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***Bullera taiwanensis* sp. nov. and *Bullera formosensis* sp. nov., two new ballistoconidium-forming yeast species isolated from plant leaves in Taiwan**

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Two strains of ballistoconidiogenous yeasts that contain xylose and form Q-10 ubiquinone were isolated from plant leaves collected in Taiwan and were found to represent two new species. The taxonomic properties of both coincide with the genus *Bullera* so they are described as *Bullera taiwanensis* sp. nov. and *Bullera formosensis* sp. nov. In a phylogenetic tree based on the nucleotide sequences of 18S ribosomal DNAs, these two species are distant from the clusters where the remaining members of the genus *Bullera* are located, i.e., *Bullera taiwanensis* is located in the *Filobasidium* lineage (Filobasidiales clade) and *Bullera formosensis* is located in the *Cryptococcus humicola*-*Trichosporon* lineage (Trichosporonales clade).

Key Words—*Bullera formosensis* sp. nov.; *Bullera taiwanensis* sp. nov.; new ballistoconidiogenous yeasts; new yeasts from Taiwan

Introduction

Ballistoconidium-forming yeasts are a heterogeneous group of basidiomycetous yeasts widely distributed in the three classes of the Basidiomycota: Urediniomycetes, Hymenomycetes and Ustilaginomycetes (Fell et al., 2000; Nakase, 2000). In the 4th edition of The Yeasts, a Taxonomic Study, hymenomycetous ballistoconidium-forming anamorphic yeasts were included in two genera, *Bullera* (teleomorph, *Bullero-*

myces) and *Kockovaella* (Boekhout, 1998). The former genus is differentiated from the latter by the lack of stalked conidia. The genus *Udeniomyces* was proposed for three species of *Bullera* that produce large ballistoconidia whose shapes are more or less elongate bodies (Nakase and Takematsu, 1992). This genus was included in *Bullera* (Boekhout and Nakase, 1998) but is now regarded as a distinct genus based on molecular phylogenetic analyses (Fell et al., 2000; Takashima and Nakase, 1999). Recently, the orange-colored *Bullera* species, *B. aurantiaca* and *B. crocea*, were transferred to *Dioszegia* by emending the diagnosis of the genus to include ballistoconidium-forming yeasts (Takashima et al., 2001a).

Takashima and Nakase (1999) deduced the phylogenetic relationships among hymenomycetous yeasts based on sequence analysis of the 18S ribosomal

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DNAs (18S rDNAs) and showed that these yeasts are located in seven phylogenetic lineages: *Filobasidium*, *Cryptococcus humicola-Trichosporon*, *Cryptococcus luteolus*, *Filobasidiella*, *Bulleromyces*, *Sterigmatosporidium* lineage and *Cystofilobasidium*. The ballistoconidium-forming yeasts were found in five lineages: *Cryptococcus luteolus*, *Filobasidiella*, *Bulleromyces*, *Sterigmatosporidium* and *Cystofilobasidium* (Takashima et al., 2001b). Ballistoconidium-forming yeasts were not found in the *Filobasidium* lineage nor in the *C. humicola-Trichosporon* lineage. Based on the sequence analysis of the D1/D2 domain of 26S ribosomal DNAs (26S rDNAs), Fell et al. (2000) showed that ballistoconidium-forming yeasts were distributed in several clusters in the Tremellales and Cystofilobasidiales clades but not in the Trichosporonales (*C. humicola-Trichosporon* lineage) or Filobasidiales clades (*Filobasidium* lineage).

In the course of a survey of ballistoconidium-forming yeasts in the phyllosphere of Taiwan, China, 154 strains were isolated from 21 samples of plants collected in a protected subtropical rain forest. One hundred and forty-six of the isolates produced ballistoconidia and were assigned to the genera *Bullera* (91 strains), *Kockovaella* (1 strain), *Sporobolomyces* (53 strains) and the yeast-like fungus *Tilletiopsis* (1 strain). Among strains of *Bullera*, two strains were found to be closely related to the Filobasidiales clade (*Filobasidium* lineage) and the Trichosporonales clade (*C. humicola-Trichosporon* lineage), respectively, based on sequence analysis of 18S rDNA sequences. This paper describes these yeasts as two new species in the genus *Bullera*.

Materials and Methods

Isolation of yeast strains employed. Plant samples for yeast isolation were collected in Fu-Shan Experimental Forest, Taiwan Forestry Research Institute, Taiwan, China, in May 1997. Yeasts were isolated by an improved ballistoconidia-fall method previously described (Nakase and Takashima, 1993) using YM agar (Difco Lab., Detroit, MI, USA) without any antibacterial agents. The isolation was carried out for consecutive six days at 17°C. YM agar plates were replaced by new ones after 24, 48, 72, 96, and 144 h, and then agar plates that collected ballistoconidia were incubated at 17°C for three weeks. Colonies produced on agar plates were examined every day with a stereomi-

croscope and yeast colonies with different appearances were isolated. Isolated strains were preserved at -80°C suspended in YM broth supplemented with 10% (w/v) glycerol immediately after purification by conventional streaking technique. Strains FK-12 and FK-116, which are described in the present paper, were isolated from leaves of *Pteris wallichiana* Ag. and *Lophaterum gracile* Brongn., respectively.

Examination of morphological, physiological, and biochemical characteristics. Most of the morphological, physiological, and biochemical characteristics were examined according to Yarrow (1998). The assimilation of nitrogen compounds was investigated on solid media using starved inoculums according to Nakase and Suzuki (1986a). Vitamin requirements were determined according to the method of Komagata and Nakase (1967). The maximum growth temperature was determined in YM broth (Difco Lab.) using metal block baths.

Ubiquinone system. Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in early stationary growth phase. The cells were washed three times with distilled water. The extraction, purification and identification of ubiquinones were carried out according to Nakase and Suzuki (1986b).

Xylose in the cells. Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in the early stationary phase. The cells were washed three times with distilled water and dried with acetone. Cell hydrolysates were prepared according to the procedures described by Suzuki and Nakase (1988). Xylose in cell hydrolysates was analyzed by High Performance Liquid Chromatograph Reducing Sugar Analysis System (Shimadzu, Kyoto).

Isolation and purification of nuclear DNA. Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in the logarithmic growth phase. The cells were washed three times with distilled water and then freeze-dried. Isolation and purification of nuclear DNA were done according to Takashima and Nakase (2000).

DNA base composition. The DNA base composition was determined by HPLC after enzymatic digestion of DNA to deoxyribonucleosides as described by Tamaoka and Komagata (1984). A DNA-GC Kit (Yamasa Shoyu Co., Ltd., Chiba) was used as the quanti-

tative standard.

Nucleotide sequences and phylogenetic analysis. Nuclear DNA was extracted by the method of Makimura et al. (1994). The 18S rDNA and ITS regions including 5.8S rDNA were amplified by PCR according to Sugita and Nakase (1999). The D1/D2 region of 26S rDNA was amplified following Kurtzman and Robnett (1998). The PCR products were directly sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Stafford, UK) and analyzed by an Applied Biosystems sequencer model 310. The sequences of 18S rDNA determined in this study are deposited in the DDBJ database under the following accession numbers: FK-12 (JCM 11143) 18S rDNA (AB072234), D1/D2 domain of 26S rDNA (AB079065), FK-116 (JCM 11142) 18S rDNA (AB072235), D1/D2 domain of 26S rDNA (AB090946). Reference sequences used for the phylogenetic analysis, shown in Fig. 1, were obtained from the database. Generated sequences were aligned with the species of *Trichosporon* and *Cryptococcus* and related genera using the CLUSTAL W ver. 1.74 computer program (Thompson et al., 1994). The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbor-joining method (Saitou and Nei, 1987) in the PHYLIP computer program. Sites where gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein, 1985) were performed from 1,000 random resamplings. *Cystofilobasidium capitatum* was used as an outgroup.

Results and Discussion

Strains FK-12 and FK-116 contained xylose in the cells and had Q-10 as the major component of ubiquinones. The strains produced ballistoconidia and budding yeast cells but not non-ballistoconidiogenous stalked conidia. These characteristics coincided with those of the genus *Bullera* (Boekhout and Nakase, 1998).

In the phylogenetic tree based on 18S rDNA sequences, FK-12 is located in the Filobasidiales clade (*Filobasidium* lineage) where all of the species of *Filobasidium* and several species of *Cryptococcus* are located (Fig. 1). The 18S rDNA of FK-12 demonstrated less than 98.3% sequence similarity to closely related species of the Filobasidiales clade. This low sequence similarity clearly suggests that FK-12 represents a dis-

tinct species from other members of this clade. Recently, Fonseca et al. (2000) found that the ubiquitous anamorphic basidiomycetous yeast species *Cryptococcus albidus* comprised at least 12 species based on analysis of rDNA sequence data and physiological, biochemical, and other molecular characteristics. They described eight new species for strains formerly included in this species. These species are located in the Filobasidiales clade. Since the 18S rDNA sequence data for these new species were not available for comparison, we compared sequences of the D1/D2 domain of 26S rDNA between FK-12 and the proposed new species. FK-12 is most closely related to a strain (CBS 9005) of *C. cylindricus* in D1/D2 domain (Fig. 2), but 9 nucleotides (1.5%) are different. The difference of 18 nucleotides (3%) found between FK-12 and the type strain of *C. cylindricus* clearly indicated that FK-12 represented a different species from *C. cylindricus*. Apparently, CBS 9005 does not belong to *C. cylindricus*. Probably, this strain belongs to an undescribed species of *Cryptococcus*. Recently, D1/D2 sequences of three unidentified strains of *Cryptococcus* were open to the public from DNA data banks. These strains clustered with FK-12 in the phylogenetic tree based on the D1/D2 domain (Fig. 2). *Cryptococcus* sp. CBS 9089 is the nearest to FK-12 and only 1 nucleotide is different from the latter. The DNA reassociation experiment is necessary for making clear the relationship of these two strains. *Cryptococcus* sp. PYCC 4949 and PYCC 5266 differ from FK-12 in 8 and 12 nucleotides, respectively, and are considered to represent different species from FK-12. Strain FK-12 represents the first ballistoconidium-forming species found in this clade. It produced typical rotationally symmetrical ballistoconidia usually found in the genus *Bullera* (Fig. 2B).

Strain FK-116 produced bilaterally symmetrical ballistoconidia that could be described as ellipsoidal, kidney-shaped or comma-shaped, so that it is clearly distinguished from other species of *Bullera* in this respect (Fig. 3B). In the phylogenetic tree based on 18S rDNA, FK-116 is associated with the Trichosporonales clade (*C. humicola*-*Trichosporon* lineage), which includes most species of *Trichosporon* and several species of *Cryptococcus* (Fig. 1). The 18S rDNA sequence similarity of FK-116 is near *Cryptococcus humicola*, but the similarity value is not high, i.e., 97.8% to the type strain of this species. This clearly indicates that FK-116 represents a distinct species from *C. humicola*. Furthermore, FK-116 has Q-10 as the major compo-

Fig. 1

nent of ubiquinones whereas the type strain of *C. humicola* has Q-9. In the phylogenetic tree based on the D1/D2 domain of 26S rDNA, FK-116 also clustered with Trichosporonales species though the bootstrap value is not very high. The fact that FK-116 showed a 93% sequence similarity in this domain to *Trichosporon inkin*, the nearest species, clearly suggested the distinctness of FK-116. Apparently, FK-116 is a new species related to Trichosporonales. For the deduction of correct phylogenetic position of this yeast, the isolation of several related strains is required.

Takashima and Nakase (1999) suggested inclusion of *C. humicola* in the *Trichosporon* lineage based on 18S rDNA sequences. Fell et al. (2000) showed that *C. humicola* was closely related to the Trichosporonales (*C. humicola*-*Trichosporon* lineage) based on analysis of the D1/D2 domain of 26S rDNA, but they did not include *C. humicola* in this order because of a low bootstrap value, 66%. Takashima et al. (2001b), however, obtained a high bootstrap value for this lineage after the reclassification of the *C. humicola* complex and confirmed the inclusion of *C. humicola* in the Trichosporonales, together with five new species and a new name from the *C. humicola* complex. It is interesting that *Cryptococcus ramirezgomezianus*, one of these new species from *C. humicola* complex, was reported to produce ballistoconidia when it was first described as the new species *Sporobolomyces albidus*, which was based on a strain isolated from rotted toadstool (Ramírez-Gómez, 1957). *Sporobolomyces albidus* was characterized by the production of non-pigmented colonies and asymmetrical (bilaterally symmetrical) ballistoconidia. However, this species had been regarded as a synonym of *Candida humicola* (Van Uden and Buckley, 1970; Meyer et al., 1984), and then regarded as a synonym of *Cryptococcus humicola* (Golubev, 1981; Fell and Statzell-Tallman, 1998) accompanied by the transfer of *Candida humicola* to the genus *Cryptococcus*, because no one observed ballistoconidia production after the description of this species.

It is well known, however, that the ability to form ballistoconidia tends to be lost during culture maintenance. Furthermore, even in fresh isolates, ballisto-

conidium formation may be poor or not be detected at all (Nakase and Suzuki, 1985). Of course, it is not easy to judge whether *S. albidus* really produced ballistoconidia when it was freshly isolated. However, the facts that strain FK-116 produces bilaterally symmetrical ballistoconidia and that it is located at Trichosporonales clade (*C. humicola*-*Trichosporon* lineage), suggests that Ramírez-Gómez (1957) really observed ballistoconidium formation in this species. It is assumed that ballistoconidium-forming species in the Trichosporonales are characterized by bilaterally symmetrical ballistoconidia different from *Bullera* species located in other orders and clusters in the class Hymenomycetes. Further isolation studies are required to confirm this assumption.

Strains FK-12 and FK-116 are located at positions distant from the position where the type species of *Bullera* (*Bullera alba*, anamorph of *Bulleromyces albus*) is located. Therefore, these two strains should be placed in different genera in the Filobasidiales and Trichosporonales, respectively. However, we decided to include them in the genus *Bullera* until the reclassification of this genus is performed to avoid confusion of the systematics of hymenomycetous yeasts. The names *Bullera taiwanensis* and *Bullera formosensis* are proposed for FK-12 and FK-116, respectively.

Descriptions

Bullera taiwanensis Nakase, Tsuzuki et Takashima, sp. nov.

In liquido YM: Post dies 3 ad 25°C cellulae ovoideae, ellipsoideae et elongatae, (2.2–6.5) × (2.8–10.5) µm, singulae aut binae; post unum mensem ad 17°C pellicula, annulus, et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura flavoalbida, glabra, nitida, mollis et margine glabra. Mycelium et pseudomycelium non formantur. Ballistospores in CMA formatae, globosae, napiformes, knob-formes aut irregularis, (3.5–5) × (3.5–5.5) µm.

Fermentatio nulla. Glucosum, galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum (lente), melibiosum, raffinotum, melezitotum, amyllum solubile, D-xylosum, L-arabinosum, D-

Fig. 1. Phylogenetic tree for *Bullera taiwanensis* sp. nov. and *Bullera formosensis* sp. nov. constructed by the neighbor-joining method based on 18S rDNA sequences.

The numerals represent the percentages from 1,000 replicate bootstrap resamplings (A frequency of less than 50% is not shown). Sequences were retrieved from the DDBJ databases under the accession numbers indicated.

Fig. 2. Phylogenetic tree for *Bullera taiwanensis* sp. nov. and *Bullera formosensis* sp. nov. constructed by the neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences.

The numerals represent the percentages from 1,000 replicate bootstrap resamplings (A frequency of less than 50% is not shown). Sequences were retrieved from the DDBJ databases under the accession numbers indicated.

arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, xylitolum, L-arabinitolum, α -methyl-D-glucosidum, salicinum, glucono- δ -lactonum, acidum D-

gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (lente et exiguum), acidum succinicum, acidum citricum (exiguum), acidum D-glucuronicum, acidum D-galacturonicum et inositolum assimilantur at non inulinum, methanolum,

Fig. 3. Morphology of *Bullera taiwanensis* sp. nov. FK-12.

A: Vegetative cells grown in YM broth for 3 days at 25°C. B: Ballistoconidia formed on corn meal agar after 16 days at room temperature. Scale bar=10 µm.

acidum saccharicum, hexadecanum, propane 1,2 diolum nec butane 2,3 diolum. Kalium nitricum, natrium nitrosum, L-lysinum (exiguum), ethylaminum et cadaverinum assimilantur. Maxima temperatura crescentiae: 28–29°C. Ad crescentiam thiaminum necessarium est. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleino: 44.1 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum cellulis praesens.

Holotypus: Isolata ex folio *Pteris wallichianae*, Formosa, JCM 11143/CCRC 22877 (originaliter ut FK-12) conservatur in collectionibus culturarum quas "Japan Collection of Microorganisms," Wako, Saitama, Japan et "Culture Collection and Research Center, Food Industry Research and Development Institute," Sinchu, Taiwan, China sustentat.

Growth in YM broth: After 3 days at 25°C, the vegetative cells are ovoidal, ellipsoidal and elongate, (2.2–6.5)×(2.8–10.5) µm, single or in pairs (Fig. 3A). A sediment is formed. After one month at 17°C, a creeping ring and a heavy sediment are present.

Growth on YM agar: After one month at 17°C, the streak culture is yellowish-white, shining, smooth, soft and has an entire margin.

Slide culture on corn meal agar: Mycelia and pseudomycelia are not produced.

Formation of ballistoconidia: Limited production of ballistoconidia was observed on corn meal agar. They are globose, napiform, knob-shaped, and sometimes irregular, (3.5–5)×(3.5–5.5) µm (Fig. 3B).

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
Galactose	+	Ribitol	+
L-Sorbose	+	Galactitol	+
Sucrose	+	D-Mannitol	+
Maltose	+	D-Glucitol	+
Cellobiose	+	Xylitol	+
Trehalose	+	L-Arabinitol	+
Lactose	+	α-Methyl-D-glucoside	+
	(latent)		
Melibiose	+	Salicin	+
Raffinose	+	Glucono-δ-lactone	+
Melezitose	+	D-Gluconic acid	+
Inulin	–	2-Ketogluconic acid	+
Soluble starch	+	5-Ketogluconic acid	+
D-Xylose	+	DL-Lactic acid	+
		(latent and weak)	
L-Arabinose	+	Succinic acid	+
D-Arabinose	+	Citric acid	+
		(weak)	
D-Ribose	+	Saccharic acid	–
L-Rhamnose	+	D-Glucuronic acid	+
D-Glucosamine	+	D-Galacturonic acid	+
N-Acetyl-D-glucosamine	+	Inositol	+
Methanol	–	Hexadecane	–
Ethanol	+	Propane 1,2 diol	–
Glycerol	+	Butane 2,3 diol	–

Assimilation of nitrogen compounds:

Nitrate	+	L-Lysine	+
			(weak)
Nitrite	+	Cadaverine	+
Ethylamine	+		

Vitamin required: Thiamine.

Production of starch-like substances: Negative.

Growth on 50% (w/w) glucose-yeast extract agar: Negative.

Maximum growth temperature: 28–29°C.

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Negative.

Diazonium blue B color reaction: Positive.

Urease: Positive.

Hydrolysis of fat: Negative.

G+C content of nuclear DNA: 44.1 mol% (by HPLC).

Major ubiquinone: Q-10.

Xylose in the cells: Present.

Type strain: FK-12, isolated from a leaf of *Pteris wallichiana* Ag. collected in May 1997, in Fu-Shan Experimental Forest, Taiwan Forestry Research Institute, Taiwan, is the type strain of this species. It was deposited at the Culture Collection and Research Center, Food Industry Research and Development Institute, Sinchu, Taiwan, as CCRC 22877, and at the Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351–0198, as JCM 11143.

Bullera formosensis Nakase, Tsuzuki et Takashima, sp. nov.

In liquido YM: Post dies 3 ad 25°C cellulae sphaeroideae, ovoideae, ellipsoideae et elongatae, (2–6.2) × (2.5–7.5) μm, singulae aut binae; post unum mensem ad 17°C pellicula incompleta, annulus incompleta et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura subflava, semi-nitida, rugosa, butyracea et margine erosa aut fimbriata. Mycelia et pseudomycelia formantur. Ballistoconidia in CMA formatae, ellipsoideae, leniformes, aut comma-formes, (2.5–4.5) × (4.5–8) μm.

Fermentatio nulla. Glucosum, galactosum, L-sorbosum (lente), sucrosum, maltosum, cellobiosum, trehalosum, lactosum (lente), raffinose, melezitose, amylosum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum (lente), L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, glycerolum (lente et exiguum), erythritolum (lente), ribitololum, galactitololum (lente), D-mannitololum, D-glucitololum, xylitololum, L-arabinitolum (lente et exiguum), α-methyl-D-glucosidum (lente), salicinum, glucono-δ-lactonum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (lente et exiguum), acidum succinicum, acidum citricum (exiguum), acidum D-glucuronicum, acidum D-galacturonicum et inositololum (exiguum) assimilantur at non melibiosum, inulinum, methanololum, ethanololum, hexadecanum, acidum saccharicum, propanae 1,2 diolum nec butanae 2,3 diolum. Natrium nitrosum, L-lysinum, ethylaminum et cadaverinum assimilantur at non kalium nitricum. Maxima temperatura crescentiae: 29–30°C. Ad crescentiam thiaminum necessarium est. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleino: 57.8 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum cellulul praesens.

Holotypus: Isolata ex folio *Lophateri gracile*, Formosa, JCM 11142/CCRC 22878 (originaliter ut FK-116) conservatur in collectionibus culturarum quas "Japan Collection of Microorganisms," Wako, Saitama, Japan et "Culture Collection and Research Center, Food Industry Research and Development Institute," Sinchu, Taiwan, China sustentat.

Growth in YM broth: After 3 days at 25°C, the vegetative cells are spherical, ovoidal, ellipsoidal and elongate, (2–6.2) × (2.5–7.5) μm, single or in pairs (Fig. 4A). A sediment is formed. After one month at 17°C, an incomplete fragile pellicle, an incomplete ring and a sediment are present.

Growth on YM agar: After one month at 17°C, the streak culture is pale to pale yellow, semi-shining, wrinkled, butyrous and has an erose and sporadically fimbriate margin.

Growth on YM agar: After one month at 17°C, the streak culture is pale to pale yellow, semi-shining, wrinkled, butyrous and has an erose and sporadically fimbriate margin.

Slide culture on corn meal agar: Mycelia and pseudomycelia are produced. Mycelia are flexuose and primitive cymose branching.

Formation of ballistoconidia: Ballistoconidia are abundantly formed on corn meal agar. They are ellipsoidal, kidney-shaped and comma-shaped, measuring (2.5–4.5) × (4.5–8) μm (Fig. 4B).

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Erythritol	+
			(latent)
Galactose	+	Ribitol	+
L-Sorbose	+	Galactitol	+
	(latent)		(latent)
Sucrose	+	D-Mannitol	+

Fig. 4. Morphology of *Bullera formosensis* sp. nov. FK-116.

A. Vegetative cells grown in YM broth for 3 days at 25°C. B. Ballistoconidia formed on corn meal agar after 3 days at room temperature. Scale bar=10 μm.

Maltose	+	D-Glucitol	+	Ethylamine	+
Cellobiose	+	Xylitol	+	Vitamin required: Thiamine.	
Trehalose	+	L-Arabinitol	+	Production of starch-like substances: Negative.	
		(latent and weak)		Maximum growth temperature: 29–30°C.	
Lactose	+	α-Methyl-D-glucoside	+	Growth on 50% (w/w) glucose-yeast extract agar: Negative.	
	(latent)		(latent)		
Melibiose	–	Salicin	+	Liquefaction of gelatin: Negative.	
Raffinose	+	Glucono-δ-lactone	+	Hydrolysis of fat: Negative.	
Melezitose	+	D-Gluconic acid	+	Acid production on chalk agar: Negative.	
Inulin	–	2-Ketogluconic acid	+	Diazonium blue B reaction: Positive.	
Soluble starch	+	5-Ketogluconic acid	+	Urease: Positive.	
D-Xylose	+	DL-Lactic acid	+	G+C content of nuclear DNA: 57.8 mol% (by HPLC).	
		(latent and weak)		Major ubiquinone: Q-10.	
L-Arabinose	+	Succinic acid	+	Xylose in the cells: Present.	
D-Arabinose	+	Citric acid	+	Type strain: FK-116, isolated from a leaf of	
		(weak)		<i>Lophaterum gracile</i> Brongn. collected in May 1997, in	
D-Ribose	+	Saccharic acid	–	Fu-Shan Experimental Forest, Taiwan Forestry Re-	
	(latent)			search Institute, Taiwan, is the type strain of this	
L-Rhamnose	+	D-Glucuronic acid	+	species. It was deposited at the Culture Collection and	
D-Glucosamine	+	D-Galacturonic acid	+	Research Center, Food Industry Research and Devel-	
N-Acetyl-D-glucosamine	+	Inositol	+	opment Institute, Sinchu, Taiwan, as CCRC 22878,	
		(weak)		and at the Japan Collection of Microorganisms, RIKEN	
Methanol	–	Hexadecane	–	(The Institute of Physical and Chemical Research),	
Ethanol	–	Propane 1,2 diol	–	Wako, Saitama 351–0198, as JCM 11142.	
Glycerol	+	Butane 2,3 diol	–		
	(latent and weak)				
Assimilation of nitrogen compounds:					
Nitrate	–	L-Lysine	+	Etymology: The specific epithet was derived from	
Nitrite	+	Cadaverine	+	“Formosa” meaning “Beautiful Island” which was given	
				to Taiwan by European people who reached this island	
				in the 16th century.	

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