Bensingtonia changbaiensis sp. nov. and *Bensingtonia sorbi* sp. nov., novel ballistoconidium-forming yeast species from plant leaves

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Six ballistoconidium-forming yeast strains that were isolated from plant leaves collected on Changbai Mountain, north-east China, were assigned to the genus *Bensingtonia* Ingold emend. Nakase & Boekhout due to the formation of asymmetrical ballistoconidia, cream-coloured colonies and Q-9 as the major ubiquinone. Two separate groups, representing two novel *Bensingtonia* species, were recognized among these yeasts by 26S rDNA D1/D2 domain, internal transcribed spacer (ITS) region and 18S rDNA sequence analyses. The names *Bensingtonia changbaiensis* sp. nov. (type strain, CB 346^T=AS 2.2310^T=CBS 9497^T) and *Bensingtonia sorbi* sp. nov. (type strain, CB 286^T=AS 2.2303^T=CBS 9498^T) are proposed for these two species.

In a survey of ballistoconidium-forming yeast diversity of the phyllosphere in north-east China, approximately 250 yeast strains were isolated from 39 leaf samples of various plants collected on Changbai Mountain, Jilin Province, north-east China. Among the yeasts, six strains were classified into one group by conventional and chemotaxonomic characterization. Molecular phylogenetic analyses based on the large-subunit (26S) rDNA D1/D2 domain, the internal transcribed spacer (ITS) region and the small-subunit (18S) rDNA indicated that these strains represent two undescribed species in the genus *Bensingtonia* Ingold emend. Nakase & Boekhout [see Nakase & Boekhout (1988)]. They are hereby described as *Bensingtonia changbaiensis* sp. nov. and *Bensingtonia sorbi* sp. nov.

The six strains studied were isolated from wilting leaves of *Aconitum coreanum* (CB 228), *Betula ermanii* (CB 255 and CB 346^T) and *Sorbus pohuashanensis* (CB 284, CB 286^T and CB 287) by using the improved ballistoconidia-fall method (Nakase & Takashima, 1993). Leaf samples were collected on Changbai Mountain in October 1998. Morphological, physiological and biochemical characteristics were

examined according to standard methods (Yarrow, 1998). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986).

Nuclear DNA was extracted by the method of Makimura *et al.* (1994). The ITS (including 5.8S rDNA) and 26S rDNA D1/D2 domain sequences were determined by a method described previously (Bai *et al.*, 2002). 18S rDNA sequences were determined according to Sugita & Nakase (1999) with the following modifications: cycle sequencing was performed by using an ABI BigDye Cycle Sequencing kit and electrophoresis and data collection were performed on an ABI PRISM 3100 genetic analyser. Sequences were aligned with the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic trees were constructed from evolutionary distance data calculated with Kimura's two-parameter model (Kimura, 1980) by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (Felsenstein, 1985) was performed from 1000 random resamplings.

Morphology and chemotaxonomy

The six yeast strains (CB 228, CB 255, CB 284, CB 286^T, CB 287 and CB 346^T) were classified together by their formation of asymmetrical ballistoconidia and cream-coloured colonies. Their major ubiquinone was Q-9. Sexual structures were not observed in cultures of single strains or in mating tests. According to the current taxonomy of basidiomycetous yeasts (Boekhout, 1998; Boekhout & Nakase, 1998), these strains could be assigned to the genus *Bensingtonia*.

Published online ahead of print on 6 June 2003 as DOI 10.1099/ijs.0.02736-0.

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the 18S rDNA, internal transcribed spacer (ITS) region and 26S rDNA D1/D2 domain sequences determined in this study are AY233339–AY233345.

Phylogenetic trees based on ITS and 18S rDNA sequences are available as supplementary data in IJSEM Online.

Molecular phylogenetic analysis

Two groups were recognized among the six strains by ITS and D1/D2 sequence comparison. Strains CB 286^T and CB 287 had identical sequences in both regions. CB 228, CB 255, CB 284 and CB 346^T were classified into another group that also had identical sequences in both regions except for CB 284, which differed from the other three strains by 1 nt in the D1/D2 region. The two groups differed from each other by 34 nt (5%) in the D1/D2 region and 143 nt (~25%) in the ITS–5.8S rDNA region.

In the phylogenetic tree drawn from D1/D2 sequences (Fig. 1), the two groups were located in the *Kondoa* clade, *Agaricostilbum* lineage of urediniomycetous yeasts (Scorzetti *et al.*, 2002). They clustered together with *Bensingtonia yuccicola* and an undescribed *Bensingtonia* species represented by strain PYCC 5562 (Fig. 1). The Changbai strains differed from the latter two taxa by approximately 3–4% of nucleotides in the D1/D2 region. More than 20% nucleotide difference was found in the ITS region among closely related *Bensingtonia* taxa.

Phylogenetic positions of the two groups depicted in the D1/D2 tree (Fig. 1) were different from those depicted in the ITS tree. Such discordances have been reported in other groups of basidiomycetous yeasts (Bai *et al.*, 2002; Scorzetti *et al.*, 2002). In order to confirm the phylogenetic positions of the two groups, 18S rDNA sequences of the representative strains CB 286^T and CB 346^T were determined and aligned with those of reference taxa, including all described *Bensingtonia* species and related urediniomycetous yeast taxa. The two strains clustered together with five *Bensingtonia* and one *Kondoa* species in the *Agaricostilbum*/*Bensingtonia* lineage (Hamamoto & Nakase, 2000) with 100% bootstrap support. CB 286^T was located with *Bensingtonia miscanthi*, whereas CB 346^T showed a close relationship to *B. yuccicola*.

The ITS and 18S rDNA trees are available as supplementary data in IJSEM Online.

The results suggest that the two yeast groups from Changbai Mountain represent two novel *Bensingtonia* species, for which the names *Bensingtonia changbaiensis* sp. nov. and *Bensingtonia sorbi* sp. nov. are proposed.

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

In YM (Difco) liquido post dies 5 ad 17 °C, cellulae vegetativae ellipsoideae, 2.0–3.6 × 4.5–8.6 µm, singulae. Sedimentum formantur. Post unum mensem ad 17 °C, annulus et sedimentum formantur. In agar YM post unum mensem ad 17 °C, cultura brunnea-cremea, glabra aut rugosa, nitida aut non-nitida, butyracea, margine glabra. Pseudomycelium non formantur. Ballistospores reniformia vel allantoidia, 2.7–4.1 × 5.6–10.9 µm. Fermentatio nulla. Glucosum, saccharosum, maltosum, cellobiosum (lente), trehalosum, raffinose (lente), inulinum (lente et exigue), amyllum solubile (lente), D-xylosum (lente et exigue), glycerolum, ribitolium (lente et exigue), D-mannitolium et D-glucitolium (lente) assimilantur at non galactosum, L-sorbose, lactosum, melibiosum, melezitosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolium, ethanolium, erithritolum, galactitolium, methyl-α-D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolium nec hexadecanum. Ammonium sulfatum, kalium nitricum, natrum nitrosum et L-lysinum assimilantur at non ethylaminum nec cadaverinum. Vitaminae externae ad crescentiam necessaria sunt. Maxima temperatura crescentiae: 27 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-9.

Typus: isolatus ex folio *Betula ermanii*, CB 346^T, depositus in

Fig. 1. Phylogenetic tree drawn from neighbour-joining analysis of 26S rDNA D1/D2 domain sequences, depicting relationships of the *Bensingtonia* strains from north-east China with closely related taxa. Bootstrap percentages >50% from 1000 bootstrap replicates are shown. Reference sequences were retrieved from GenBank under the accession numbers indicated.

collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2310^T).

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

Bensingtonia changbaiensis (chang.bai.en'sis. N.L. fem. adj. *changbaiensis* referring to the geographical origin of the type strain of the species).

In YM broth after 5 days at 17 °C, cells are ellipsoidal, 2.0–3.6 × 4.5–8.6 µm (Fig. 2), single, budding is polar and sediment is formed. After 1 month at 17 °C, sediment and a ring are present. On YM agar after 1 month at 17 °C, the streak culture is brownish-cream, butyrous, smooth or slightly wrinkled and shining or dull. The margin is entire. In Dalmau plate culture on corn-meal agar, pseudohyphae are not formed. Ballistoconidia are produced on sterigmata of 5–12 µm long; they are ellipsoidal, reniform or allantoid and 2.7–4.1 × 5.6–10.9 µm (Fig. 2). Fermentation is negative.

Fig. 2. *Bensingtonia changbaiensis* sp. nov. CB 346^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 20 °C (b). Bars, 10 µm.

Glucose, sucrose, maltose, cellobiose (delayed), trehalose, raffinose (delayed), inulin (delayed and weak), soluble starch (delayed), D-xylose (delayed and weak), glycerol, ribitol (delayed and weak), D-mannitol and D-glucitol (delayed) are assimilated. Galactose, L-sorbose, lactose, melibiose, melezitose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, galactitol, methyl α -D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite and L-lysine are assimilated. Ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 27 °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. Major ubiquinone is Q-9.

The type strain of *Bensingtonia changbaiensis*, CB 346^T, was isolated from a wilting leaf of *Betula ermanii* Cham collected on Changbai Mountain, north-east China, in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2310^T (= CBS 9497^T).

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

In YM (Difco) liquido post dies 5 ad 17 °C, cellulae vegetativae ovoideae et globosa, 2.7–6.4 × 2.7–8.6 µm, singulae. Annulus et sedimentum formantur. Post unum mensem ad 17 °C, annulus et sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura brunneusa-cremea, glabra aut rugosa, nitida aut non-nitida, butyracea, margine glabra. Pseudomycelium non formantur. Ballistosporae ovoideae, 2.7–4.5 × 4.1–7.3 µm. Fermentatio nulla. Glucosum, L-sorbosum, galactosum (lente), saccharosum, maltosum, trehalosum, raffinsum, D-xylosum, L-arabiosum (lente et exigue), glycerolum, ribitolum (lente et exigue), D-mannitolum et D-glucitolum assimilantur at non cellobiosum, lactosum, melibiosum, melezitosum, inulinum, amyllum solubile, D-arabiosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, erithritolum, galactitolum, methyl- α -D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum, L-lysinum et cadaverinum assimilantur at non kalium nitricum, natrum nitrosum nec ethylaminum assimilantur. Vitaminae externae ad crescentiam necessariae sunt. Maxima temperatura crescentiae: 27 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-9.

Typus: isolatus ex folio Sorbus pohuashanensis, CB 286^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2303^T).

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

Bensingtonia sorbi (sor'bi. N.L. gen. adj. *sorbi* of *Sorbus*, referring to the genus name of the plant from which the type strain was isolated).

In YM broth after 5 days at 17 °C, cells are ovoidal or globose, 2·7–6·4 × 2·7–8·6 µm (Fig. 3), single, budding is polar, sediment and a ring are formed. After 1 month at 17 °C, sediment and a ring are present. On YM agar after 1 month at 17 °C, the streak culture is brownish-cream, butyrous, smooth or slightly wrinkled and shining or semi-dull. The margin is entire. In Dalmau plate culture on corn-meal agar, pseudohyphae are not formed. Ballistoconidia are produced on corn-meal agar, ovoidal, 2·7–4·5 × 4·1–7·3 µm (Fig. 3). Fermentation is negative. Glucose, L-sorbose, galactose (delayed), sucrose, maltose, trehalose, raffinose, D-xylose, L-arabinose (delayed and weak), glycerol, ribitol (delayed and weak), D-mannitol and D-glucitol are assimilated. Cellobiose, lactose, melibiose, melezitose,

Fig. 3. *Bensingtonia sorbi* sp. nov. CB 286^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 20 °C (b). Bars, 10 µm.

inulin, soluble starch, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, galactitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, L-lysine and cadaverine dihydrochloride are assimilated. Potassium nitrate, sodium nitrite and ethylamine hydrochloride are not assimilated. Maximum growth temperature is 27 °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. Major ubiquinone is Q-9.

The type strain of *Bensingtonia sorbi*, CB 286^T, was isolated from a wilting leaf of *Sorbus pohuashanensis* (Hance) Hedl collected on Changbai Mountain, north-east China, in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2303^T (= CBS 9498^T).

In addition to differences in molecular data, the two novel *Bensingtonia* species are distinguishable from each other and from other described species of the genus by some physiological properties, as shown in Table 1. With the inclusion of the recently described species *Bensingtonia thailandica* Fungsin *et al.* (2001) and the anamorph of *Mastigobasidium intermedium* Golubev (1999) (*Bensingtonia intermedia*), 14 species are now included in the genus *Bensingtonia* (Table 1). Although the heterogeneity of this genus, as well as a number of other basidiomycetous yeast

Table 1. Physiological characteristics that differentiate *B. changbaiensis* and *B. sorbi* from described species

Species: 1, *B. changbaiensis*; 2, *B. sorbi*; 3, *Bensingtonia ciliata*; 4, *Bensingtonia ingoldii*; 5, *Bensingtonia intermedia*; 6, *B. miscanthi*; 7, *Bensingtonia musae*; 8, *Bensingtonia naganoensis*; 9, *Bensingtonia phyllada*; 10, *Bensingtonia sakaguchii*; 11, *Bensingtonia subrosea*; 12, *B. thailandica*; 13, *Bensingtonia yamatoana*; 14, *B. yuccicola*. Abbreviations: +, positive; L, delayed positive; W, weakly positive; S, slow positive; –, negative; V, variable.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Assimilation of:														
L-Sorbose	–	+	W	–	+	L	+	–	+	–	L	–	–	+
Sucrose	+	+	–	+	V	+	–	+	+	–	+	+	+	–
Maltose	+	+	–	–	V	–	–	+	+	+	+	+	+	–
Cellobiose	L	–	–	+	+	+	–	+	+	+	+	+	–	+
Trehalose	+	+	+	+	+	+	+	–	+	+	+	+	+	+
Lactose	–	–	–	+	–	L	–	–	W	+	+	–	–	–
Melibiose	–	–	–	+	–	–	–	+	–	–	–	+	–	–
Raffinose	L	+	–	L	–	L	–	+	–	+	+	+	–	–
Soluble starch	L	–	–	S	–	–	–	+	+	+	+	+	V	–
D-Xylose	– (W)	+	–	+	V	L	+	–	–	–	L	–	L	–
L-Arabinose	–	–	–	+	–	–	L	–	W	–	–	–	–	–
Nitrate	L	–	+	+	–	+	–	+	+	–	–	+	–	+

genera, has been demonstrated by rRNA gene sequence analyses (Takashima *et al.*, 1995; Fell *et al.*, 2000; Hamamoto & Nakase, 2000; Scorzetti *et al.*, 2002), redefinitions of these genera would be premature at present. Relationships among lineages and clades of these yeasts should become clearer as novel species are added to the phylogenetic trees, which is hoped to make the demarcation of genera more reliable.

Acknowledgements

This study was supported by grants no. 30170002 from the National Natural Science Foundation of China (NSFC) and no. KSCX2-SW-101C from the Chinese Academy of Sciences.

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