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**Full Paper**

# *Bullera hoabinhensis* sp. nov., a new ballistoconidiogenous yeast isolated from a plant leaf collected in Vietnam

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**VY-68, a ballistoconidiogenous yeast strain, isolated from a plant leaf at Cuc Phuong National Park of Ninh Binh Province, Vietnam, was assigned to the genus *Bullera* based on morphological and chemotaxonomical characteristics. Based on the sequence analyses of 18S rDNA, D1/D2 region of 26S rDNA, and internal transcribed spacer regions (ITS), VY-68 was phylogenetically closely related to *Bullera pseudoalba* and *Cryptococcus cellulolyticus*. DNA-DNA reassociation experiments among VY-68, *B. pseudoalba* and *C. cellulolyticus* revealed that strain VY-68 is a distinct species, and the latter two are conspecific. *Bullera hoabinhensis* is proposed for VY-68.**

**Key Words——***Bullera hoabinhensis* sp. nov.; systematics

## Introduction

One hundred and twenty-one strains of ballistoconidiogenous yeasts were isolated from plant materials collected in Cuc Phuong National Park of Ninh Binh Province, Vietnam. Eighty-five strains selected for the morphology of their ballistoconidia and colony appearances were assigned to four genera: *Bullera* (39 strains), *Kockovaella* (5 strains), *Sporobolomyces* (39 strains) and *Tilletiopsis* (2 strains) based on their morphological and chemotaxonomical characteristics.

Five strains of the genus *Kockovaella* represented 4 new species, which have already been described as *K. calophylli*, *K. cucphuongensis*, *K. litseae* and *K. vietnamensis* (Luong et al., 2000). Fifteen of 39 strains of genus *Bullera* seemed to represent undescribed species. Of these, *Bullera hoabinhensis* VY-68$^T$, belonging to the *Bulleromyces* clade of Scorzetti et al. (2002), is discussed in this report, based on a single isolate.

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## Materials and Methods

*Yeast strain.* The yeast strain used in this study was isolated from a plant leaf collected in Cuc Phuong National Park of Ninh Binh Province, Vietnam (Table 1), using the ballistoconidium-fall method on YM agar as reported by Nakase and Takashima (1993).

*Morphological, physiological and biochemical characteristics.* Most methods used for the examination of morphological, physiological and biochemical characteristics were described by Yarrow (1998). The determination of maximum growth temperature was made in YM broth, using metal block baths. The assimilation of nitrogen compounds was determined by the method of Nakase and Suzuki (1986b). The vita-

min requirement followed the method of Komagata and Nakase (1967).

*Chemotaxonomic characteristics.* Extraction, purification and identification of ubiquinones were carried out according to the method of Nakase and Suzuki (1986b). The presence or absence of xylose in the cells was analyzed by thin-layer chromatography (Nakase et al., 1976) after hydrolyzing the cells with trifluoroacetic acid (Suzuki and Nakase, 1988).

*Sequencing and phylogenetic analysis.* The sequences of 18S rDNA, ITS regions including 5.8S rDNA and the D1/D2 region of 26S rDNA, were determined after amplifying the DNA using PCR. Both strands were sequenced directly (Kurtzman and Robnett, 1997; Takashima and Nakase, 1999). Generated sequences were aligned with related species by using the CLUSTAL W ver. 1.74 computer program (Thompson et al., 1994). Reference sequences used for the phylogenetic study were obtained from the database. 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 gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein, 1985) were performed from 1,000 random resamplings. For a comparison of ITS regions among closely related species, pairwise sequences were aligned by sight, and the sequence similarity including gaps was calculated.

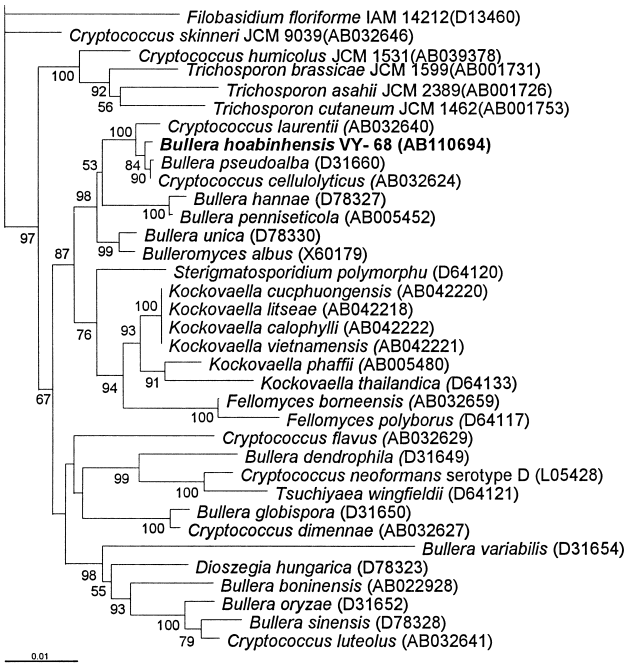


Fig. 1. Phylogenetic tree of *Bullera hoabinhensis* VY-68$^T$ and related species based on 18S rDNA sequences.

The tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbour-joining method (Saitou and Nei, 1987) with bootstrapping (Felsenstein, 1985). The numerals represent the results from 1,000 replicate bootstrap samplings. Reference sequences were retrieved from GenBank/DDBJ under the accession numbers indicated.

*DNA-DNA relatedness.* Isolation and purification of nuclear DNA was carried out according to Takashima and Nakase (2000). The DNA base composition was determined by HPLC after enzymatic digestion of DNA to deoxyribonucleosides (Tamaoka and Komagata, 1984). The DNA-GC Kit (Yamasa Shoyu Co., Ltd., Chiba, Japan) was used as a quantitative standard. DNA-DNA reassociation experiments were performed by a membrane-filter method (Hamamoto and Nakase, 1995).

## Results and Discussion

### *Taxonomic position of VY-68*

A ballistoconidiogenous yeast strain, VY-68, was characterized based on the presence of xylose in the cells, of Q-10 as a major ubiquinone and the production of symmetrical ballistoconidia and budding cells.

Table 1. Strains used in this study.

| Species | Strain | Source | DDBJ accession numbers | | |
|---|---|---|---|---|---|
| | | | 18S rDNA | ITS & 5.8S | D1/D2 |
| *Bullera hoabinhensis* sp.nov. | VY-68$^T$ | *Anadendrum montanum* | AB110694 | AB110695 | AB193347 |
| *B. pseudoalba* | JCM 5290$^T$ | Dead leaf of *Oryza sativa* | | | |
| *Cryptococcus cellulolyticus* | JCM 9707$^T$ | Decayed wood | | | |
| *C. laurentii* | JCM 9066$^T$ | Palmwine | | | |

$^T$ Type strain.

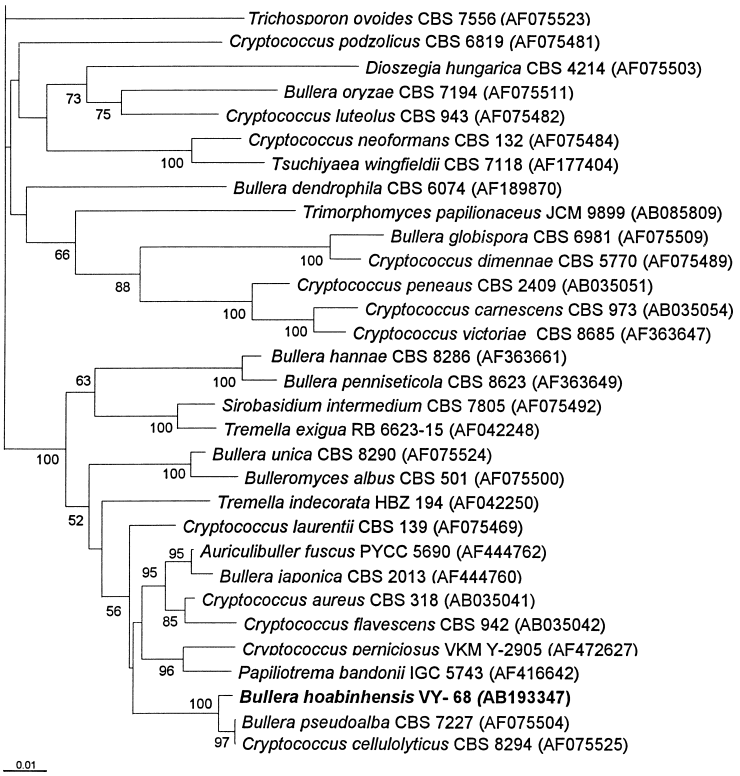


Fig. 2. Phylogenetic tree of *Bullera hoabinhensis* VY-68$^T$ and related species based on the sequence of the D1/D2 region of 26S rDNA.

The tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbour-joining method (Saitou and Nei, 1987) with bootstrapping (Felsenstein, 1985). The numerals represent the results from 1,000 replicate bootstrap samplings. Reference sequences were retrieved from GenBank/DDBJ under the accession numbers indicated.

Based on these results, the isolate was assigned to the genus *Bullera* (Boekhout and Nakase, 1998).

A phylogenetic tree was constructed based on the 18S rDNA sequences of the strain and 34 species of the genera *Bullera*, *Bulleromyces*, *Cryptococcus*, *Dioszegia*, *Fellomyces*, *Filobasidium*, *Kockovaella*, *Sterigmatosporidium*, *Trichosporon* and *Tsuchiyaea*. *Bullera hoabinhensis* VY-68$^T$ was located at the *Bulleromyces* clade, and made a cluster with *Bullera pseudoalba*, *Cryptococcus cellulolyticus* (84% bootstrap value) that connected with *C. laurentii* with 100% bootstrap support.

Fell et al. (2000), Kurtzman and Robnett (1998), and Sugita and Nishikawa (2003) demonstrated that yeast strains could be identified to the species level by molecular phylogenetic analysis using D1/D2 sequences of 26S rDNA. A phylogenetic tree was constructed based on the D1/D2 sequences of 26S rDNA of *B. hoabinhensis* VY-68$^T$ and 30 species of the genera *Auriculibuller*, *Bullera*, *Bulleromyces*, *Cryptococcus*, *Dioszegia*, *Papiliotrema*, *Sirobasidium*, *Tremella*, *Trichosporon*, *Trimorphomyces* and *Tsuchiyaea* (Fig. 2). *B. hoabinhensis* VY-68$^T$ clustered with *B. pseudoalba* and *C. cellulolyticus*, which is strongly supported (100%) by bootstrap analysis.

The sequences of ITS regions were determined and a phylogenetic tree of *B. hoabinhensis* VY-68$^T$ and 28 species of the genera *Auriculibuller*, *Bullera*, *Bulleromyces*, *Cryptococcus*, *Fellomyces*, *Kockovaella*, *Papiliotrema*, *Sirobasidium*, *Tremella* and *Trichosporon* was constructed (Fig. 3). *B. hoabinhensis* VY-68$^T$ also made a cluster with *B. pseudoalba* and *C. cellulolyticus*, which is strongly supported (100%) by bootstrap analysis. The sequence similarity between *B. hoabinhensis* VY-68$^T$ and phylogenetically closely related species was calculated. The result showed that the sequence similarity between the ITS1 region of VY-68$^T$ and that of *B. pseudoalba* and *C. cellulolyticus* was 94.7%, and that in the case of the ITS2 region was 91.6–92.1%. These results indicated that *B. hoabin-*

*hensis* VY-68$^T$ was distinct from the known *Bullera* species.

Further experiments were made to confirm the taxonomic position of *B. hoabinhensis* VY-68$^T$, *B. pseudoalba* and *C. cellulolyticus*. The G+C content of cells of *B. hoabinhensis* VY-68$^T$ was 54 mol% different from those of *B. pseudoalba* (52 mol%), *C. cellulolyticus* (52 mol%) and *C. laurentii* (58 mol%). DNA-DNA reassociation experiments (Table 2) showed that *B. hoabinhensis* VY-68$^T$ had a low reassociation value to reference species (7–14%). Based on these facts, strain VY-68$^T$ is considered to be a new species. Hence, *Bullera hoabinhensis* sp. nov is described.

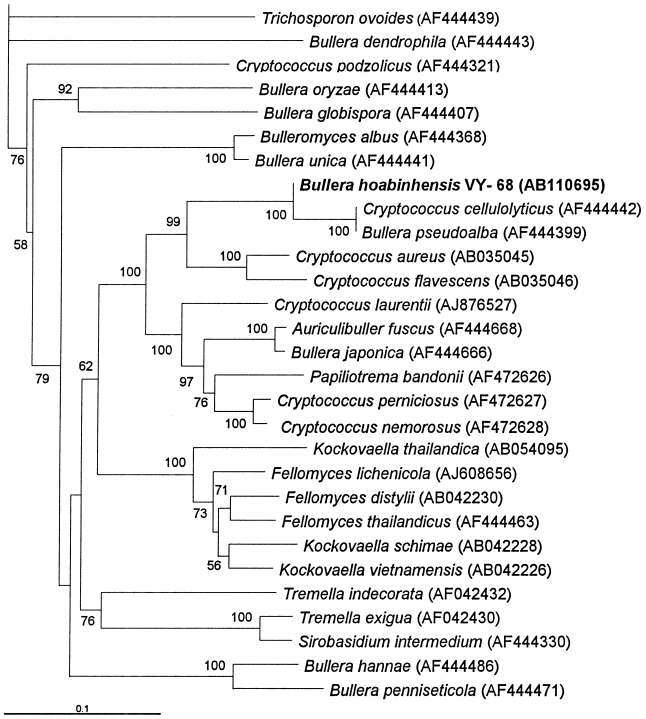


Fig. 3. Phylogenetic tree of *Bullera hoabinhensis* VY-68$^T$ and related species based on the sequence of the ITS regions of rDNA.

The tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbour-joining method (Saitou and Nei, 1987) with bootstrapping (Felsenstein, 1985). The numerals represent the results from 1,000 replicate bootstrap samplings. Reference sequences were retrieved from GenBank/DDBJ under the accession numbers indicated.

Differential physiological and biochemical characteristics for new and known species of *Bullera* are listed in Table 3. *Bullera hoabinhensis* sp. nov. is easily distinguished from reference species by the assimilation of melibiose, D-glucitol, sodium nitrite, and by growth in the medium containing 50% glucose.

### *Cryptococcus cellulolyticus is a synonym of Bullera pseudoalba*

As shown in Table 2, DNA-DNA reassociation experiments showed 70–78% DNA relatedness between *B. pseudoalba* (Nakase and Suzuki, 1986a) and *C. cellulolyticus* (Nakase et al., 1996), indicating that they are conspecific. Scorzetti et al. (2002) reported that *C. cellulolyticus* and *B. pseudoalba* had identical D1/D2 and ITS sequences, which would suggest conspecificity

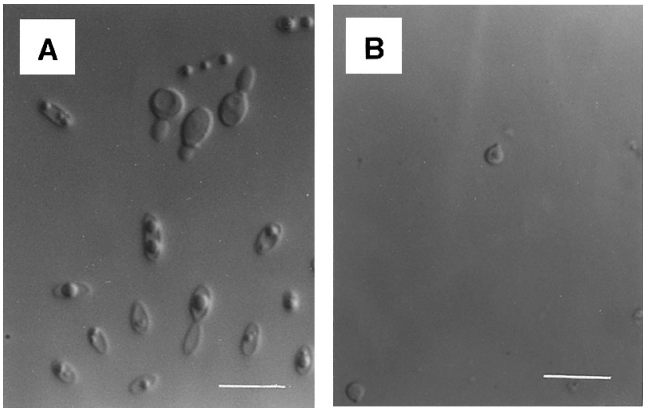


Fig. 4. *Bullera hoabinhensis* VY-68$^T$.

A, Vegetative cells grown in YM broth for 5 days at 17°C. B, Ballistoconidia produced on corn meal agar after 3 days at 17°C.

Table 2. DNA-DNA reassociation experiment among *Bullera hoabinhensis* VY-68$^T$ and related species.

| Species | Strain | Mol% G+C | % relative binding of DNA from | | | |
|---|---|---|---|---|---|---|
| | | | VY-68 | JCM 5290 | JCM 9707 | JCM 9066 |
| *Bullera hoabinhensis* sp. nov. | VY-68$^T$ | 54 | 100 | 14 | 13 | 14 |
| *B. pseudoalba* | JCM 5290$^T$ | 52 | 12 | 100 | 78 | 15 |
| *Cryptococcus cellulolyticus* | JCM 9707$^T$ | 52 | 11 | 71 | 100 | 13 |
| *C. laurentii* | JCM 9066$^T$ | 58 | 7 | 8 | 7 | 100 |

$^T$ Type strain.

Table 3. Salient characteristics of *Bullera hoabinhensis* and related species.

| Species | Strain | Formation of ballistoconidia | Assimilation of[a] | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Carbon source | | | | | | | | | | | | | | | | | Nitrogen source | | | | | |
| | | | Lactose | Melibiose | Inulin | D-Xylose | D-Arabinose | D-Ribose | L-Rhamnose | Ethanol | Glycerol | Erythritol | Ribitol | D-Mannitol | D-Glucitol | α-Methyl-D-glucoside | Salicin | Citric acid | Inositol | Potassium nitrate | Sodium nitrite | Growth in medium containing 50% glucose | Acid production from glucose | Gelatin liquefaction | Maximum growth temperature (°C) |
| *Bullera hoabinhensis* sp. nov. | VY-68[T] | + | l | − | − | + | l | s | + | s | + | − | lw | + | − | w | + | + | + | − | + | w | − | w | 32–33 |
| *B. pseudoalba* | JCM 5290[T], JCM 9707 | + | + | w/+ | −/w | l/+ | w/+ | s | s/+ | w/+ | s/w | −/+ | −/+ | +/l | l/+ | l/+ | + | + | s/+ | lw | lw | − | −/w | w/− | 32–35 |
| *C. laurentii* | JCM 9066[T] | − | l | l | − | + | w | + | + | + | − | − | + | + | + | w | + | s | l | lw | lw | − | − | w | 32–33 |

[T] type strain.

[a] +, positive; −, negative; l, latent; s, slow positive; w, weak; lw, latent and weak.

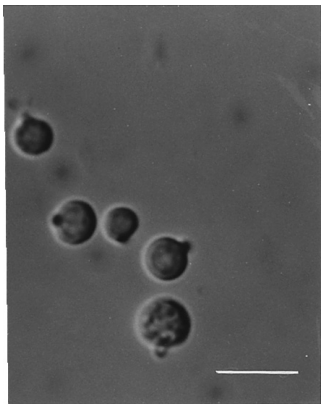

Fig. 5. Ballistoconidia of *B. pseudoalba* JCM 9707 (formerly the type strain of *C. cellulolyticus*) produced on corn meal agar after 14 days at 17°C.

and the loss of ballistoconidial formation by the type strain of the former species. We examined the ballistoconidium-forming activity of *B. pseudoalba* JCM 9707 (formerly the type strain of *Cryptococcus cellulolyticus*) and found that it definitely produced ballistoconidia (Fig. 5). They were spherical to pyriform like those of typical *Bullera*. We assume that the authors of *Cryptococcus cellulolyticus* missed this property.

**Description of New Taxa**

*Latin diagnosis of* ***Bullera hoabinhensis*** *Luong, Takashima, Ty, Dung et Nakase, sp. nov.*

In liquido "YM" post dies 5 ad 17°C, cellulae vegetativae sphaericae vel ovoideae aut elongatae, 3.0–6.0×5.0–10.0 μm, singulae, binae, aut in catenis. Post unum mensem ad 17°C, pellicula fragilis, completa et sedimentum formantur. Cultura in agaro "YM," subflava, glabra, nitida, mollis vel mucosa et margine glabra. Mycelium et pseudomycelium non formantur. Ballistoconidia apiculata, 1.5–2×1.5–2 μm.

Fermentatio nulla. Glucosum, galactosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum (lente), raffinosum, melezitosum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum (lente), D-ribosum (exiguum), L-rhamnosum, ethanolum (exiguum), glycerolum, ribitolum (lente et exiguum), galactitolum, D-mannitolum, α-methyl-D-glucosidum (exiguum), salicinum, glucuno-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum succinicum, acidum citricum et inositolum assimilantur at non L-sorbosum, melibiosum, inulinum, erythritolum, D-glucitolum nec acidum DL-lacticum. Ammonium sulfatum, natrium nitrosum, L-lysinum, cadaverinum et ethylaminum assimilantur at non kalium nitricum. Maxima temperatura crescentiae: 32–33°C. Ad crescentiam thiaminum necessarium est. Materia amyloidea iodophila formantur. Ureum hydrolysatur. Diazonium caeruleum B: Positivum. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 54 mol% per HPLC. Systema ubiquini: Q-10. Xylosum in cellulis presens.

Holotypus: Isolatus ex folio *Anadendrum montanum*, Vietnam, JCM 10835/VTCC2 0181 (originaliter ut VY-68) conservatur in collectionibus culturarum quas "Japan Collection of Microorganisms," Wako, Saitama et "Vietnam Type Culture Collection" sustentat.

*Description of* ***Bullera hoabinhensis*** *Luong, Takashima, Ty, Dung et Nakase, sp. nov.*

*Bullera hoabinhensis* (*hoabinh* is the place name where the plant leaf was collected in Cuc Phuong National Park)

Growth in YM broth: After 5 days at 17°C, the vegetative cells are spherical to ovoidal or elongated and measure 3.0–6.0×5.0–10.0 μm. They occur singly, in pairs or in groups, reproduce by budding (Fig. 4). A complete ring, fragile pellicle and a sediment are formed after 1 month at 17°C.

Growth in YM agar: After 1 month at 17°C, the streak culture is pale yellow, smooth, shining, mucous, soft and has an entire margin.

Dalmau plate culture on corn meal agar: Mycelium or pseudomycelium is not formed after 2 weeks of incubation at 17°C.

Formation of ballistoconidia: Ballistoconidia are formed abundantly on corn meal agar after 7 days incubation at 17°C (Fig. 4). They are globose to napiform, measuring 1.5–2.0×1.5–2.0 μm.

Fermentation: Absent.

Assimilation of carbon compounds:

| | | | |
|---|---|---|---|
| Glucose | + | Ethanol | + (slow) |
| Galactose | + | Glycerol | + |
| L-Sorbose | − | Erythritol | − |
| Sucrose | + | Ribitol | + (latent and weak) |
| Maltose | + | Galactitol | + |
| Cellobiose | + | D-Mannitol | + |
| Trehalose | + | D-Glucitol | − |
| Lactose | + (latent) | α-Methyl-D-glucoside | + (weak) |

| | | | |
|---|---|---|---|
| Melibiose | − | Salicin | + |
| Raffinose | + | Glucono-δ-lactone | + |
| Melezitose | + | 2-Ketogluconic acid | + |
| Inulin | − | 5-Ketogluconic acid | + |
| Soluble starch | + | D-Glucuronic acid | + |
| D-Xylose | + | D-Galacturonic acid | + |
| L-Arabinose | + | DL-Lactic acid | − |
| D-Arabinose | + (latent) | Succinic acid | + |
| D-Ribose | + (slow) | Citric acid | + |
| L-Rhamnose | + | Inositol | + |
| Assimilation of nitrogen compounds: | | | |
| Ammonium sulfate | + | Ethylamine hydrochloride | + |
| Potassium nitrate | − | L-Lysine hydrochloride | + |
| Sodium nitrite | + | Cadaverine dihydrochloride | + |

Maximum growth temperature: 32–33°C.

Vitamin required: Thiamine.

Production of starch-like substances: Positive.

Diazonium blue B color reaction: Positive.

Urease: Positive.

Splitting of fat: Negative.

Liquefaction of gelatin: Weak.

Growth in medium containing 50% glucose: Weak.

Acid production on chalk agar: Negative.

C+G content of nuclear DNA: 54 mol% (by HPLC).

Ubiquinone system: Q-10.

Xylose in the cells: Present.

Strain examined: *Bullera hoabinhensis* sp. nov. (VY-68) was isolated by Dao Thi Luong in February 1999, from a leaf of *Anadendrum montanum* Schott, collected by Takashi Nakase and Dao Thi Luong at Cuc Phuong National Park of Ninh Binh, Vietnam. This strain has been deposited in JCM and VTCC, with the accession number JCM 10835 and VTCC2 0181, respectively.

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**References**

Boekhout, T. and Nakase, T. (1998) *Bullera* Derx. *In* The Yeasts, A Taxonomic Study, 4th ed., ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, pp. 731–741.

Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G., and Statzell-Tallman, A. (2000) Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int. J. Syst. Evol. Microbiol.*, **50**, 1351–1371.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**, 783–791.

Hamamoto, M. and Nakase, T. (1995) Ballistosporous yeasts found on the surface of plant materials collected in New Zealand. 1. Six new species in the genus *Sporobolomyces. Antonie van Leeuwenhoek*, **67**, 151–171.

Kimura, M. (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.*, **16**, 111–120.

Komagata, K. and Nakase, T. (1967) Reitoshokuhin no biseibutsu ni kansuru kenkyu. V. Shihan reitoshokuhin yori bunri shita kobo no seijo (Microbiological studies on frozen foods. V. General properties of yeast isolaed from frozen foods). *Shokuhin Eiseigaku Zasshi*, **8**, 53–57 (in Japanese).

Kurtzman, C. P. and Robnett, C. J. (1997) Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. *J. Clin. Microbiol.*, **35**, 1216–1223.

Kurtzman, C. P. and Robnett, C. J. (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek*, **73**, 331–371.

Luong, D. T., Takashima, M., Ty, P. V., Dung, N. L., and Nakase, T. (2000) Four new species of *Kockovaella* isolated from plant leaves collected in Vietnam. *J. Gen. Appl. Microbiol.*, **46**, 297–301.

Nakase, T., Komagata, K., and Fukazawa, Y. (1976) *Candida pseudointermedia* sp. nov. isolated from “Kamaboko,” a traditional fish-paste product in Japan. *J. Gen. Appl. Microbiol.*, **22**, 177–182.

Nakase, T. and Suzuki, M. (1986a) *Bullera derxii* sp. nov. and *Bullera pseudoalba* sp. nov. isolated from dead leaves of *Oryza sativa* and *Miscanthus sinensis. J. Gen. Appl. Microbiol.*, **32**,125–135.

Nakase, T. and Suzuki, M. (1986b) *Bullera megalospora*, a new species of yeast forming large ballistospores isolated from dead leaves of *Oryza sativa*, *Miscanthus sinensis*, and *Sasa* sp. in Japan. *J. Gen. Appl. Microbiol.*, **32**, 225–240.

Nakase, T., Suzuki, M., Hamamoto, M., Takashima, M., Hatano, T., and Fukui, S. (1996) A taxonomic study on cellulolytic yeasts and yeast-like microorganisms isolated in Japan. II. The genus *Cryptococcus. J. Gen. Appl. Microbiol.*, **42**, 7–15.

Nakase, T. and Takashima, M. (1993) A simple procedure for

the high frequency isolation of new taxa of ballistosporous yeasts living on the surfaces of plants. *RIKEN Rev.*, **3**, 33–34.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.

Scorzetti, G., Fell, J. W., Fonseca, A., and Statzell-Tallman, A. (2002) Systematics of basidiomycetous yeasts: A comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res.*, **2**: 495–517.

Sugita, T. and Nishikawa, A. (2003) Fungal identification method based on DNA sequence analysis: Reassessment of the methods of the Pharmaceutical Society of Japan and the Japanese Pharmacopoeia. *J. Health Sci.*, **49**, 531–533.

Suzuki, M. and Nakase, T. (1988) The distribution of xylose in the cells of ballistosporous yeasts—Application of high performance liquid chromatography without derivatization to the analysis of xylose in whole cell hydrolysates. *J. Gen. Appl. Microbiol.*, **34**, 95–103.

Takashima, M. and Nakase, T. (1999) Molecular phylogeny of the genus *Cryptococcus* and related species based on the sequences of 18S rDNA and internal transcribed spacer regions. *Microbiol. Cult. Coll.*, **15**, 33–45.

Takashima, M. and Nakase, T. (2000) Four new species of the genus *Sporobolomyces* isolated from leaves in Thailand. *Mycoscience*, **41**, 357–369.

Tamaoka, J. and Komagata, K. (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Lett.*, **25**, 125–128.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Yarrow, D. (1998) Methods for the isolation, maintenance and identification of yeasts. *In* The Yeasts, A Taxonomic Study, 4th ed., ed. by Kurtzman, C. P. and Fell, J. W., Elsevier, Amsterdam, pp. 77–100.