulphate and 1.8% agar) in pure and mixed cultures at 17 and 25°C. The cultures were examined weekly for up to 4 weeks.

Genomic DNA was extracted using Dr. GenTLE (from Yeast) High Recovery in accordance with the manufacturer’s protocol (Sangon Biotech). The D1/D2 domains of the LSU rRNA gene and ITS regions were amplified by PCR, and sequenced using primers NL1 and NL4 [10] and ITS1 and ITS4 [11], respectively. PCR conditions recommended in the references for each primer pair were adopted. Both DNA strands were sequenced using a dye terminator cycle sequencing kit (Applied Biosystems).

Pairwise sequence comparisons were made using Basic Local Alignment Search Tool (BLAST) search [12] and aligned with the sequences of related species retrieved from GenBank by using the multiple alignment program CLUSTAL_X version 1.81 [13]. A phylogenetic tree based on the D1/D2 domains of the LSU rRNA gene sequences was reconstructed using the neighbour-joining method in MEGA 5.0 [14]. The evolutionary distances were calculated using the two-parameter model of Kimura [15] for the neighbour-joining analyses. Candida melibiosica CBS 5814\(^T\) was used as the outgroup. The confidence levels of the clades were estimated through bootstrap analysis (1000 replicates) [16]. Only values greater than 50% were recorded on the resulting tree. Reference sequences were retrieved from GenBank under the accession numbers indicated on the tree.

**SPECIES DELINEATION, CLASSIFICATION AND ECOLOGY**

The results of phylogenetic analysis of D1/D2 LSU rRNA genes indicated that the seven novel strains represented members of the *Kodamaea* clade and could be classified into two taxa representing novel species. The D1/D2 and ITS sequences were identical for members within each taxon, but clearly distinguishable from those of other undescribed or previously known species.

The novel species represented by strains NYNU 167139\(^T\), NYNU 16831, NYNU 1679, NYNU 16845 and NYNU 16855 was most closely related to *Candida* sp. GJ20M04, *Candida kaohsiungensis* CBS 11435\(^T\) and *Candida hsintzibiensis* CBS 11427\(^T\) (Fig. 1). The species differed by only five substitutions from its close relative *Candida* sp. GJ20M04 in the D1/D2 domain but differed by 15 substitutions in the ITS region. The differences observed in the ITS sequences were found to be significant enough for this novel species and *Candida* sp. GJ20M04 to be considered two distinct species. In addition, five strains of the novel species differed by 10 substitutions from the type strain of *C. kaohsiungensis* and by 16 substitutions from the type strain of *C. hsintzibiensis*. The ITS sequences of the five strains differed by 27 substitutions from the type strain of *C. kaohsiungensis* but could not be successfully aligned with the sequences of *C. hsintzibiensis*, which contained ITS sequences not currently available from either the NCBI GenBank database or the CBS database. Additionally, the five strains were separated from *C. kaohsiungensis* and *C. hsintzibiensis* [3] on the basis of carbon assimilation and other physiological characteristics. They differed from *C. kaohsiungensis* by their ability to assimilate inulin and soluble starch and growth in the presence of 0.01% cycloheximide and at 30°C and their inability to assimilate DL-glucono-1,5-lactone and ethanol, and from *C. hsintzibiensis* by their ability to assimilate inulin and soluble starch and growth at 30°C and their inability to assimilate ethanol. The results of molecular and phenotypic comparisons indicated that these five strains represent a novel species of the *Kodamaea* clade, but they have not been observed to produce ascospores on common sporulation media. The name *Kodamaea neixiangensis* f.a., sp. nov. is proposed to accommodate the strains of this novel species.

The other novel species, represented by strains NYNU 167162\(^T\) and NYNU 16897, formed a well-supported clade with *Candida fukazawae* CBS 9137\(^T\) and five undescribed yeast strains in a tree reconstructed using the D1/D2 LSU rRNA gene sequences (Fig. 1). The species distinctly differed from the most closely related species, *Candida fukazawae* CBS 9137\(^T\), by 14 substitutions in the D1/D2 domain and by 15 substitutions in the ITS region. Five undescribed yeast strains could be classified into three species group (Fig. 1). Each species group shared similar D1/D2 sequences (0–2 substitutions), which differed by 13–15 substitutions from the novel species represented by strains NYNU 167162\(^T\) and NYNU 16739, indicating that they are not conspecific. The physiological characteristics of the two strains of the novel species can be obviously differentiated from the most closely related species with a validly published name, *C. fukazawae* [7], by their ability to ferment maltose and sucrose and to assimilate galactose, inulin and soluble starch and their inability to assimilate DL-lactate and ethanol. On the basis of the evidence from molecular and phenotypic comparison, we concluded that these two strains represent a novel species of the *Kodamaea* clade, although formation of ascospores was not observed. The name *Kodamaea jinghongensis* f.a., sp. nov. is proposed for strains NYNU 167162\(^T\) and NYNU 16897.

Three undescribed yeast species cluster near the type strain of *K. jinghongensis* sp. nov. (Fig. 1). Among the sequences of these undescribed species, *Kodamaea* sp. NYNU 14731, deposited by the authors of this study, has not yet been satisfactorily characterized. Therefore, in this paper, only *K. neixiangensis* sp. nov. and *K. jinghongensis* sp. nov. are proposed for the seven strains isolated from rotting wood in China.
All strains of the two novel species in this study were isolated from rotting wood samples in China. Four strains of *K. neixiangensis* sp. nov. were isolated in Baotianman Nature Reserve, Henan Province, but another one was found in Xishuangbanna Tropical Rainforest in Yunnan Province. The two localities were separated from each other by approximately 2189 km. The existence of strains of *K. neixiangensis* sp. nov. in two such widely separated locations indicates a broad range of occurrence. Species of the *Kodamaea* clade have been isolated mostly from flowers, fruits, mushrooms and associated insects [1, 3–7]. The two novel species, *K. neixiangensis* sp. nov. and *K. jinghongensis* sp. nov., were clustered into subclade A [1, 3]. The previously described members of the subclade such as ‘Endomyces scopularum’, *Candida sagamina*, *Candida fukazawae*, *C. hsintzibuenis*, *C. kaohsiungensis* and *K. laetipori* were recovered from mushrooms or basidiocarp-feeding beetles [1, 3, 4, 7]. The presence of the novel species from rotting wood in this study may be a consequence of these yeasts being carried to rotting wood by visiting basidiocarp-feeding beetles. Therefore, insect-associated sources such as rotten wood are a source for further investigation of yeasts in this clade.

**DESCRIPTION OF KODAMAEA NEIXIANGENSIS**

HUI AND ZHENG SP. NOV

*Kodamaea neixiangensis* (nei.xiang.en‘sis. N.L. fem. adj. neixiangensis of or belonging to the county of Neixiang,
Henan Province, PR China, the collection locality of the type strain of the species).

In YM broth after 3 days at 25°C, the cells are mostly ellipsoidal (2.5–5×6–9 µm) and occur singly or in pairs. Budding is multilateral (Fig. 2a). Sediment and pellicle are formed after 1 month. On YM agar after 6 days at 25°C, colonies are cream, butyrous and rough, with filamentous margins. After 2 weeks in Dalmau plate culture on corn meal agar at 25°C, pseudohyphae and true mycelia are formed. Asci or signs of conjugation are not seen on sporulation media. Glucose and trehalose are fermented but not galactose, maltose, sucrose, melibiose, lactose, cellubiose, melezitose, raffinose, inulin, soluble starch or xylene. Glucose, inulin, sucrose, trehalose, maltose, melezitose, methyl α-D-glucoside, soluble starch (weakly), cellubiose, salicin, D-xylose (weakly), glycerol, ribitol, mannnitol, glucitol, succinate, citrate, D-glucosamine, 2-keto-D-gluconate and arbutin are assimilated. No growth occurs in raffinose, melibiose, galactose, lactose, L-sorbose, L-rhamnose, L-arabinose, D-arabinose, D-ribose, manhanol, ethanol, erythritol, galactitol, myo-inositol, DL-lactate, D-glucunate, xylitol, D-glucuronate, D-glucuno-1,5-lactone, L-arabinol or 5-keto-D-glucunate. With respect to the assimilation of nitrogen compounds, L-lysine, glucosamine and D-trytophan are assimilated, whereas nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine and imidazole are not assimilated. Growth is observed at 30°C but not at 35°C. Growth in the presence of 0.01% cycloheximide is positive, but growth in the presence of 0.1% cycloheximide, 10% NaCl plus 5% glucose and 1% acetic acid is negative. Starch-like compounds are not produced. Urease activity and diazoinium blue B reactions are also negative.

Four yeast strains, NYNU 167162T, NYNU 16831, NYNU 16845 and NYNU 16855 were isolated from rotting wood collected in Baotianman Nature Reserve, Henan Province, PR China. The strain NYNU 1679 was isolated from rotting wood collected in Xishuangbanna Tropical Rainforest in Yunnan Province, PR China. The type strain is NYNU 167162T, permanently preserved in a metabolically inactive state in the China Centre of Industrial Culture Collection (CICC), Beijing, PR China, as strain CICC 33170T. The type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 14699T. The MycoBank number is MB 820935.

**DESCRIPTION OF KODAMAEA JINGHONGENSIS HUI AND ZHENG SP. NOV**

Kodamea jinghongensis (jing.hong.en’sis. N.L. fem. adj. jinghongensis of or belonging to the of Jinghong, Yunnan Province, PR China, where the type strain of the species was isolated).

In YM broth after 3 days at 25°C, the cells are ellipsoidal or ovoidal (1.5–4×3–9 µm) and occur singly or in pairs. Budding is multilateral (Fig. 2b). Sediment is formed after 1 month, but no pellicle is observed. On YM agar after 6 days at 25°C, colonies are white, convex and smooth with a sector edge. On Dalmau plates after 2 weeks on cornmeal agar, pseudohyphae and true mycelia are formed. Asci or signs of conjugation are not seen on sporulation media. Glucose, maltose, sucrose and trehalose are fermented but not galactose, melibiose, lactose, cellubiose, melezitose, raffinose, inulin, soluble starch or xylene. Glucose, inulin, sucrose, galactose (weakly), trehalose, maltose, methyl α-D-glucoside, soluble starch (weak), cellubiose, salicin, D-xylose (weakly), glycerol, erythritol, ribitol, mannnitol, glucitol, succinate, citrate, D-glucosamine, 2-keto-D-glucunate, D-glucuno-1,5-lactone and arbutin are assimilated. No growth occurs in raffinose, melibiose, lactose, cellubiose, melezitose, raffinose, inulin, soluble starch or xylene. Glucose, inulin, sucrose, galactose (weakly), trehalose, maltose, methyl α-D-glucoside, soluble starch (weak), cellubiose, salicin, D-xylose (weakly), D-ribose (weakly), glycerol, erythritol, ribitol, mannnitol, glucitol, succinate, citrate, D-glucosamine, 2-keto-D-glucunate, D-glucuno-1,5-lactone and arbutin are assimilated. No growth occurs in raffinose, melibiose, lactose, melezitose, L-sorbose, L-rhamnose, L-arabinose, D-arabinose, methanol, ethanol, galactitol, myo-inositol, DL-lactate, D-glucunate, xylitol, D-glucuronate, L-arabinol or 5-keto-D-glucunate. With respect to the assimilation of nitrogen compounds, L-lysine, glucosamine and D-tryptophan are assimilated, whereas nitrate, nitrite, ethylamine, cadaverine, creatine, creatinine and imidazole are not assimilated. Growth is observed at 35°C but not at 37°C. Growth in the presence of 0.1% cycloheximide is positive, but growth in the presence of 10% NaCl plus 5% glucose and 1% acetic acid is negative. Starch-like compounds are not produced. Urease activity and diazoinium blue B reactions are also negative.

Two yeast strains, NYNU 167162T and NYNU 16897 were isolated from rotting wood collected in Xishuangbanna Tropical Rainforest in Yunnan Province, PR China. The type strain is NYNU 167162T, permanently preserved in a metabolically inactive state in the China Centre of Industrial Culture Collection (CICC), Beijing, PR China, as strain CICC 33171T. The type culture has been deposited in the collection of the Yeast Division of the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, as strain CBS 14700T. The MycoBank number is MB 820939.

**Funding information**

This work was supported by the National Natural Science Foundation of China (grant numbers 31570021 and 31370073).
Conflicts of interest
The authors have declared that there is no conflict of interest.

References

Five reasons to publish your next article with a Microbiology Society journal
1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at microbiologyresearch.org.