

Bullera sakaeratica sp. nov., a new species of ballistoconidium-forming yeast found in Thailand

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Two strains of ballistoconidium-forming yeasts were isolated from leaves collected in a protected rain forest in Thailand. They were found to represent a new anamorphic hymenomycetous yeast species based on analysis of the nucleotide sequences of small subunit (SSU) rDNA, internal transcribed spacer regions including 5.8S rDNA and the D1/D2 domain of large subunit (LSU) rDNA, and was described as *Bullera sakaeratica* Fungsin, Takashima et Nakase sp. nov. In phylogenetic trees based on the sequences of SSU and the D1/D2 domain of LSU rDNA, it constitutes a cluster with *Trimorphomyces papilionaceus*.

Key words : *Bullera sakaeratica* sp. nov. ; new ballistoconidium-forming yeast from Thailand ; new yeast from a leaf

INTRODUCTION

During a survey of ballistoconidium-forming yeasts in the tropical protected rain forest in Northeastern Thailand, we isolated two strains of *Bullera* which can not be assigned to any known species. These yeasts were found to represent a single new species and described as *Bullera sakaeratica* sp. nov. in the present paper.

MATERIALS AND METHODS

Isolation of yeast strain employed. Plant samples for yeast isolation were collected in a protected rain

forest in Sakaerat, Nakhon Ratchasima Province, Thailand, in November 1996. The two strains of yeasts, TY-194 and TY-231, employed in the present study were isolated from leaves of *Seteria pallidifusca* Stapt. and *Urena lobata* Linn. var. *sinuata* King, respectively. They were isolated by ballistoconidia-fall method previously reported (8) with YM agar (Difco Lab., Detroit) without any antibacterial or antifungal agents. Immediately after the purification by conventional streaking technique, the isolates were preserved at -80 °C suspended in YM broth supplemented with 10% (w/v) glycerol.

Examination of morphological, physiological and biochemical characteristics, ubiquinone systems,

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xylose in the cells, isolation and purification of nuclear DNA, and DNA base composition. All of these were carried out according to the procedures described in the previous paper (5).

Sequencing and phylogenetic analysis. The nucleotide sequences of SSU (18S) rDNA and the internal transcribed spacer regions (ITS 1 and 2) including the 5.8S rDNA were directly determined using PCR products according to Sugita and Nakase (10). The nucleotide sequences of the D1/D2 domain of LSU (26S) rDNA were directly determined using PCR products according to Kurtzman and Robnett (7) and Fell et al. (4) and Fungsin et al. (5). The sequences of SSU rDNA, ITS regions including 5.8S rDNA and the D1/D2 domain of LSU rDNA determined in this study were deposited in the GenBank database under the following accession numbers: TY-194 (TISTR 5803 = JCM 11900) SSU rDNA (AY211544); ITS region including 5.8 rDNA (AY217651); D1/D2 domain of LSU rDNA (AY211546); and TY-231 (TISTR 5804 = JCM 11901) SSU rDNA (AY211545); ITS region including 5.8 rDNA (AY217652); D1/D2 domain of LSU rDNA (AY211547). Reference sequences used for the phylogenetic study, shown in Figs. 1 and 2, were obtained from the database. Generated sequences were aligned with related species in hymenomycetous yeasts using the CLUSTAL W ver. 1.74 computer program (13). The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (6) using the neighbor-joining method (9) in the PHYLIP computer program. Sites where any gaps existed in any sequences were excluded. Bootstrap analyses (3) were performed from 1,000 random resamplings.

RESULTS AND DISCUSSION

TY-194 and TY-231 formed rotationally symmetrical ballistoconidia, did not produce stalked-conidia, had Q-10 as the major ubiquinone isoprenologue and had xylose in the cells. These characteristics coincided well with the genus *Bullera* (2). Therefore, these strains were assigned to this genus.

The SSU rDNA sequence of TY-194 and TY-231

were determined in the present study. A phylogenetic tree was constructed for these two strains based on the SSU rDNA sequences and the related species by the neighbor-joining method (Fig. 1). In this tree, TY-194 and TY-231 are located at a closely related position from each other. The sequence similarity value in SSU rDNA between the two strains is 99.8%. *Trimorphomyces papilionaceus* and *Bullera miyagiana* are also located in the same cluster, however, the phylogenetic distances from the isolates are not short.

The ITS region (including 5.8S rDNA) sequences of the strains TY-194 and TY-231 were determined. The total length of ITS1-5.8S rDNA-ITS2 regions of these strains is 456 bases. The length of its ITS1 and ITS2 regions are 115 and 185 bases, respectively. In this region, only one nucleotide (0.18%) is different between a two strains. According to Sugita et al. (11, 12), conspecific strains have a nucleotide difference of less than 1% in this region, meanwhile, Bai et al. (1) considered that this value was 2%. Therefore the two strains, TY-194 and TY-231, are decided to be conspecific. The BLAST analysis of the ITS region showed that the most closely related species was *Trimorphomyces papilionaceus*, however, the nucleotide difference in their ITS1 and ITS2 regions overall between the former two strains and the latter species is high, 46 bases (8.9%).

The sequences of the D1/D2 domain of LSU rDNA of TY-194 and TY-231 were determined and a phylogenetic tree was constructed based on the aligned sequence of the two strains with related species of *Bullera* and other hymenomycetous yeast taxa (Fig. 2). The tree showed that TY-194 and TY-231 are located at closely related positions. The nucleotide difference is one (0.18%) in the domain between the two strains. In the tree, the two strains are most closely related to *Trimorphomyces papilionaceus*, however, the nucleotide difference in this domain between the two strains and the latter species is high, 43 bases (7.9%). Based on the facts mentioned above, we concluded that TY-194 and TY-231 represented a single new species of the genus *Bullera*, which is described as *Bullera sakaeratica* sp. nov. This species is located at a cluster that is distant

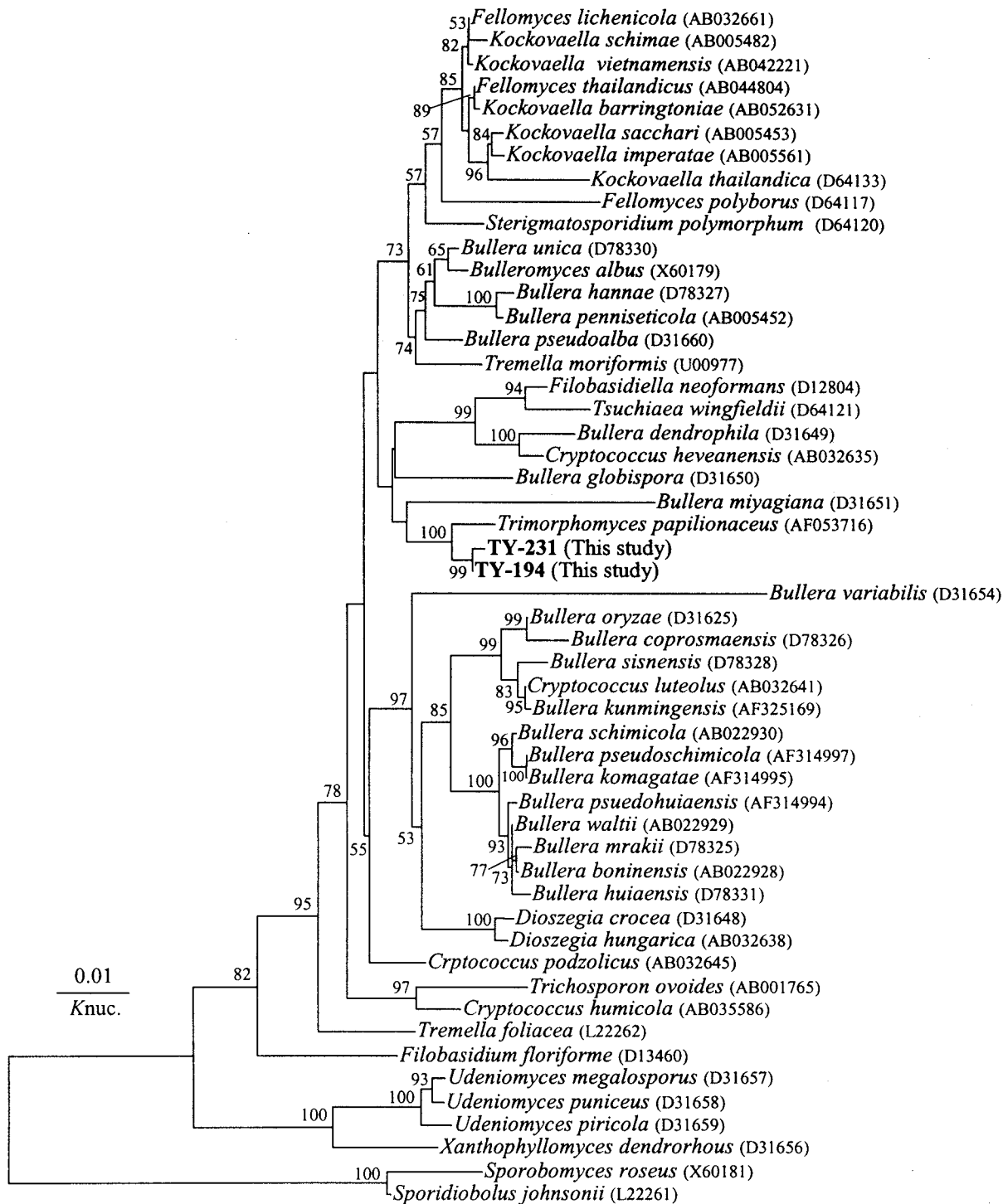


Fig. 1. Phylogenetic tree for strains TY-194 and TY-231 and related taxa based on the SSU rDNA sequences. The tree was constructed from the evolutionary distance data according to Kimura (6), using the neighbor-joining method (9) with bootstrapping (3). The numerals represent results from 1,000 replicated bootstrap samplings (a frequency of less than 50% is not indicated).

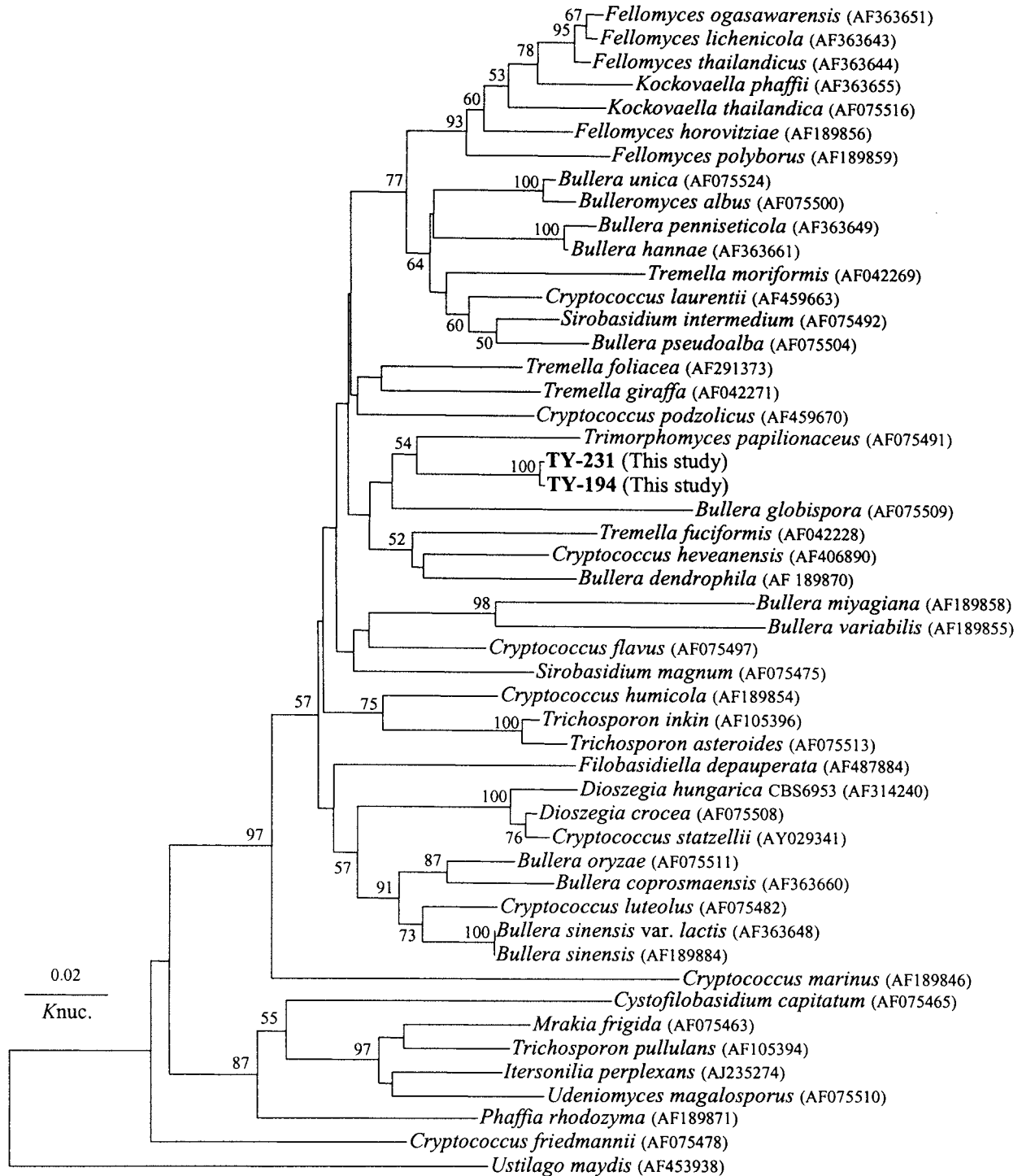


Fig. 2. Phylogenetic tree for strains TY-194 and TY-231 and related taxa based on the D1/D2 domain of LSU rDNA sequences. The tree was constructed from the evolutionary distance data according to Kimura (6), using the neighbor-joining method (9) with bootstrapping (3). The numerals represent results from 1,000 replicated bootstrap samplings (a frequency of less than 50 % is not indicated).

from the cluster where *Bulleromyces albus*, a teleomorph of the type species of *Bullera*, *B. alba*, is located (Figs. 1 and 2).

Phenotypically, *Bullera sakaeratica* is close to *Bullera miyagiana*, however, it can be distinguished from the latter species in the assimilation of L-lysine and cadaverine as the nitrogen sources, and the lack of production of starch-like compounds. Practically, *Bullera sakaeratica* can be distinguished from others of *Bullera* by the characteristic of carbon assimilation of L-sorbose, glycerol and ribitol.

Description of *Bullera sakaeratica* Fungsin, Takashima et Nakase sp. nov.

In liquido "YM", post dies 3 ad 25 °C, cellulae subglobosae, ovoideae, aut ellipsoideae 2.5-6.3 × 5.3-10.3 μm, Sedimentum formatur. In agar "YM", post unum mensem ad 17 °C, cultura crenea, nitida, margine undulata. Mycelium et pseudomycelium non formantur. Ballistosporae globosae aut subglobosae, 5.3-7.8 × 5.3-7.8 μm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, erythritolum, galactitolum, D-mannitolum, D-glucitolum (lente et exiguum), α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum D-glucuronicum, acidum D-galacturonicum, acidum succinicum et inositolum assimilantur at non inulinum, ethanolum, glycerolum nec ribitolum. Ammonium sulfatum, natrium nitrosum, L-lysinum et cadaverinum (vel nullum) assimilantur at non kalium nitricum nec ethylaminum. Maxima temperatura crescentiae : 27-28 °C. Ad crescentiam thiaminae necessarium est. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleico : 51.3 mol% (ex HPLC). Ubiquinonum majus : Q-10. Teleomorphosis ignota.

Holotypus : TY-194 isolata ex folio *Seteria pallide-fusca* Stapt. In collectionibus culturarum quas 'Thailand Institute of Scientific and Technological Research', Chatuchak, Bangkok, sustentat, no.

TISTR 5803, JCM deposita et in collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama, sustentat, no. JCM 11900.

Growth in YM broth : After 3 days at 25 °C cells are subglobose, ovoidal or ellipsoidal, 2.5-6.3 × 5.3-10.3 μm (Fig. 3A). After 1 month at 17 °C, a sediment is formed.

Growth on YM agar : After 1 month at 17 °C, the streak culture is cream, smooth, dull and has an undulate margin.

Dalmu plate culture on corn meal agar : Mycelia and pseudomycelia are not formed.

Production of ballistoconidia : Fairly good production of ballistoconidia is observed on the corn meal agar. They are globose or subglobose, 5.3-7.8 × 5.3-7.8 μm (Fig. 3B).

Fermentation : Absent

Assimilation of carbon compounds :

Glucose	+	Ethanol	-
Galactose	+	Glycerol	-
L-Sorbose	+	Erythritol	+
Sucrose	+	Ribitol	-
Maltose	+	Galactitol	+
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
			(Delay & Weak)
Lactose	+	α-Methyl-D-glucoside	+
Melibiose	+	Salicin	+
Raffinose	+	Glucono-α-lactone	+

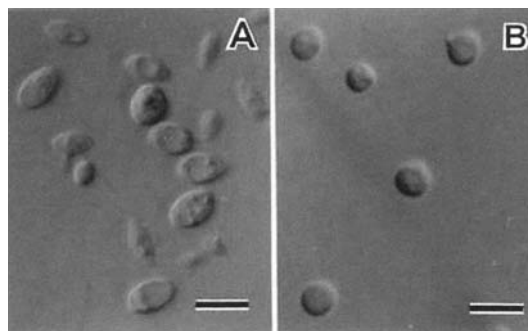


Fig. 3. Morphology of *Bullera sakaeratica*

A : Vegetative cells of *B. sakaeratica* TY-194 grown in YM broth for 3 days at 25 °C.

B : Ballistoconidia of *B. sakaeratica* TY-194 produced on corn meal agar after 7 days at 25 °C.

Scale bars indicate 10 μm.

Melezitose	+	2-Ketogluconic acid	+
Inulin	-	5-Ketogluconic acid	+
Soluble starch	+	DL-Lactic acid	+
		(Delay & Weak)	
D-Xylose	+	D-Glucuronic acid	+
L-Arabinose	+	D-Galacturonic acid	+
D-Arabinose	+	Succinic acid	+
D-Ribose	+	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: 27–28 °C.

Vitamin required : Thiamine.

Production of starch-like substances : Negative.

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

Urease : Positive.

Liquefaction of gelatin : Negative.

Diazonium Blue B reaction : Positive.

G + C content of nuclear DNA : 51.3 mol % (by HPLC).

Major ubiquinone : Q-10.

Xylose in the whole cell hydrolysate : Present.

Holotype : TY-194, isolated by B. Fungsin, M. Takashima and T. Nakase from a leaf of *Seteria pal-lide-fusca* Stapt. which was collected at a tropical rain forest in Sakaerat Environmental Research Station, Nakhon Ratchasima Province, Thailand, in November 1996. This strain is deposited at the Thailand Institute of Scientific and Technological Research, Bangkok as TISTR 5803, and the Japan Collection of Microorganisms, Wako, Saitama, as JCM 11900. Strain TY-231 was also deposited in the culture collection mentioned above as TISTR 5804 = JCM 11901.

Etymology : The specific epithet of this species is derived from the name of the place where this species was isolated.

ACKNOWLEDGEMENTS

We thank Ms. Poonsook Atthasampunna and Dr. Jiraporn Sukhumavasi, Thailand Institute of

Scientific and Technological Research (TISTR), Dr. Makiko Hamamoto, Japan Collection of Microorganisms (JCM), RIKEN, and Dr. Morakot Tanticharoen, National Center for Genetic Engineering and Biotechnology (BIOTEC), NSTDA, for their invaluable suggestions for the present study, and Ms. Sasitorn Jindamorakot, Ms. Phuntip Poonpairoj and Hathairat Jan-ngam, BIOTEC, for their technical assistance on several experiments. We are indebted to Ms. Tippa Sadakorn, BK Herbarium, Department of Agriculture, Ministry of Agriculture and Cooperative of the Thai Government, for the identification of plants. We also thank Dr. Worapot Suntornsuk, Department of Microbiology, King Mongkut Institute of Technology (Tonburi), for his help in collecting plant samples. This study was supported in part by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

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タイ国産射出胞子形成酵母の新種 *Bullera sakaeratica* sp. nov

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タイの保護熱帯雨林で採集した植物から射出胞子形成酵母2株を分離した。SSU rDNA, 5.8S rDNAを含むITS領域およびLSU rDNAのD1/D2領域の塩基配列の解析から、これらの酵母は菌草綱に属するアナモルフ酵母の新種であることを明らかにし、*Bullera sakaeratica* Fungsin, Takashima & Nakase, sp. nov. として記載した。本種はSSU rDNA およびLSU rDNAのD1/D2領域の配列に基づき作成した分子系統樹では *Trimorphomyces papilionaceus* と系統枝を形成する。