Bensingtonia rectispora sp. nov. and *Bensingtonia bomiensis* sp. nov., ballistoconidium-forming yeast species from Tibetan plant leaves

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Five yeast strains isolated from plant leaves collected in south-east Tibet formed cream to brownish colonies and produced asymmetrical ballistoconidia and CoQ-9 as the major ubiquinone. Sequence analysis of the 26S rRNA D1/D2 domain and the internal transcribed spacer region indicated that these strains represented two novel species of the genus *Bensingtonia*. The names *Bensingtonia rectispora* sp. nov. (type strain XZ 4C5^T=CGMCC 2.02635^T=CBS 10710^T) and *Bensingtonia bomiensis* sp. nov. (type strain XZ 33D1^T=CGMCC 2.02670^T=CBS 10713^T) are proposed for the two novel species, which are phylogenetically closely related to *Bensingtonia naganoensis*, *Bensingtonia pseudonaganoensis* and the type species of the genus, *Bensingtonia ciliata*.

The genus *Bensingtonia* was described by Ingold (1986) and emended by Nakase & Boekhout (1988) to accommodate basidiomycetous yeasts characterized by the production of ballistoconidia, the formation of CoQ-9 as the major ubiquinone and the absence of xylose in the cell wall (Nakase *et al.*, 2011). The genus is highly polyphyletic though only 15 species are currently recognized (Takashima *et al.*, 1995; Fell *et al.*, 2000; Hamamoto & Nakase, 2000; Scorzetti *et al.*, 2002; Wang *et al.*, 2003, 2006; Nakase *et al.*, 2011). Thirteen *Bensingtonia* species, including the type species *Bensingtonia ciliata*, belong to the Agaricostilbales in the Agaricostilbomycetes, whereas the other two species, *Bensingtonia yamatoana* and *Bensingtonia intermedia* (*Mastigobasidium intermedium*), are located in separate lineages of the Microbotryomycetes (Boekhout *et al.*, 2011; Nakase *et al.*, 2011). The *Bensingtonia* species in the Agaricostilbales occur in at least four different lineages or clades intermingled with species of *Agaricostilbum*, *Kondoa* and *Sterigmatomyces* (Fell *et al.*, 2000; Scorzetti *et al.*, 2002; Wang *et al.*, 2003, 2006; Boekhout *et al.*, 2011; Nakase *et al.*, 2011). Only two species, *Bensingtonia naganoensis* and *Bensingtonia pseudonaganoensis*, cluster together with the type species (*B. ciliata*) in a strongly supported clade (Wang *et al.*, 2006; Nakase *et al.*, 2011). Reclassification of *Bensingtonia* and related genera will be necessary in the

future and the addition of novel species to the *B. ciliata* clade will be valuable for both phenotypic and phylogenetic redefinition of the genus.

In recent years, we have been investigating phyllosphere yeast diversity. Of the ballistoconidium-forming yeast strains isolated from Tibet, five strains that formed bilaterally symmetrical ballistoconidia and possessed CoQ-9 as the major ubiquinone were isolated; studies revealed that these strains represent two novel *Bensingtonia* species closely related to the type species *B. ciliata*.

Strains XZ 4C5^T, XZ 5C1-D, XZ 9C3-D, XZ 14B3-2 and XZ 33D1^T (Table 1) were all isolated from wilting plant leaves collected from mixed coniferous and broad leaved forests in Nyingchi (coordinates: 30° 17' N 94° 40' E) and Bomi (coordinates: 30° 48' N 95° 44' E) counties, south-east Tibet, China, in July 2004, by using the improved ballistoconidia-fall method as described by Nakase & Takashima (1993). The two counties are adjacent, are approximately 3000 m above sea-level and have similar climates, with mean annual temperatures of 8–9 °C and annual precipitation of 600–900 mm. Most of the morphological, physiological and biochemical characteristics were examined according to standard methods (Kurtzman *et al.*, 2011). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973).

Nuclear DNA was extracted by using the method of Makimura *et al.* (1994). The internal transcribed spacer

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the ITS region and 26S rRNA gene D1/D2 domain sequences of strains XZ 4C5^T, XZ 5C1-D, XZ 9C3-D, XZ 14B3-2 and XZ 33D1^T are JN620351–JN620355, respectively.

33D1^T were located in a well-supported clade together with *B. ciliata*, *B. naganoensis* and *B. pseudonaganoensis* (Fig. 1). The new strains were more closely related to the latter two species than to *B. ciliata*. Strain XZ 4C5^T differed from *B. naganoensis* and *B. pseudonaganoensis* by 8 (1.2 %) and 11 (1.6 %) substitutions in the D1/D2 domain, respectively. Strain XZ 33D1^T differed from the two described species by 16 (2.4 %) and 17 (2.5 %) substitutions in the D1/D2 domain, respectively. In the ITS region, the two novel strains XZ 4C5^T and XZ 33D1^T differed from the two closely related described species by 6–13 % mismatches. The results suggest that the two groups represent two novel *Bensingtonia* species, for which the names *Bensingtonia rectispora* sp. nov. and *Bensingtonia bomiensis* sp. nov. are proposed.

It is apparent that more extensive molecular studies aiming to clarify the fine phylogenetic relationships among *Bensingtonia* and related basidiomycetous yeast species will be necessary for the reclassification of *Bensingtonia* as well as other polyphyletic basidiomycetous yeast genera. The addition of novel species to the phylogenetic trees will be helpful for the recognition of monophyletic groups, as shown in our previous studies (Wang & Bai, 2008; Wang *et al.*, 2011). The present study strengthens the monophyletic nature of the *B. ciliata* clade, on which redefinition of the genus *Bensingtonia* will be based. Although *B. bomiensis* is only represented by a single strain at present, its clear phylogenetic isolation suggests that the proposal of the novel species is beyond question.

Morphology and physiology

All the strains studied formed cream to brownish colonies and ellipsoidal, elongate or cylindrical vegetative cells (Fig. 2a and Fig. 3a). The major ubiquinone of all these strains was CoQ-9. They produced bilaterally symmetrical ballistoconidia on corn meal agar. The above characteristics are typical for the genus *Bensingtonia*. The ballistoconidia formed by *B. rectispora* strains were nearly straight (Fig. 2b), whereas those formed by *B. bomiensis* strain XZ 33D1^T were usually curved (Fig. 3b). Sexual cycles were not observed in the cultures of single strains of the two novel species or mixed strains of *B. rectispora* compared on various media.

Although *B. rectispora* is distinguishable from its close relatives by its ballistoconidium morphology, this novel species exhibited similar physiological characters to *B. bomiensis*, *B. naganoensis* and *B. pseudonaganoensis* (Table 2). These four closely related species clearly differed from *B. ciliata* in the assimilation reactions of sucrose, melzitose, ethanol and citric acid (Table 2).

Latin diagnosis of *Bensingtonia rectispora* F.-Y. Bai et Q.-M. Wang sp. nov.

In YM (Difco) liquido post dies 5 ad 17 °C, cellulae vegetativae longae ellipsoideae vel cylindricae, 2.5–5.6 × 5.0–11.0 µm,

Fig. 2. *Bensingtonia rectispora* sp. nov. XZ 4C5^T vegetative cells grown in YM broth for 5 days at 17 °C (a) and ballistoconidia produced on corn meal agar after 5 days at 17 °C (b). Bars, 10 µm.

singulae. Sedimentum formatur. Post unum mensem ad 17 °C, sedimentum formatur. In agaro YM post unum mensem ad 17 °C, cultura brunnea-cremea, glabra, butyracea, margine glabra. Pseudomycelium non formatur. Ballistosporae longae ellipsoideae vel allantoidia, 2.2–5.5 × 5.0–11.0 µm. Glucosum, saccharosum, maltosum, trehalosum, melezitosum, glycerolum (vel lente) et D-mannitolum (lente vel exigue) assimilantur at non galactosum, L-sorbosum (lente vel exigue),

Fig. 3. *Bensingtonia bomiensis* sp. nov. XZ 33D1^T vegetative cells grown in YM broth for 5 days at 17 °C (a) and ballistoconidia produced on corn meal agar after 5 days at 17 °C (b). Bars, 10 µm.

cellobiosum, lactosum, melibiosum, raffinsum, inulin, amyllum solubile, D-xylosum (vel exigue), L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, erithritolum, ribitolum, galactitolum, glucitolum, methyl α-D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum, kalium nitricum et cadaverinum

Table 2. Physiological characteristics that differentiate *B. rectispora* sp. nov. and *B. bomiensis* sp. nov. from closely related species

Taxa: 1, *B. rectispora* sp. nov.; 2, *B. bomiensis* sp. nov.; 3, *B. naganoensis* (Nakase *et al.*, 2011); 4, *B. pseudonaganoensis* (Wang *et al.*, 2006); 5, *B. ciliata* (Nakase *et al.*, 2011). +, Positive; –, negative; l, latent; s, slowly positive; w, weak.

Assimilation reaction	1	2	3	4	5
Sucrose	+	+	+	+	–
Cellobiose	–	–	+/s	+/w	–
Trehalose	+	+	+/-	+	+/s
Melibiose	–	–	+/s	+/w	–
Raffinose	–	–	+/s	+/w	–
Melezitose	+	+	+	+	–
Ethanol	–	–	–	–	+/s
Ribitol	–	–	+/-	+	+/w
D-Glucitol	–	–	+/s	+	+/s
Methyl α-D-glucoside	–	l	–	–	–
Succinic acid	–	–	+/-	–	+
Citric acid	–	–	–	–	+

assimilantur at non natrum nitrosum, L-lysinum nec ethylaminum. Ad crescentiam vitaminum necessarium est. Maxima temperatura crescentiae: 23 °C. Materia amyloidea iodophila non formatur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: CoQ-9. Typus: isolatus ex folio bamboo, XZ 4C5^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (=CGMCC 2.02635^T=CBS 10710^T).

Description of *Bensingtonia rectispora* F.-Y. Bai & Q.-M. Wang sp. nov.

Bensingtonia rectispora [rec.ti.spo'ra. L. adj. *rectus* straight; N.L. n. *spora* (from Gr. n. *spora* a seed) spore; N.L. n. *rectispora* (nominative in apposition) straight spore, referring to the ballistoconidium morphology of this species].

In YM broth, after 5 days at 17 °C, cells are long ellipsoids or cylindrical, 2.5–5.6 × 5.0–11.0 µm, and occur singly; budding is polar and sediment is formed. After 1 month at 17 °C, sediment is present. On YM agar, after 1 month at 17 °C, the streak culture is brownish cream, butyrous and smooth. The margin is entire. In Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Ballistoconidia are long ellipsoids or allantoid, 2.2–5.5 × 5.0–11.0 µm. Fermentation is negative. Glucose, sucrose, maltose, trehalose, melezitose, glycerol (may be delayed) and D-mannitol (delayed or weak) are assimilated. Galactose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, ribitol, galactitol, D-glucitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated; assimilation of L-sorbose may be negative, delayed or weak and assimilation of D-xylose may be negative or weak. Ammonium sulfate,

potassium nitrate and cadaverine dihydrochloride are assimilated. Sodium nitrite, L-lysine and ethylamine hydrochloride are not assimilated. Maximum growth temperature is 23 °C. Growth in vitamin-free medium is negative. Starch-like substances are not produced. Growth on 50 % (w/w) glucose-yeast extract agar is negative. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is CoQ-9.

The type strain is XZ 4C5^T (=CGMCC 2.02635^T=CBS 10710^T), isolated from a leaf of bamboo collected in Nyingchi county, Tibet, China, in July 2004.

Latin diagnosis of *Bensingtonia bomiensis* F.-Y. Bai et Q.-M. Wang sp. nov.

In YM (Difco) liquido post dies 5 ad 17 °C, cellulae vegetativae ellipsoideae vel cylindrica, 2.6–5.8 × 5.2–11.5 µm, singulae. Sedimentum formatur. Post unum mensem ad 17 °C, annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura brunneusa-cremea, glabra, butyracea, margine glabra. Pseudomycelium non formatur. Ballistospores allantoidia vel reniformia, 2.5–6.5 × 6.0–12.0 µm. Fermentatio nulla. Glucosum, saccharosum, maltosum, trehalosum, melezitosum, L-rhamnosum (exigue), glycerolum, D-mannitolum (lente) et methyl α-D-glucosidum (lente) assimilantur at non galactosum, L-sorbosum, cellobiosum, lactosum, melibiosum, raffinolum, inulin, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, D-glucosaminum, methanolum, ethanolum, erithritolum, ribitolum, galactitolum, glucitolum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum, kalium nitricum et cadaverinum assimilantur at non natrum nitrosum, L-lysinum nec ethylaminum. Ad crescentiam vitaminum non necessarium est. Maxima temperatura crescentiae: 22 °C. Materia amyloidea iodophila non formatur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: CoQ-9. Typus: isolatus ex folio plantae incognitae, XZ 33D1^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (=CGMCC 2.02670^T=CBS 10713^T).

Description of *Bensingtonia bomiensis* F.-Y. Bai & Q.-M. Wang sp. nov.

Bensingtonia bomiensis (bo.mi.en'sis. N.L. fem. adj. *bomiensis* pertaining to Bomi county, Tibet, referring to the geographical origin of the type strain of this species).

In YM broth, after 5 days at 17 °C, cells are long ellipsoids or cylindrical, 2.6–5.8 × 5.2–11.5 µm, and occur singly; budding is polar and sediment is formed. After 1 month at 17 °C, a ring and sediment are present. On YM agar after 1 month at 17 °C, the streak culture is brownish cream, butyrous and smooth. The margin is entire. In Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Ballistoconidia are allantoid or reniform, 2.5–6.5 × 6.0–12.0 µm. Fermentation is negative. Glucose, sucrose, maltose, trehalose, melezitose, L-rhamnose (weak),

glycerol, D-mannitol (delayed) and methyl α-D-glucoside (delayed) are assimilated. Galactose, L-sorbose, cellobiose, lactose, melibiose, raffinose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, D-glucosamine, methanol, ethanol, erythritol, ribitol, galactitol, D-glucitol, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate and cadaverine dihydrochloride are assimilated. Sodium nitrite, L-lysine and ethylamine hydrochloride are not assimilated. Maximum growth temperature is 22 °C. Growth in vitamin-free medium is positive (weak). Starch-like substances are not produced. Growth on 50 % (w/w) glucose-yeast extract agar is negative. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is CoQ-9.

The type strain is XZ 33D1^T (=CGMCC 2.02670^T=CBS 10713^T), isolated from a leaf of an unidentified plant collected in Bomi county, Tibet, China, in July 2004.

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