Bullera cylindrica sp. nov., Bullera hubeiensis sp. nov. and Bullera nakasei sp. nov., ballistoconidium-forming yeast species from plant leaves

Qi-Ming Wang,1 Feng-Yan Bai,1 Hui-Zhong Lu,1 Jian-Hua Jia1 and Masako Takashima2

1Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China
2Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama, 351-0198, Japan

Among yeasts isolated from plant leaves collected in different regions of China that form whitish or yellowish colonies and symmetrical ballistoconidia, four strains were shown to represent three novel Bullera species by conventional and molecular taxonomic characterization. The novel species are described as Bullera cylindrica sp. nov. (type strain CB 169T = AS 2.2308T = CBS 9744T), Bullera hubeiensis sp. nov. (type strain HX 19.3T = AS 2.2466T = CBS 9747T) and Bullera nakasei sp. nov. (type strain HX 15.5T = AS 2.2435T = CBS 9746T). These three species, and another eight previously described Bullera species represented by Bullera mrakii, formed a strongly supported distinct clade among the hymenomycetous yeasts in each of the phylogenetic trees drawn from the 26S rDNA D1/D2 domain and the internal transcribed spacer region sequences.

INTRODUCTION

In the Cryptococcus luteolus lineage of hymenomycetous yeasts defined on the basis of 18S rDNA sequence analysis (Takashima & Nakase, 1999), two species isolated from plants collected in New Zealand, Bullera mrakii and Bullera hulaensis (Hamamoto & Nakase, 1996), form a distinct clade. Three additional Bullera species isolated from the Ogasawara Islands, Japan, were added to the clade by Sugita et al. (1999). The number of species in the clade was increased to eight by Bai et al. (2001), who identified three novel Bullera species among strains originally assigned to Bullera variabilis and assigned these species to the B. mrakii clade by 18S rRNA gene and internal transcribed spacer (ITS) region sequence analyses. None of the species in this clade were studied by Fell et al. (2001) or Scorzetti et al. (2002).

Recently, three novel Bullera species belonging to the B. mrakii clade were found among ballistoconidium-forming yeast strains isolated from plant leaves collected in China. The present study supports the distinct nature of the B. mrakii clade in basidiomycetous yeasts of the Tremellales group.

METHODS

Yeast strains and phenotypic characterization. Four strains were examined in the present study. Strain CB 169T was isolated from a wilting leaf of Brachythecium variiformis collected on Changbai Mountain, Jilin Province, in October 1998. Strains HX 15.5T and HX 19.3T were isolated from wilting leaves of Litsea sp. and Tilia sp., respectively, collected in Hubei Province in October 2002. Strain ST 19.14 was isolated from a wilting leaf of Pinus armandii collected in Shanxi Province in October 2002. Yeast strain isolation was performed using the improved ballistoconidia-fall method (Nakase & Takashima, 1993).

Morphological, physiological and biochemical characteristics were examined according to standard methods (Yarrow, 1998). 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).

Sequence analysis. Nuclear DNA was extracted by the method of Makimura et al. (1994). Sequencing of the ITS (including 5.8S rDNA) and 26S rDNA D1/D2 domain and molecular phylogenetic analysis were performed using methods described previously (Bai et al., 2002a).

RESULTS AND DISCUSSION

Morphology and chemotaxonomy

The four strains studied formed whitish colonies and reproduced asexually by polar or multilateral budding and...
production of rotationally symmetrical ballistoconidia. Stalked conidia were not produced. The major ubiquinone was Q-10. Sexual cycles were not observed in single strains or mixed cultures. According to the current taxonomy of basidiomycetous yeasts (Boekhout, 1998; Boekhout & Nakase, 1998), these strains were assigned to genus *Bullera*.

**rDNA sequence analysis**

Three taxa were recognized among the four strains studied from the rDNA sequence comparisons. Strains HX 15.5T and ST 19.14 had identical D1/D2 and ITS sequences. CB 169T and HX 19.3T differed markedly from each other and from the other two strains in the same rDNA regions.

In the phylogenetic tree drawn from the D1/D2 sequence alignment, the three taxa formed three distinct branches in the *B. mrakii* clade (Fig. 1). This clade was strongly supported (100%) by bootstrap analysis. In the tree drawn from ITS sequences, the *B. mrakii* clade was also resolved with 100% bootstrap support (phylogenetic tree available as supplementary material in IJSEM Online). Strains CB 169T and HX 15.5T clustered in a subclade with *B. mrakii* and four other described species in both the D1/D2 and ITS trees. Strain CB 169T differed from the other taxa in the subclade by 7–19 nt (1–4–3–1%) and 29–40 nt (>7%) in the D1/D2 and ITS regions, respectively. Strain HX 15.5T differed from the other taxa in the same subclade by 13–19 nt (2–1–3–1%) and 39–58 nt (>10%) in the D1/D2 and ITS regions, respectively.

Strain HX 19.3T occupied a basal position in the *B. mrakii* clade in the D1/D2 tree (Fig. 1). It differed from the other taxa in this clade by 19–24 nt (3–0–4–0%). In the ITS tree, the position of HX 19.3T was slightly different. This strain formed a less well supported subclade together with *Bullera komagatae*, *Bullera schimicola* and *Bullera pseudoschimicola* in the ITS tree (available as supplementary material in IJSEM Online) and differed from these species by more than 100 nt in the ITS region.

These data indicated that strains CB 169T, HX 15.5T and HX 19.3T represent three novel *Bullera* species, for which the names *Bullera cylindrica* sp. nov., *Bullera nakasei* sp. nov. and *Bullera hubeiensis* sp. nov. are proposed.
Characteristics of the *B. mrakii* clade

The *B. mrakii* clade recognized in the present study belongs to the Tremellales in the Hymenomycetes (Fell et al., 2000; Scorzetti et al., 2002). This clade is closely related to taxa in the Luteolus clade of the Tremellales (Scorzetti et al., 2002). Interestingly, the *B. mrakii* clade is composed exclusively of species belonging to the single genus *Bullera*, although these species are only distantly related to the type species *Bullera alba* (an amorph of *Bulleromyces albus*), as showed in Fig. 1. This could imply that species in the *B. mrakii* clade should be moved to a different genus. The precedent is that the genus *Dioszegia* was reinstated and redefined by rDNA sequence analysis to accommodate both ballistoconidium-forming and other yeast species in a sister clade of the *B. mrakii* clade (Takashima et al., 2001). In addition to the signature sequences in the 18S rDNA, *Dioszegia* species form orange-coloured colonies (Bai et al., 2002b; Takashima et al., 2001; Wang et al., 2003). Though a sequence signature (AAGCTC) was found near the 3′ end of the ITS2 regions of all the species in the *B. mrakii* clade (data not shown), no particular morphological or physiological characteristics could be found to discriminate species in this clade from other *Bullera* species. Therefore, it is thought to be premature to propose a new genus for this clade based only on rDNA sequence data at present.

Physiological differences between species in the *B. mrakii* clade are shown in Table 1. *B. cylindrica* sp. nov. can be distinguished from closely related species by its ability to assimilate nitrate and to grow in vitamin-free medium. Therefore, it is thought to be premature to propose a new genus for this clade based only on rDNA sequence data at present.

Morphologically, *B. cylindrica* sp. nov. and *B. nakasei* sp. nov. are also distinguishable from other species in the *B. mrakii* clade. The former forms characteristic cylindrical vegetative cells and the latter forms relatively large, somewhat apiculate vegetative cells.

### Latin diagnosis of *Bullera cylindrica* Bai, Wang et Takashima sp. nov.


### Description of *Bullera cylindrica* Bai, Wang & Takashima sp. nov.

*Bullera cylindrica* (cy. lin’ dri.ca. L. nom. fem. adj. cylindrica referring to the cylindrical shaped vegetative cells of the species).

In YM broth, after 5 days at 20 °C, cells are ovoid, ellipsoidal or cylindrical, 2.5–4.5 × 5.5–7.5 μm, and occur singly or in pairs (Fig. 2a). Budding is polar. A sediment and a ring are formed. On YM agar, after 1 month at 20 °C, the streak culture is yellowish cream, butyrous, dull, smooth or somewhat wrinkled. The margin is entire. In Dalmau plate culture on cornmeal agar, pseudohyphae are formed. Ballistoconidia are produced on cornmeal agar and are
Lum: Q-10. *Ubiquinonum majus* 1880

**Description of Bullera hubeiensis Bai, Wang & Takashima sp. nov.**

In *YM* (Difco) liquid post dies 5 ad 20°C, *cellulae vegetativae ovoideae* aut *ellipsoideae*, 2-5-4-5 × 5-0-7-5 μm, *singulae*, *binae* aut *adhaerentes*. Annulus et sedimentum formantur. Ballistoconidia *napiformia* vel *subglobosa*, 3-7-5-0 × 4-9-6-2 μm. Fermen
ingenum majus*: Q-10. Typus: *HX* 19.3T (= AS 2.2466T = CBS 9747T), isolatus ex folio *Tilia* sp.

**Latin diagnosis of Bullera hubeiensis Bai, Wang & Takashima sp. nov.**

Fig. 2. *Bullera cylindrica* sp. nov. CB 169T vegetative cells grown in YM broth for 5 days at 20°C (a) and ballistoconidia produced on cornmeal agar after 5 days at 20°C (b). Bars, 10 μm.

Fig. 3. *Bullera hubeiensis* sp. nov. HX 19.3T vegetative cells grown in YM broth for 5 days at 20°C (a) and ballistoconidia produced on cornmeal agar after 5 days at 20°C (b). Bars, 10 μm.

Fig. 4. *Bullera nakasei* sp. nov. HX 15.5T vegetative cells grown in YM broth for 5 days at 20°C (a) and ballistoconidia produced on cornmeal agar after 5 days at 20°C (b). Bars, 10 μm.

Three novel *Bullera* species from plant leaves
Description of *Bullera nakasei* Bai, Wang & Takashima sp. nov.

*Bullera nakasei* (na.k.a.se.i. L. gen. masc. n. nakasei in honour of Dr Takashi Nakase, Japan, for his outstanding contributions to the progress of systemsatics of ballistoconidium-forming yeasts).

In YM broth, after 5 days at 20 °C, cells are ovoid, ellipsoidal or apiculate, 2–0·6–0·6 × 2·5–14·5 μm, and occur singly or in pairs (Fig. 4a). Budding is multilateral. Sediment and a ring are formed. On YM agar, after 1 month at 20 °C, the streak culture is cream to light yellow, butyrous, semi-shiny to dull, smooth and somewhat wrinkled. The margin is entire or eroded. In Dalmau plate culture on cornmeal agar, true hyphae are formed. Ballistoconidia are produced on cornmeal agar and are napiform, subglobose or somewhat trigonal, 3–2–7·4 × 4·5–8·7 μm (Fig. 4b). Fermentation of glucose is negative. Glucose, galactose, L-sorbitose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch (weak), D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol (delayed and weak), D-mannitol (delayed and weak), D-glucitol, methyl α-D-glucoside, salicin, succinic acid and inositol are assimilated. Lactose, inulin, D-glucosamine, methanol, ethanol, glycerol, ribitol, galactitol, DL-lactic acid, citric acid and hexadecane are not assimilated. Ammonium sulfate, L-lysine and ethylamine hydrochloride are assimilated. Potassium nitrate, sodium nitrite 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. The major ubiquinone is Q-10. The type strain, HX 15.5T (=AS 2.2435T =CBS 9746T), was isolated from a wilting leaf of *Litsea* sp. collected in Xingshan County, Hubei Province, China in October, 2002.

ACKNOWLEDGEMENTS

We thank Professor J.-Y. Zhuang, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, for identifying the plant samples. This study was supported by grants no. 30170002 from the National Natural Science Foundation of China (NSFC) and no. 2001AA227131 of the ‘863 program’ from the Ministry of Science and Technology, China.

REFERENCES


