**BENSINGTONIA MUSAE SP. NOV. ISOLATED FROM
A DEAD LEAF OF MUSA PARADISIACA AND ITS
PHYLOGENETIC RELATIONSHIP AMONG
BASIDIOMYCETOUS YEASTS**

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A new species of ballistoconidium-forming yeast, *Bensingtonia musae*, was isolated from a dead leaf of *Musa paradisiaca* collected in the southeast seacoast of Bangkok, Thailand. *B. musae* showed physiological and biochemical characteristics similar to *B. ingoldii* and *B. intermedia*. DNA-DNA reassociation experiments, however, showed that it was distinct from these two species. *B. musae* is easily distinguished from *B. ingoldii* by the assimilation of sucrose, cellobiose, lactose, melezitose, soluble starch and nitrate, and from *B. intermedia* by the assimilation of cellobiose, L-arabinose, erythritol and salicin, and the requirement of *p*-aminobenzoic acid and pyridoxine. In the phylogenetic tree constructed based on small subunit rRNA gene sequences, *B. musae* was located at a cluster which was composed of *B. ciliata*, the type species of the genus, *B. ingoldii*, *B. miscanthi*, *B. naganoensis*, *B. phylladus*, *B. subrosea* and *B. yuccicola*. Among these species, *B. musae* was the most closely related to *B. ingoldii*.

The genus *Bensingtonia* was described as a new ballistoconidium-forming genus by Ingold (3) based on a single species *B. ciliata*. Ingold regarded this fungus as a hyphomycete and concluded that it represented a new genus because it lacked clamp-connections and formed non-curved ballistospores (ballistoconidia), differing in these aspects from *Itersonilia* and *Tilletiopsis*, respectively. In 1988, Nakase and Boekhout (6) proposed the emendation of the diagnosis of the genus *Bensingtonia* to include all ballistoconidium-forming yeasts which were characterized

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by Q-9 as the major ubiquinone and the lack of xylose in the cells. They proposed the following six new combinations: *B. intermedia*, *B. miscanthi*, *B. naganoensis*, *B. subrosea*, *B. yamatoana* and *B. yuccicola*. Later, *B. ingoldii* was described as a new species by Nakase et al. (7), and *Sporobolomyces phylladus* was transferred to the genus *Bensingtonia* by Yamada et al. (18).

In 1990, a ballistoconidium-forming yeast was isolated from a dead leaf of *Musa paradisiaca* collected in Thailand. This strain was assigned to the genus *Bensingtonia*, because it was characterized by Q-9 as the major ubiquinone, the absence of xylose in the cells, and the production of kidney-shaped ballistoconidia. It showed physiological and biochemical characteristics similar to *B. intermedia* and *B. ingoldii*. DNA-DNA reassociation experiments, however, revealed that this yeast represented the 10th species of the genus *Bensingtonia*. It is described as *Bensingtonia musae* Takashima, Suh et Nakase sp. nov.

MATERIALS AND METHODS

The strain K-304 reported in this paper was isolated from a dead leaf of *Musa paradisiaca*, which was collected in the southeast seacoast of Bangkok, Thailand, using ballistoconidium-fall method with YM agar plates.

Most of the morphological, physiological and biochemical characteristics were examined according to van der Walt and Yarrow (17). Assimilation of nitrogen compounds was investigated on solid media with starved inoculum according to Nakase and Suzuki (9). Vitamin requirements were investigated according to Komagata and Nakase (5). The maximum growth temperature was determined in YM broth (Difco Labs, Detroit, MI, U.S.A.) using metal block baths. Phenotypic similarity analysis was performed using the NTSYS-pc program package Ver. 1.60 (12) based on the physiological and biochemical characteristics.

Extraction, purification and identification of ubiquinones was carried out according to Nakase and Suzuki (11). Xylose in the cells was analyzed as described by Suzuki and Nakase (14). Isolation and purification of DNA was carried out according to Nakase and Suzuki (9). The DNA base composition (mol% G + C) was determined by high-performance liquid chromatography according to Nakase et al. (7), and was also calculated from the thermal denaturation temperature (T_m) using DNA of *Candida parapsilosis* JCM 1785 (=CBS 604) as the reference whose mol% G + C was 40.8%. DNA-DNA reassociation experiments were carried out using the membrane-filter method according to Nakase and Suzuki (8).

The small subunit (SSU) rDNA sequence was determined after amplifying the gene using the polymerase chain reaction (PCR). Purification of PCR products and direct sequencing of amplified DNA were carried out as described in the previous paper (15).

Generated sequences were aligned by the Clustal V computer program (2). The SSU rDNA sequences of yeasts and yeast-like fungi employed for phylogenetic analysis were obtained from the nucleotide sequence libraries (EMBL, GenBank

and DDBJ) as shown in Table 1. The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (4) using the neighbor-joining method (13) in the Clustal V computer program. Sites where gaps existed in any sequences were excluded. Bootstrap analysis (1) was performed from 1,000 random resamplings.

RESULTS AND DISCUSSION

Comparison in physiological and biochemical characteristics and DNA homologies

The dendrogram, which was constructed based on assimilation tests of 34 carbon compounds and 6 nitrogen compounds, and requirement of 9 vitamins of *Bensingtonia* sp. K-304 and the type strains of the genus *Bensingtonia*, is shown in Fig. 1, along with mol% G+C. *Bensingtonia* sp. K-304 was located in a cluster which was composed of *B. intermedia*, *B. ingoldii*, *B. naganoensis* and *B. yamatoana*. Some of the species of this cluster showed similar G+C values to *Bensingtonia* sp. K-304. The remaining five species of *Bensingtonia*, *B. ciliata*, *B. phylladus*, *B. miscanthi*, *B. yuccicola* and *B. subrosea*, comprised another cluster which was far from *Bensingtonia* sp. K-304. The G+C values of the latter cluster were lower than those of the former one. Therefore, DNA-DNA reassociation experiments were carried out among the species in the former cluster to determine the taxonomic position of *Bensingtonia* sp. K-304. As shown in Table 2, *Bensingtonia* sp. K-304 showed low DNA relatedness (4–15%) with *B. ingoldii*, *B. intermedia*, *B. nagano-*

Table 1. SSU rDNA sequences used for the phylogenetic analysis.

Species	Accession No. ^a	Species	Accession No.
<i>Genus Bensingtonia</i>			
<i>B. ciliata</i>	D38233	<i>B. phylladus</i>	D38237
<i>B. ingoldii</i>	D38234	<i>B. subrosea</i>	D38238
<i>B. intermedia</i>	D38235	<i>B. yamatoana</i>	D38239
<i>B. miscanthi</i>	D38236	<i>B. yuccicola</i>	D38367
<i>B. naganoensis</i>	D38366		
<i>Other basidiomycetous fungi</i>			
<i>Bullera alba</i>	X60179	<i>Sporidiobolus johnsonii</i>	L22261
<i>Cystofilobasidium capitatum</i>	D12801	<i>Sporobolomyces roseus</i>	X60181
<i>Erythrobasidium hasegawianum</i>	D12803	<i>Tilletia caries</i>	U00972
<i>Filobasidiella neoformans</i>	X60183	<i>Tremella moriformis</i>	U00977
<i>Filobasidium floriforme</i>	D13460	<i>Tremella globospora</i>	U00976
<i>Leucosporidium scottii</i>	X53499	<i>Tremella foliacea</i>	L22262
<i>Mrakia frigida</i>	D12802	<i>Ustilago hordei</i>	U00973
<i>Rhodospodium toruloides</i>	X60180	<i>Ustilago maydis</i>	X62396
<i>Outgroup</i>			
<i>Candida albicans</i>	X53497	<i>Neurospora crassa</i>	X04971
<i>Saccharomyces cerevisiae</i>	J01353		

^a EMBL, GenBank and DDBJ accession numbers.

Fig. 1. Dendrogram based on the physiological and biochemical characteristics among *Bensingtonia* sp. K-304 and type strains of the genus *Bensingtonia*.

The similarity value was calculated using SIMQUAL in NTSYS-pc (12). Clustering was performed by UPGMA using SAHN in NTSYS-pc. ^a, data from van der Walt et al. (16); ^b, data from Nakase and Suzuki (10); ^c, data from Nakase and Suzuki (11).

Table 2. DNA relatedness among *Bensingtonia* sp. K-304 and phenotypically similar *Bensingtonia* species.

Species	Strain	% Relative binding of DNA from	
		K-304	JCM 5291
<i>Bensingtonia</i> sp.	K-304	100	7
<i>B. ingoldii</i>	JCM 7445	15	8
<i>B. intermedia</i>	JCM 5291	5	100
<i>B. naganoensis</i>	JCM 5978	8	7
<i>B. yamatoana</i>	JCM 2896	4	14

ensis and *B. yamatoana*. These results clearly indicate the separation of K-304 from the other four species. Strain K-304 is described as a new species.

Description

Bensingtonia musae Takashima, Suh et Nakase sp. nov.

In liquido 'YM,' post dies 3 ad 25°C, cellulae ovoideae, ellipsoideae, elongatae aut cylindraceae, (1.6–2.4) × (5.4–12.6) μm, singulae aut binae. Sedimentum formatur. Post unum mensem ad 17°C, pellicula fragilis et incompleta, et sedimentum formatur. In agar 'YM,' post unum mensem ad 17°C, cultura olivaceo-flava aut atroflava, glabra aut rugosa, butyracea, margine erosa. Pseudomycelium primitivum formatur. Ballistospores reniformes, (1.6–3.3) × (5.0–7.5) μm. Fermentatio nulla. Glucosum, L-sorbosum, trehalosum, D-xylosum, L-arabiosum (lente), D-arabiosum (lente), D-ribosum, ethanolum, glycerolum, erythritolum, ribitolum, D-mannitolum, D-glucitolum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum (lente), acidum succinicum (lente et exiguum) et acidum citricum

Fig. 2. *Bensingtonia musae*.

(A) Vegetative cells grown in YM broth for 3 days at 25°C. (B) Ballistoconidia produced on corn meal agar after 5 days at 25°C. Scales = 10 µm.

(exiguum) assimilantur at non galactosum (vel lente et exiguum), saccharosum, maltosum, cellobiosum, lactosum, melibiosum, raffinose, melezitose, inulinum, amyllum solubile, L-rhamnosum, galactitolum, α -methyl-D-glucosidum, acidum DL-lacticum nec inositolum. Ammonium sulfatum, ethylaminum, L-lysinum et cadaverinum assimilantur at non kalium nitricum nec natrium nitrosum. Maxima temperatura crescentiae: 31–32°C. Ad crescentiam acidum *p*-aminobenzonicum, pyridoxinum et thiaminum necessarium sunt. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 52.3 mol% (per HPLC), 55.2 mol% (ex T_m). Ubiquinonum majus: Q-9. Xylosum in cellulis absens. Holotypus: Isolatus ex folio emortuo *Musae paradisiacae*, JCM 8801 (originaliter ut K-304) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms,' Wako, Saitama sustentat.

Growth in YM broth: After 3 days at 25°C, cells are ovoidal, ellipsoidal, elongate or cylindrical, $(1.6\text{--}2.4) \times (5.4\text{--}12.6)$ µm, single or in pairs. A sediment is formed. After one month at 17°C, an incomplete fragile pellicle and a sediment are present.

Growth on YM agar: After one month at 17°C, the streak culture is olive yellow to dark yellow (carry yellow), dull, smooth to delicately wrinkled, butyrous, and has an erose margin.

Dalmau plate culture on corn meal agar: Primitive pseudomycelia are produced.

Production of ballistoconidia: Ballistoconidia are abundantly produced on corn meal agar. They are kidney-shaped, $(1.6\text{--}3.3) \times (5.0\text{--}7.5)$ µm (Fig. 2).

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Ethanol	+
Galactose	— or + (latent and weak)	Glycerol	+
		Erythritol	+
L-Sorbose	+	Ribitol	+
Sucrose	—	Galactitol	—
Maltose	—	D-Mannitol	+
Cellobiose	—	D-Glucitol	+
Trehalose	+	α -Methyl-D-glucoside	—
Lactose	—	Salicin	+
Melibiose	—	Glucono- δ -lactone	+
Raffinose	—	2-Ketogluconic acid	+
Melezitose	—	5-Ketogluconic acid	+
Inulin	—	D-Glucuronic acid	+
Soluble starch	—	D-Galacturonic acid	+ (latent)
D-Xylose	+	DL-Lactic acid	—
L-Arabinose	+ (latent)	Succinic acid	+ (latent and weak)
D-Arabinose	+ (latent)		
D-Ribose	+	Citric acid	+ (weak)
L-Rhamnose	—	Inositol	—

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+
Potassium nitrate	—	L-Lysine hydrochloride	+
Sodium nitrite	—	Cadaverine dihydrochloride	+

Maximum growth temperature: 31–32°C.

Vitamins required: *p*-Aminobenzoic acid, pyridoxine and thiamine.

Production of starch-like substances: Negative.

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

Urease: Positive.

Liquefaction of gelatin: Negative.

Hydrolysis of fat: Negative.

Diazonium Blue B reaction: Positive.

G+C content of nuclear DNA: 52.3 mol% (by HPLC), 55.2 mol% (from T_m).

Major ubiquinone: Q-9.

Xylose in the cells: Absent.

Type strain: K-304, isolated from a dead leaf of *Musa paradisiaca* collected by T. Nakase in Dec. 1990, Thailand. This strain has been deposited in the Japan Collection of Microorganisms, Wako, Saitama as JCM 8801.

Practical discrimination

Bensingtonia musae is easily distinguished from *B. ingoldii* by the assimilation

Table 3. Salient characteristics of *Bensingtonia musae*, *B. ingoldii* and *B. intermedia*.

Species	Carbon assimilation													
	Galactose	L-Sorbose	Sucrose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Sol. starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose
<i>Bensingtonia musae</i>	lw/−	+	−	−	+	−	−	−	−	−	+	L	L	+
<i>B. ingoldii</i>	lw	lw/−	+	+	+	+	lw	lw	+	+	+	+	lw/−	lw
<i>B. intermedia</i>	L	L	−	L	L	−	−	−	−	−	L	−	L	L

Species	Carbon assimilation							Nitrogen assimilation		Vitamins required
	Erythritol	Ribitol	Glucitol	Salicin	Glucono- δ-lactone	Succinic acid	Citric acid	Nitrate	L-Lysine	
<i>Bensingtonia musae</i>	+	+	+	+	+	lw	W	−	+	PABA, pyridoxine, thiamine
<i>B. ingoldii</i>	+	+	W	W	+	W	W	+	+	PABA, pyridoxine, thiamine
<i>B. intermedia</i>	−	L	+	−	L	+	+	−	L	Thiamine

+, positive; -, negative; L, latent; W, weak; lw, latent and weak.

Fig. 3. Phylogenetic tree based on SSU rDNA sequences for the *Bensingtonia* species and related taxa.

The tree was constructed from the evolutionary distance data according to Kimura (4) using the neighbor-joining method (13) with bootstrapping (1). The numerals represent the percentages from 1,000 replicate bootstrap sampling.

of sucrose, cellobiose, lactose, melezitose, soluble starch and nitrate, and from *B. intermedia* by the assimilation of cellobiose, L-arabinose, erythritol and salicin, and the requirement of *p*-aminobenzoic acid and pyridoxine (Table 3).

Phylogenetic analysis

A total of 1739 positions of SSU rDNA of *Bensingtonia musae* were determined. The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases, with the accession number D43946.

After alignment, the phylogenetic tree of *B. musae* and 28 species of ascomycetous and basidiomycetous yeasts and yeast-like fungi was constructed by the neighbor-joining method based on 1,561 bases of the sequences (Fig. 3). The designations of clusters was the same as the previous paper (15).

Bensingtonia musae was located at cluster 2, which was composed of both ballistoconidiogenous and non-ballistoconidiogenous yeasts. Species in this cluster were characterized by the absence of xylose in the cells. In cluster 2, *B. musae* was located at cluster 2a which was composed of *B. ciliata*, the type species of the genus, *B. naganoensis*, *B. ingoldii*, *B. phylladus*, *B. yuccicolla*, *B. subrosea* and *B. miscanthi*. Among these species, *B. musae* constituted a cluster with *B. ingoldii*. *Bensingtonia intermedia* was located at cluster 2b in spite of the close resemblance of phenotypical characteristics to *B. musae*. It is apparent that the similarity in physiological and biochemical characteristics traditionally employed to classify yeasts do not necessarily reflect the phylogenetic relationship.

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