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FULL PAPER

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Bullera begoniae sp. nov. and *Bullera setariae* sp. nov., two new species of ballistoconidium-forming yeasts in the *Bullera variabilis (Bulleribasidium)* cluster isolated from plants in Taiwan

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Abstract Two strains of xylose-containing and Q-10-having ballistoconidiogenous yeasts isolated from plant leaves collected in Taiwan were found to represent two new species of the genus *Bullera.* In the phylogenetic trees based on the sequence analysis of 18S rDNA and D1/D2 domain of 26S rDNA, these species are located in the *Bullera variabilis (Bulleribasidum)* cluster in Hymenomycetes. They are described as *Bullera begoniae* sp. nov. and *Bullera setariae* sp. nov., respectively.

Key words *Bullera begoniae* sp. nov. · *Bullera setariae* sp. nov. · New ballistoconidium-forming yeasts · New yeasts from Taiwan

Introduction

Nakase and Suzuki (1987) described *Bullera variabilis* based on 17 strains isolated in Japan and Canada. They reported that the electrophoretic pattern of enzymes differed from strain to strain, suggesting that this species comprised several different species. However, they found no distinct groups based on enzyme pattern or other taxonomic characteristics. Takashima and Nakase (1999) reported that *Bullera variabilis* showed a relationship to *Cryptococcus luteolus* lineage in the phylogenetic tree based on the sequence of 18S rDNAs, but its phylogenetic position was not stable depending on the methods of analysis, neighbor joining and maximum parsimony. Bai et al. (2001) clarified the taxonomy of *Bullera variabilis* based on the comparison of the rDNA internal transcribed spacer (ITS) regions and DNA–DNA hybridization experiments. Of the 20 strains studied, they retained 5 strains in *B. variabilis*, reassigned 6 strains to *Bullera mrakii*, and proposed 3 novel species for 8 of the 9 remaining strains, i.e., *Bullera pseudohuiaensis*, *Bullera komagatae*, and *Bullera pseudoschimicola.* In the phylogenetic tree based on the sequences of the D1/D2 domain of 26S rDNA, *B. variabilis* clustered with *Bullera miyagiana* in the Tremellales, although the bootstrap confidence level was low (Fell et al. 2000). A large phylogenetic distance was found between *B. variabilis* and the remaining species of hymenomycetous yeasts.

Bai et al. (2002) found a new ballistoconidium-forming yeast among the strains isolated from leaves collected in Yunnan Province, China, and described it as *Bullera pseudovariabilis* that clustered with *B. variabilis* in the phylogenetic tree based on the 18S rDNA sequences. They showed that the *B. variabilis* cluster was separated from the position of other hymenomycetous yeasts.

Sampaio et al. (2002) proposed the genus *Bulleribasidium* comprising a single species, *Bulleribasidium oberjochense*, as a second teleomorphic counterpart of the genus *Bullera* different from the genus *Bulleromyces* (Boekhout et al. 1991). *Bulleribasidium oberjochense* was initially identified as *B. variabilis* and clustered with this species in a phylogenetic tree based on D1/D2 domain sequences of 26S rDNA.

Fungsin et al. (2003) reported the isolation of two new ballistoconidium-forming yeasts from plants in Thailand and described them as *Bullera panici* and *Bullera siamensis*, respectively. They found that these new species were located in the *B. variabilis* cluster and showed that the *B.*

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variabilis cluster was a well-separated group in the Hymenomycetes.

In the course of a survey of ballistoconidiogenous yeasts in the subtropical phyllosphere of Taiwan, two strains of hymenomycetous yeasts isolated from leaves collected in a protected subtropical rain forest were found to represent two new species of the *B. variabilis* cluster based on the sequence analysis of 18S rDNA, internal transcribed spacer (ITS) regions, and the D1/D2 domain of 26S rDNA. They are described as *Bullera begoniae* sp. nov. and *Bullera setariae* sp. nov. as the sixth and seventh species of the *B. variabilis* cluster, respectively.

Materials and methods

Isolation of yeast strains employed

Plant samples for yeast isolation were collected in a protected subtropical rain forest in Fu-Shan Experimental Forest, Taiwan Forestry Research Institute, Taiwan, in May 1997. Yeasts were isolated by an improved ballistoconidia-fall method previously described (Nakase and Takashima 1993) using YM agar (Difco) without any antibacterial agents. The isolation was carried out for 6 consecutive days at 17°C. YM agar plates were replaced by new ones after 24, 48, 72, 96, and 144 h, and then agar plates with collected ballistoconidia were incubated at 17°C for up to 3 weeks. Colonies produced on agar plates were examined every day with a stereomicroscope and yeast colonies with different appearance were isolated. Isolates were preserved at −80°C suspended in YM broth supplemented with 10% (w/v) glycerol immediately after purification by the conventional streaking technique. Strain FK-71 was isolated from a leaf of *Begonia formosana* (Hayata) Masamune and strain FK-77 from *Setaria plicata* (Lam.) T. Cooke.

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 inocula 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) using metal block baths.

Ubiquinone system

Cells grown in 500-ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 150 rpm at 25°C were harvested in the 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 grown in 500-ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 150 rpm at 25°C 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 the procedures described by Suzuki and Nakase (1988). Xylose in cell hydrolysates was analyzed by a High Performance Liquid Chromatograph Reducing Sugar Analysis System (Shimadzu, Kyoto, Japan).

Isolation and purification of nuclear DNA

Cells grown in 500-ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 150 rpm at 25°C were harvested in the logarithmic growth phase. The cells were washed three times with distilled water and 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, Chiba, Japan) was used as the quantitative standard.

Sequencing and phylogenetic analysis

The 18S rDNA and ITS regions including 5.8S rDNA were amplified by PCR according to Sugita and Nakase (1999). The nucleotide sequences of the D1/D2 domain of 26S rDNA were directly determined using polymerase chain reaction (PCR) products according to Kurtzman and Robnett (1998) and Fell et al. (2000). The rDNA sequences determined in this study were deposited in the DDBJ database under the following accession numbers: FK-71 (BCRC 22974 = JCM 12155) 18S rDNA and ITS regions including 5.8S rDNA (AB118874), D1/D2 domain of 26S rDNA (AB119462); FK-77 (BCRC 22975 = JCM 12156) 18S rDNA and ITS regions including 5.8S rDNA (AB118875), D1/D2 domain of 26S rDNA (AB119463). Reference sequences used for the phylogenetic analysis, shown in Figs. 1 and 2, were obtained from the database. Generated sequences were aligned with the species of *Bullera* and related hymenomycetous yeast taxa using the CLUSTAL X ver. 1.8 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). Sites where any gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein 1985) were performed from 1000 random resamplings.

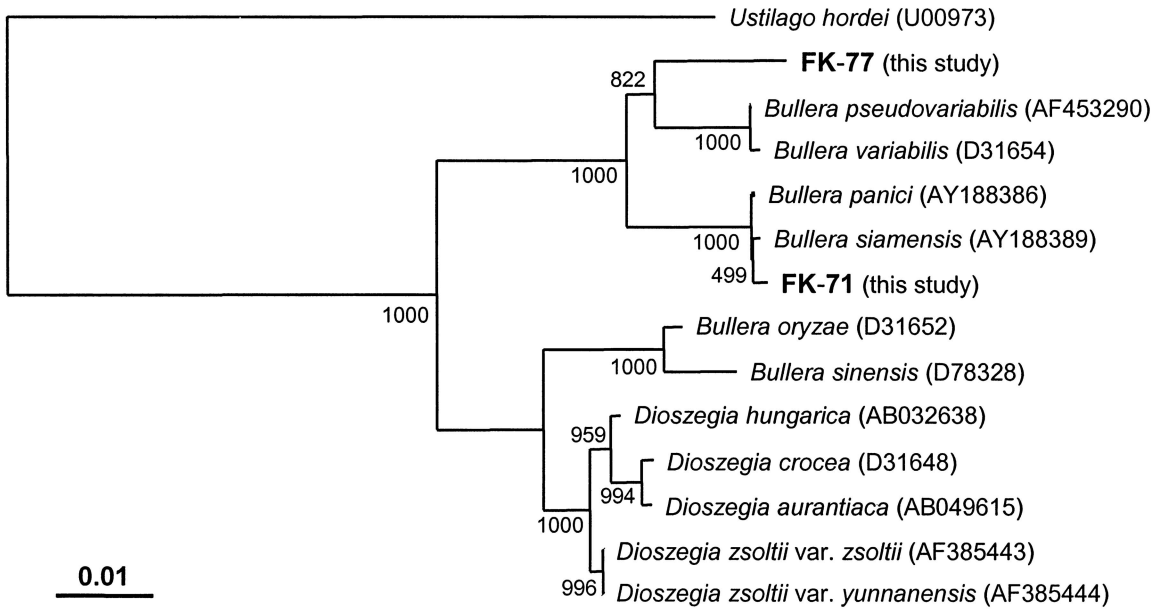

Fig. 1. Phylogenetic tree for new *Bullera* species from Taiwan constructed by neighbor-joining method based on 18S rDNA sequences. The *numerals* indicate the values from 1000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated

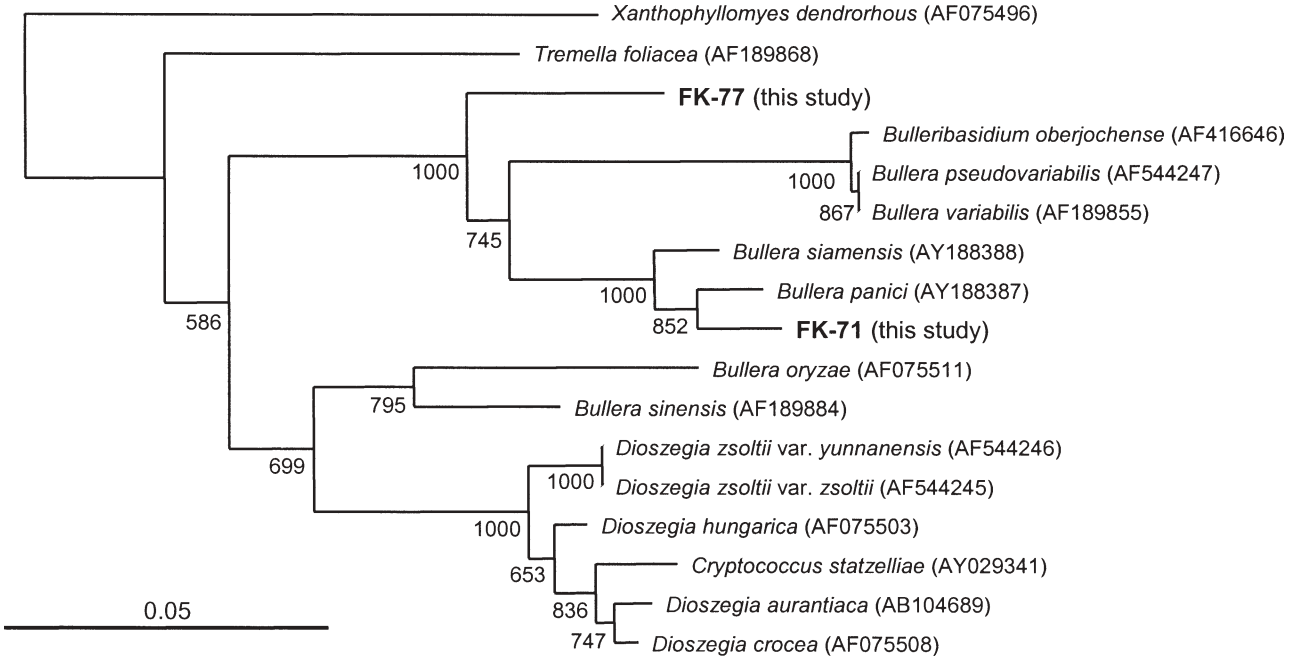

Fig. 2. Phylogenetic tree for new *Bullera* species from Taiwan constructed by neighbor-joining method based on the D1/D2 domain of 26S rDNA sequences. The *numerals* indicate the values from 1000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated

Results and discussion

Strains FK-71 and FK-77 isolated from leaves in Taiwan produced rotationally symmetrical ballistoconidia, contained cellular xylose, contained Q-10 as the major component of ubiquinones, and did not show any kind of sexual reproduction. These characteristics coincide with the genus *Bullera* (Boekhout and Nakase 1998).

In the phylogenetic tree constructed by the neighbor-joining method based on the 18S rDNA sequences, strains FK-71 and FK-77 were located in the *Bullera variabilis* cluster in the *Cryptococcus luteolus* lineage (Takashima and Nakase 1999) together with *B. pseudovariabilis*, *B.*

variabilis, *B. panici*, and *B. siamensis* (see Fig. 1). FK-71 constituted a cluster with *Bullera panici* and *B. siamensis*. This cluster connected with a cluster comprising FK-77, *B. variabilis*, and *B. pseudovariabilis* with high bootstrap value. The *Bullera variabilis* cluster is distant from other species in the *C. luteolus* lineage (Takashima and Nakase 1999).

In 18S rDNA sequences, FK-71 showed high similarities to closely related species, 99.8% to *B. siamensis* and 99.7% to *B. panici*. The sequence similarities of FK-77 are 98.5% to *B. variabilis* and *B. pseudovariabilis*, 97.3% to *B. siamensis*, 97.1% to *B. panici*, and 97.3% to FK-71.

A similar topology was found in a phylogenetic tree based on the D1/D2 domain of 26S rDNA to that based on 18S rDNA sequences. FK-71 and *B. panici* constituted a cluster that connected with a cluster comprising *B. variabilis*, *B. pseudovariabilis*, and *Bulleribasidium oberjochense* (see Fig. 2). FK-77 constituted a cluster with these six species.

In the D1/D2 domain, FK-71 is most closely related to *B. panici*, but 2.4% of nucleotides were different from this species. FK-77 differed in 6.0% of nucleotides from *B. siamensis*, the species having the smallest nucleotide difference from FK-77.

In ITS regions (ITS1 + ITS2), FK-71 and FK-77 differed in 15.0% and 39.5% of nucleotides, respectively, from *B. panici*, the species having the smallest nucleotide difference from these two isolates.

Based on the facts already mentioned, we concluded that FK-71 and FK-77 represented two different new species in the *B. variabilis* cluster. The names *Bullera begoniae* and *Bullera setariae* are proposed for these strains, respectively.

The *Bullera variabilis (Bulleribasidum)* cluster is well separated from other hymenomycetous yeasts. All seven species in the *B. variabilis* cluster possessed a four-nucleotide deletion at a site in the D1/D2 domain of 26S rDNA, compared with other yeast species in the Hymenomycetes.

Descriptions

Bullera begoniae Nakase, Tsuzuki, F.L. Lee & M. Takashima, sp. nov.

In liquido YM: Post dies 5 ad 25°C cellulae ovoideae, elongatae, cocoon-formes vel ampulliformes, 3–5.5 × 3.5–8 μm, singulae, binae, racemosae vel catenatae. Annulus incompletus et sedimentum formans. In agaro YM post 14 dies ad 17°C cultura subflava vel brunneo-glauca, glabra vel crisplata, nonnitida, butyracea, margine integra. Pseudomycelium primitivum formans. Ballistosporae in CMA formatae, napiformes, 4–8.5 × 4.5–9.5 μm.

Fermentatio:nulla. Glucosum, galactosum, L-sorbosum (lente et exiguum), sucrosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinosum (fortasse lente), melezitosum (fortasse lente), amylum solubile, D-xylosum, L-arabinosum (fortasse lente), D-arabinosum (tardum), D-ribosum (lente et exiguum), L-rhamnosum (fortasse lente), D-glucosaminum, *N*-acetyl-D-glucosaminum, galactitolum (lente), D-mannitolum (lente vel nullum), D-glucitolum (lente vel lente et exiguum), xylitolum (lente et exiguum), α-methyl-D-glucosidum (fortasse lente), salicinum, glucono-δ-lactonum (vel nullum), acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (lente vel nullum), acidum succinicum, acidum citricum (fortasse exiguum), acidum saccharicum (tardum), acidum D-glucuronicum et acidum D-galacturonicum assimilativa et lactosum, inulinum, methanolum, ethanolum, glycerolum, erythritolum, ribitolum, L-arabinitolum, inositolum, propane-1,2-diolum nec butane-2,3-diolum non assimilativ. Maxima temperatura incrementi: 28°–29°C. Pro incremento thiaminum necessum est. Commutatio coloris per diazonium caeruleum B positiva. Proportio molaris guanini + cytosine in acido deoxyribonucleinico: 55.0 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum cellulae praesens.

Holotypus: Stirps FK-71, cultura viva ex folio *Begoniae formosanae* (Hayata) Masamune, in sylva pluviali subtropica, Fu-Shan, Formosa isolatus et conservatus in Collectione Culturarum in "Bioresouce Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI)," Hsinchu, Formosa ut BCRC 22974 in statu lyophilo. Isotypus: item in collectione culturarum ut JCM 12155 in statu lyophilo quas "Japan Collection of Microorganisms (JCM), RIKEN," Wako, Saitama, Japonia conservatus.

Growth in YM broth: After 5 days at 25°C vegetative cells are ovoidal, ellipsoidal, elongate, cocoon-shaped, or bottle-shaped, single, in pairs, in small clusters or in short chains, 3–5.5 × 3.5–8 μm (Fig. 3A). Incomplete ring and sediment are formed. After 2 weeks at 25°C, a ring and a sediment are present.

Growth on YM agar: After 2 weeks at 25°C, the streak culture is pale yellow to brownish-gray, smooth to delicately wrinkled near the bottom of the tube, dull, butyrous, and has an entire margin.

Slide culture on corn meal agar: Primitive pseudomycelium is abundantly formed.

Ballistoconidium formation: Ballistoconidia are abundantly produced on corn meal agar, napiform, 4–8.5 × 4.5–9.5 μm (Fig. 3B).

Assimilation of carbon compounds:

Glucose	+
Galactose	+
L-Sorbose	+ (latent and weak)
Sucrose	+
Maltose	+
Cellobiose	+
Trehalose	+
Lactose	−
Melibiose	+
Raffinose	+ (may be latent)
Melezitose	+ (may be latent)
Inulin	−
Soluble starch	+
D-Xylose	+
L-Arabinose	+ (may be latent)

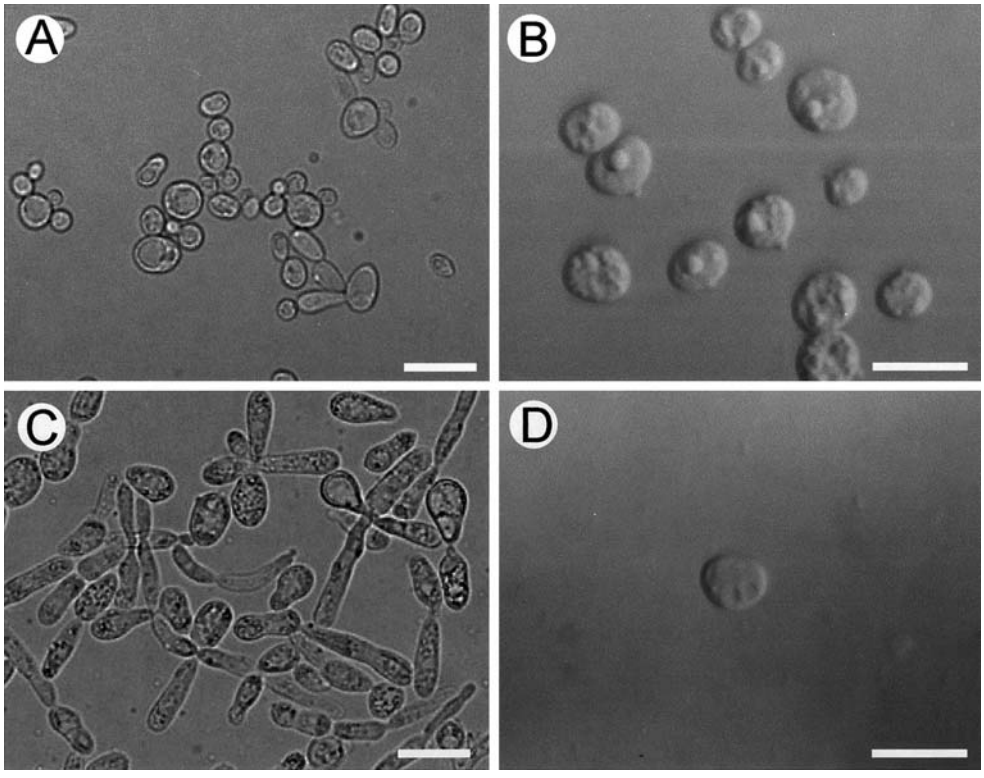

Fig. 3. Morphology of *Bullera begoniae* and *Bullera setariae*. **A** Vegetative cells of *Bullera begoniae* FK-71 grown in YM broth for 3 days at 25°C. **B** Ballistoconidia of *Bullera begoniae* FK-71 formed on corn meal agar after 16 days at room temperature. **C** Vegetative cells of *Bullera setariae* FK-77 grown in YM broth for 3 days at 25°C. **D** Ballistoconidium of *Bullera setariae* FK-77 formed on corn meal agar after 16 days at room temperature. *Bars* 10 μm

D-Arabinose	+ (slow)
D-Ribose	+ (latent and weak)
L-Rhamnose	+ (may be latent)
D-Glucosamine	+
N-Acetyl-D-glucosamine	+
Methanol	−
Ethanol	−
Glycerol	−
Erythritol	−
Ribitol	−
Galactitol	+ (latent)
D-Mannitol	+ (latent) or −
D-Glucitol	+ (latent or latent and weak)
Xylitol	+ (latent and weak)
L-Arabinitol	−
α-Methyl-D-glucoside	+ (may be latent)
Salicin	+
Glucono-δ-lactone	+ or −
D-Gluconic acid	+
2-Ketogluconic acid	+
5-Ketogluconic acid	+
DL-Lactic acid	+ (latent) or −
Succinic acid	+
Citric acid	+ (may be weak)
Saccharic acid	+ (slow)
D-Glucuronic acid	+
D-Galacturonic acid	+
Inositol	−
Propane-1,2-diol	−
Butane-2,3-diol	−

Assimilation of nitrogen compounds:

Potassium nitrate	−
Sodium nitrite	+
Ethylamine	−
L-Lysine	+
Cadaverine	+ (latent and weak)

Vitamin required: Thiamine.
Production of starchlike substances: Positive.
Growth on 50% (w/w) glucose-yeast extract agar: Negative.
0.1% cycloheximide resistance: 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: 55.0 mol% (by HPLC).
Major ubiquinone: Q-10.
Xylose in the cells: Present.

Type strain: FK-71, isolated from a leaf of *Begonia formosana* (Hayata) Masamune 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 Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, as BCRC 22974. Isotype was deposited at Japan Collection of Microorganisms, RIKEN (The Institute

of Physical and Chemical Research), Wako, Saitama 351-0198, as JCM 12155.

Etymology: The specific epithet of this species is derived from the name of the plant from which it was isolated.

In the conventional taxonomic characteristics, this species resembles *Bullera crocea* and known species of the *B. variabilis* cluster. However, the former species produces orange-colored colonies on agar media and is easily discriminated from *B. begoniae* in this respect. *Bullera begoniae* is distinguished from known species of the *B. variabilis* cluster in the lack of assimilation of inositol.

Bullera setariae Nakase, Tsuzuki, F.L. Lee & T. Takashima, sp. nov.

In liquido YM: Post dies 5 ad 25°C cellulae in sedimento subovoideae, ovoideae vel ellipsoideae, singulae, binae vel racemosae, 3–7 × 4–13–21 μm. Cellulae in annulus longeovoideae, elongatae vel longelageniformes, 1.5–6 × 4–18 μm. Annulus obsoletus et sedimentum formans. In agaro YM post 14 dies ad 25°C cultura brunneola vel fuscobrunneo-flava, glabra, subnitida, mollis, margine integra. Pseudomycelium primitivum formans. Ballistosporae in CMA formatae, subglobosae vel napiformes, 5.5–7.5 × 6.5–8.5 μm.

Fermentatio: nulla. Glucosum, galactosum, sucrosum, maltosum, cellobisosum, trehalosum, melibiosum, raffinosum, melezitosum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum (fortasse lente et exiguum), D-ribosum (tardum), L-rhamnosum, D-glucosaminum (exiguum), *N*-acetyl-D-glucosaminum, ribitolum (lente vel nullum), galactitolum, D-mannitolum, D-glucitolum (lente, vel lente et exiguum), α-methyl-D-glucosidum (lente, vel lente et exiguum), salicinum (fortasse tardum), glucono-δ-lactonum (vel nullum), acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum (lente, vel lente et exiguum), acidum succinicum, acidum citricum (fortasse exiguum), acidum saccharicum, acidum D-glucuronicum (fortasse tardum) et acidum D-galacturonicum (fortasse tardum) assimilativa et L-sorbosum, lactosum, inulinum, methanolum, ethanolum, glycerolum, erythritolum, xylitolum, L-arabinitolum, inositolum, propane-1,2-diolum nec butane-2,3-diolum non assimilativa. Maxima temperatura incrementi: 29°–30°C. Pro incremento thiaminum necessum est. Commutatio coloris per diazonium caeruleum B positiva. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 50.5 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum cellulae praesens.

Holotypus: Stirps FK-77, cultura viva ex folio *Setariae plicatae* (Lam.) T. Cooke, in sylva pluviali subtropica, Fu-Shan, Formosa; isolatus et conservatus in Collectionie Culturarum in "Bioresouce Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI)," Hsinchu, Formosa ut BCRC 22975 in statu lyophilo. Isotypus: item in collectione culturarum ut JCM 12156 in statu lyophilo quas "Japan Collection of Microorganisms (JCM), RIKEN," Wako, Saitama, Japonia conservatus.

Growth in YM broth: After 5 days at 25°C, a trace of a ring and a sediment are formed. Cells in the sediment are diverse in shape and size, short ovoidal, ovoidal, ellipsoidal, elongate, single, in pairs, in clusters, sometimes in short pseudomycelia, 3–7 × 4–13–21 μm. Cells in the ring are long oval, elongate, or long bottle-shaped, in branched chains, 1.5–6 × 5–18 μm (see Fig. 3C). After 2 weeks at 25°C, a ring and a sediment are present.

Growth on YM agar: After 2 weeks at 25°C, the streak culture is pale brown to dark brownish-yellow, smooth, dull-shining, soft, and has an entire margin.

Slide culture on corn meal agar: Pseudomycelia are produced but are not well developed.

Ballistoconidium formation: Ballistoconidium is poorly produced on corn meal agar, subglobose or napiform, 5.5–7.5 × 6.5–8.5 μm (see Fig. 3D).

Assmilation of carbon compounds:

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

Inositol	−
Propane-1,2-diol	−
Butane-2,3-diol	−

Assimilation of nitrogen compounds:

Potassium nitrate	−
Sodium nitrite	−
Ethylamine	−
L-Lysine	+
Cadaverine	−

Vitamin required for growth: Thiamine.
Production of starchlike substances: Negative.
Growth on 50% (w/w) glucose-yeast extract agar: Negative.
0.1% cycloheximide resistance: Negative.
Maximum growth temperature: 29°–30°C
Liquefaction of gelatin: Positive.
Acid production on chalk agar: Negative.
Diazonium blue B color reaction: Positive.
Urease: Positive.
Hydrolysis of fat: Negative.
G+C content of nuclear DNA: 50.5 mol% (by HPLC).
Major ubiquinone: Q-10.
Xylose in the cells: Present.

Type strain: FK-77, isolated from a leaf of *Setaria plicata* (Lam.) T. Cooke, 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 Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan, as BCRC 22975. Isotype was deposited at Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama, as JCM 12156.

Etymology: The specific epithet of this species is derived from the name of the plant from which it was isolated.

In conventional taxonomic characteristics, this species resembles *Dioszegia crocea* and *B. begoniae*, which is described in this article, and the known members of the *B. variabilis* cluster. However, *D. crocea* produces orange-colored colonies on agar media and is easily distinguished from *B. setariae* in this respect. *Bullera setariae* is distinguished from *B. begoniae* in the lack of nitrite assimilation, the lack of production of starchlike substances, and the difference of mol% G+C, and from known species of the cluster in the lack of inositol assimilation and "starch" production.

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