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ORIGINAL PAPER

Kondoa gutianensis f.a. sp. nov., a novel ballistoconidium-forming yeast species isolated from plant leaves

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Abstract Two strains, GT-165^T and GT-261, isolated from plant leaves collected from Gutian Mountain in Zhejiang province in China were identified as a novel species of the genus *Kondoa* by the sequence analysis of the internal transcribed spacer (ITS) region, the D1/D2 domains of the large subunit of rRNA (LSU rRNA) and the RNA polymerase II second largest subunit (*RPB2*), complemented by physiological tests. Phylogenetic analysis based on the concatenated sequences of ITS, D1/D2 and *RPB2* showed that the closest known relatives of the new species are three undescribed *Kondoa* species and *Kondoa thailandica*. The ITS and D1/D2 sequences of the new species differ from the closely related species by 11–22% and 2–9%, respectively. The name *Kondoa gutianensis* f.a. sp. nov. (MB 820648, holotype = CGMCC 2.5703^T; isotype: CBS 14811^T = CGMCC 2.5703^T) is proposed to accommodate the new taxon.

Keywords Basidiomycetous yeasts · *Kondoa gutianensis* · Molecular phylogeny · Plant leaves

Introduction

The genus *Kondoa* Y. Yamada, Nakagawa & Banno was erected to accommodate a single species, *Kondoa malvinella* (Fell & Hunter) Y. Yamada, Nakagawa & Banno, which was transferred from the genus *Rhodosporidium* Banno based on significant differences in the 5S and 26S rRNA nucleotide sequences to *Rhodosporidium toruloides*, the type species of the genus (Yamada et al. 1989, 1990). This species was later reinvestigated and it was concluded that teliospores were not produced. Another trait differing from typical *Rhodosporidium* species was the production of forcibly discharged basidiospores. A new species, *Kondoa aeria*, was proposed simultaneously (Fonseca et al. 2000). Phylogenetic analyses based on rRNA gene sequences indicated that some species of the anamorphic genus *Bensingtonia* and the *Kondoa* species formed a monophyletic group with strong statistical support (Nakase and Suzuki 1987, 1988; Van der Walt et al. 1989; Fungsin et al. 2001; Wang et al. 2003; Bauer et al. 2006). The family Kondoaceae

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was then proposed for this group which belongs to Agaricostilbomycetes, Pucciniomycotina (Bauer et al. 2006). Recently, the basidiomycetous yeasts in Pucciniomycotina were reclassified based on multigene phylogeny and the two genera *Kondoa* and *Bensingtonia* in *Kondoaceae* were emended (Wang et al. 2015a, b). The genus *Kondoa* was expanded to include nine species, including seven anamorphic species, namely *K. changbaiensis*, *K. miscanthi*, *K. phyllada*, *K. sorbi*, *K. subrosea*, *K. thailandica* and *K. yuccicola* that previously belonged to the genus *Bensingtonia* (Wang et al. 2015b). In a survey of phylloplane yeast diversity in subtropical evergreen broad-leaved forest of China, 49 leaf samples were collected in Gutian Mountain, Zhejiang Province in July 2010. Approximately 800 strains were isolated from those samples and classified into 52 known species of 31 genera. One novel *Kondoa* species represented by two strains was identified. The name *Kondoa gutianensis* f.a. sp. nov. is proposed.

Materials and methods

The two yeast strains, GT-165^T and GT-261, belonging to a novel species were isolated from plant leaves of *Uncaria rhynchophylla* and *Sapium sebiferum*, respectively, using the improved ballistoconidia-fall method described by Nakase and Takashima (1993). They were collected from Gutian Mountain (29°10′19.4″–29°17′41.4″N, 118°03′49.7″–118°11′12.2″E; annual precipitation 1963.7 mm; annual average temperature 15.3 °C), Zhejiang Province, China. The phenotypic and physiological characters were examined according to the standard methods used in yeast taxonomy (Kurtzman et al. 2011).

Genomic DNA was extracted from yeast cells that were actively growing on YPD medium following the protocol described by Makimura et al. (1994). The E.Z.N.A.® Gel Extraction Kit (Omega Bio-tek, USA) was used when high quality DNA templates were required for PCR amplification of protein genes. The ITS region (including the 5.8S rRNA gene) and D1/D2 domains of the LSU rRNA gene were amplified using the protocols described previously (Bai et al. 2002). The *RPB2* genes were amplified and sequenced according to Liu et al. (2015).

Multiple sequences alignment was performed using MAFFT version 7 and the G-INS-I option (Standley 2013). Maximum likelihood (ML) and Bayesian analyses were conducted for separate and combined nucleotide sequences using RAxML v8.1.X (Stamatakis 2014) with 1000 bootstrap replicates and MrBayes 3.2.2 (Ronquist et al. 2012) with 5 million generations, respectively. The best-fit evolution model was analysed with jModeltest (Posada 2008), and TIM2 + I + G model was suggested as the best model for the combined sequences. The sequence divergences were calculated using DnaSP v5.10 (Librado and Rozas 2009). The GenBank accession numbers for the sequences of the ITS region, D1/D2 domain and *RPB2* determined in this study are KY767666-KY767669, respectively.

Results and discussion

Phylogenetic analysis

The two strains, GT-165^T and GT-261, isolated from different plant leaves possessed identical D1/D2 and *RPB2* gene sequences, and differed from each other by one substitution in the ITS region. The BLAST search of the D1/D2 and ITS sequence against the GenBank database revealed that the closest hits are three undescribed *Kondoa* strains, namely TUB ZP352, CBS8379 and AS483, with similarity below 98 and 86%, respectively. Phylogenetic analyses were done based on single and concatenated sequences of the ITS, D1/D2 and *RPB2* genes from the new strains and type strains of related taxa using maximum likelihood and Bayesian inference algorithms (Figs. 1, S1). These two strains were located in the genus *Kondoa*, Kondoaceae of Agaricostilbales (Fig. 1; Wang et al. 2015b). They were found to be closely related to three undescribed *Kondoa* species represented by strains AS 483, CBS 8379 and TUB ZP352. GT-165^T differed from CBS 8379 and AS 483 by 15 nucleotides (nt) in the D1/D2 domain, and differed from strain TUB ZP352 by 14 nt. More than 11% nucleotide mismatches was found in the ITS region between GT-165^T and *Kondoa* sp. '*myxariophila*' CBS 8379. Among the described species, strain GT-165^T was most closely related to *K. thailandica*, but differed from the type strain of the latter by 9% and 22% nucleotide mismatches in the D1/D2 and ITS sequences, respectively. These results suggest that the two new strains represent a novel *Kondoa* species,

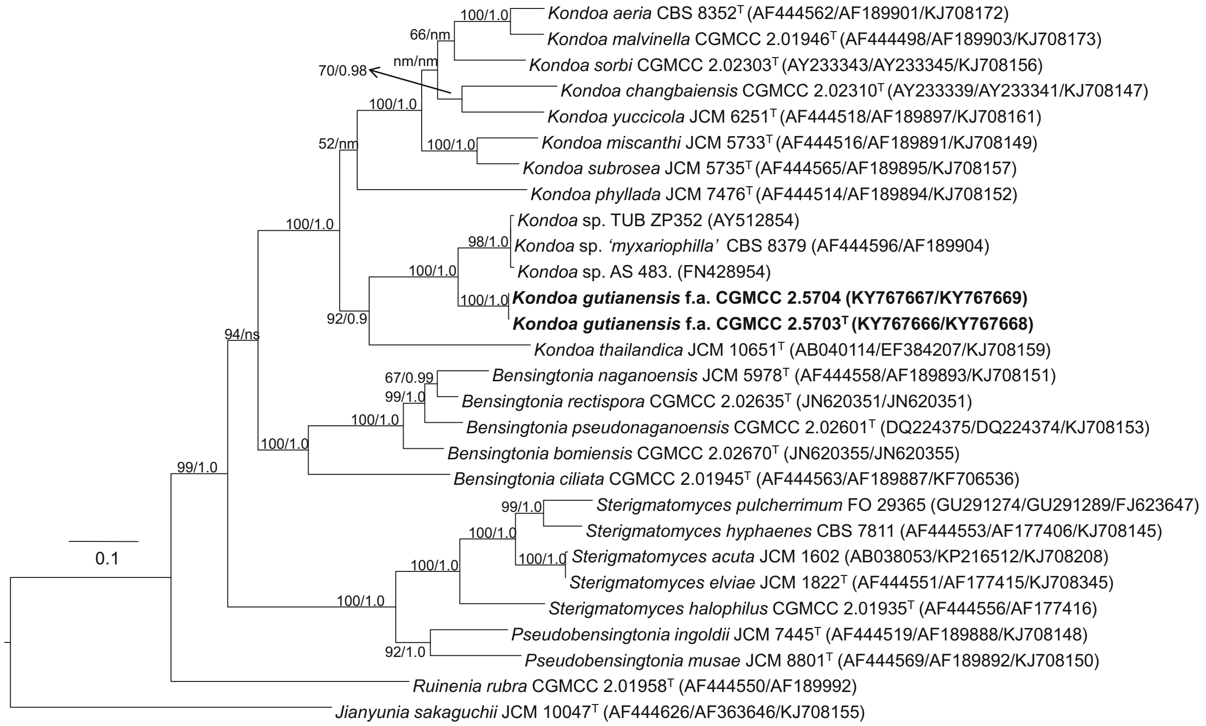

Fig. 1 The phylogenetic relationships of the novel species *Kondoa gutianensis* f.a. with related taxa in Agaricostilbales. The tree backbone was constructed using maximum likelihood analysis of the combined sequences of the ITS region (including 5.8S rRNA), LSU rRNA D1/D2 domains and *RPB2* genes. Bootstrap percentages (BP) over 50% from 1000 replicates and posterior probabilities (PP) of Bayesian inference above 0.9 are shown respectively from left to right. *Note*: *ns* not supported (BP < 50% or BP < 0.9); *nm* not monophyletic

for which the name *Kondoa gutianensis* f.a. sp. nov. is proposed.

Morphology, physiology and ecology

These two strains formed cream and butyrous colonies like other *Kondoa* species. Ballistoconidia were formed. Sexual structures were not observed in the cultures of single strains or mixed strains on CMA agar. The novel species can be distinguished from theclosely related taxon *K. thailandica* by the ability to utilize ethanol, citrate, inulin, DL-lactic acid, melibiose, raffinose, ribitol, salicin, soluble starch, succinic acid and nitrite (Table 1). In addition, the novel species can grow in vitamin-free medium, unlike the other *Kondoa* species. Thus we propose these two strains as a new species, namely *Kondoa gutianensis* f.a. sp. nov.

Plant leaves have been found to be the main habitat of *Kondoa* species (Nakase and Suzuki 1987, 1988; Van der Walt et al. 1989; Fungsin et al. 2001; Wang et al. 2003). *Kondoa* species also appear in marine environments (Fell 1970; Laurenavichene et al. 1989; Fonseca et al. 2000). The two strains of the novel species were isolated from plant leaves in a subtropical evergreen broad-leaved forest of southeast China. *Kondoa gutianensis* f.a. sp. nov. seems to be a rare member of the yeast community in the phyllosphere. These two strains were only isolated from two samples of 49 different plant leaf samples in our investigation. From the plant leaf yielding the strains GT-165^{T} and GT-261, a total of 98 and 20 yeast strains belonging to 12 and 3 described species were isolated, respectively. More than half of the strains were classified as *Sporobolomyces carnicolor* in these two plant leave samples. The following yeast species were isolated as well, namely *Bannoa ogasawarensis*, *Bullera alba*, *Bulleribasidium pseudovariabilis*, *Derxomyces mrakii*, *Derxomyces pseudoschimicola*, *Derxomyces qinlingensis*, *Dioszegia zsoltii*, *Rhodotorula mucilaginosa*, *Saitozyma podzolica*, *Sporobolomyces koalae* and *Vanrija humicola*.

Table 1 Physiological properties of the *Kondoa* species and closely related taxa

	1	2[a]	3[b]	4[c]	5[d]	6[e]	7[f]	8[g]	9[h]	10[i]
Assimilation of carbon compounds										
D-galactose	l/w	v	−	+	l	−	l	−	−	−
Melibiose	−	+	−	−	−	−	−	−	l	−
Melezitose	+	+	−	−	−	−	−	−	−	+
Raffinose	−	+	+	l/w	+	l	−	v	l	−
L-arabinose	w	−	v	v	lw	−	−	−	−	−
L-rhamnose	−	−	+	−	−	−	−	−	−	+
Ethanol	+	−	v	−	−	−	−	−	−	+
Ribitol	l	−	v	+	lw	lw	l/lw	+	+	+
Citrate	−	+	v	+	−	−	lw	−	+	−
DL-lactic acid	−	v	+	l	−	−	−	v	l	−
Succinic acid	−	+	+	+	−	−	s	+	+	+
Salicin	+	−	+	+	−	−	−	w	−	+
Inulin	+	−	−	−	−	lw	−	−	−	−
Soluble starch	−	+	+	+	−	l	−	l	−	−
Assimilation of nitrogen compounds										
Nitrate	+	+	+	l	−	+	+	−	w	+
Nitrite	−	+	+	v	−	+	+	−	w	+
Other test										
Vitamin-free	+	−	−	−	−	−	−	−	−	−

Species: 1, *Kondoa gutianensis* f.a. sp. nov.; 2, *K. thailandica*; 3, *K. malvinella*; 4, *K. aeria*; 5, *K. sorbi*; 6, *K. changbaiensis*; 7, *K. yuccicola*; 8, *K. subrosea*; 9, *K. miscanthi*; 10, *K. phyllada*

+ positive, *l* delayed positive, *w* weakly positive, *lw* delayed and weakly positive, *s* slow positive, − negative, *v* variable

[a] Results taken from Fungsin et al. (2001)

[b] Results taken from Fonseca (2011)

[c] Results taken from Fonseca et al. (2000)

[d] Results taken from Wang et al. (2003)

[e] Results taken from Wang et al. (2003)

[f] Results taken from Nakase and Suzuki (1988)

[g] Results taken from Nakase and Suzuki (1987)

[h] Results taken from Nakase and Suzuki (1987)

[i] Results taken from Van der Walt et al. (1989)

Description of *Kondoa gutianensis* X.-Z. Liu, F.-Y. Bai, M. Groenew. & T. Boekhout sp. nov., MycoBank number MB 820648

Etymology: The specific epithet *gutianensis* (gu.-tian.en'sis. N.L. fem. adj.) refers to the geographical origin of the type strain of this species.

After 8 days of growth on YM agar at 20 °C, cells are ellipsoidal or cylindrical, 2.0–5.0 μm × 6.0–9.0 μm (Fig. 2a), and occur singly. Budding is polar. The streak culture is cream-coloured, butyrous, shiny, somewhat slimy with a smooth surface and an entire margin. In Dalmau plate culture on corn meal agar, septate hyphae are formed (Fig. 2b). No clamp connections are observed. Ballistoconidia are produced on CMA agar and are reniform or allantoid, 2.0–4.5 μm × 5.5–10.0 μm (Fig. 2c). Fermentation of glucose is negative. Glucose, galactose (delayed or

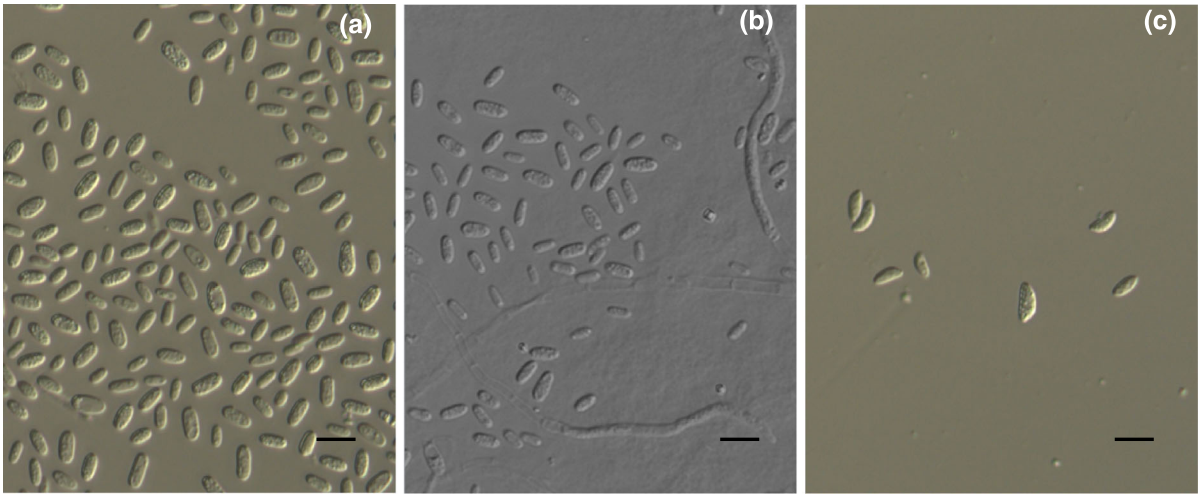

Fig. 2 Morphology of *Kondoa gutianensis* f.a. CGMCC 2.5703^T. **a** Vegetative cells grown on YM agar for 8 days at 20 °C; **b** septate hyphae grown on CMA agar for 8 days at 20 °C; **c** ballistoconidia produced on corn meal agar for 8 days at 20 °C, bars 10 μm

weak), L-sorbose (variable), cellobiose (variable), maltose, sucrose, trehalose, melezitose, D-arabinose (variable), L-arabinose (weak), D-xylose (variable), ethanol, galactitol (variable), D-glucitol (delayed), glycerol, D-mannitol (delayed), ribitol (delayed), α-methyl-D-glucoside (variable), salicin and inulin are assimilated. Lactose, melibiose, raffinose, D-ribose, L-rhamnose, erythritol, inositol, methanol, citrate, D-glucuronic acid, DL-lactic acid, succinic acid, D-glucosamine, hexadecane and soluble starch are not assimilated. Ammonium sulfate and potassium nitrate are assimilated. Sodium nitrite, L-lysine, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Growth in vitamin-free medium is positive. Starch-like compounds are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. Growth at 25 and 30 °C is positive and at 32 °C is negative. Sexual structures are not observed in the cultures of single strains or mixed strains on CMA agar. The holotype strain, GT-165^T, was isolated from a leaf of *Uncaria rhynchophylla* collected in Gutian Mountain, Zhejiang Province, China in July 2010. This strain has been permanently preserved in a metabolically inactive state in the China Collection Center (CGMCC), Academia Sinica, Beijing, China (CGMCC 2.5703^T) as lyophilized culture. The isotype culture: CBS 14811^T = CGMCC 2.5703^T.

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Conflict of interest The authors declare that they have no conflict of interest.

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