Short Communication

***Bensingtonia sakaguchii* sp. nov. isolated from a leaf of *Bischofia javanica* in the Ogasawara Islands**

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Bensingtonia Ingold is characterized by Q-9 as the major ubiquinone, the production of kidney-shaped ballistoconidia and the absence of xylose in the cells. Since Ingold (1986) established the genus *Bensingtonia* based on a single species, *B. ciliata*, ten species have been found. During a survey on ballistoconidium-forming yeasts from leaves of plants collected in the Ogasawara Islands, Japan, we isolated two strains of a hitherto undescribed yeast from a leaf of *Bischofia javanica* in Euphorbiaceae, which was collected in Chichi-jima, Ogasawara Islands, Japan. These isolates seemed to belong to the genus *Bensingtonia* because they have Q-9 as the major ubiquinone, lack xylose in the cells and produce kidney-shaped ballistoconidia. A new species, *Bensingtonia sakaguchii* Sugita, Takashima, Hamamoto et Nakase, is proposed for these two isolates. The specific epithet was chosen in honor of Prof. K. Sakaguchi who first studied yeasts in the Ogasawara Islands.

The strains reported in this paper, OK-255 and OK-259, were isolated using the ballistoconidium-fall method with YM agar (Difco Lab, MI, U.S.A.) plates. Most of the morphological, biochemical and physiological characteristics were examined using the methods described by van der Walt (1984). The assimilation of nitrogen compounds was investigated according to Nakase and Suzuki (1986). Vitamin requirements were examined using the method of Komagata and

Nakase (1967). The maximum growth temperature was determined in YM broth (Difco Lab.) using metal block baths. The extraction, purification and identification of ubiquinones was carried out according to Nakase and Suzuki (1988). Xylose in the cells was analyzed as described by Suzuki and Nakase (1988). Isolation and purification of DNA was carried out according to Nakase and Suzuki (1986). The DNA base composition (mol% G+C) was determined by nucleotide analysis using high-performance liquid chromatography (HPLC) after DNA digestion with Alkaline phosphatase (Sigma, MO, U.S.A.) and Nuclease P1 (Yamasa Shoyu, Chiba, Japan) as described by Nakase et al. (1989). DNA-DNA hybridization experiments were performed using the membrane-filter method according to Nakase and Suzuki (1985). The small subunit ribosomal RNA coding region was amplified by polymerase chain reaction (PCR) as described by Suh and Nakase (1995). The PCR products were sequenced using a SequiTherm Long-Read Cycle Sequencing Kit (Epicentre Technologies Corp., WI, U.S.A.) with the primers as described in Suh et al. (1996). Analyses of DNA sequence reactions were performed with an ALFexpress DNA sequencer (Pharmacia Biotech, Uppsala, Sweden). The sequences were aligned using the Clustal W computer program (Thompson et al., 1994). Introns were excluded from the alignment. For phylogenetic analysis, the following reference small subunit ribosomal DNA (SSU rDNA) sequences were obtained from nucleotide sequence libraries (EMBL, GenBank and DDBJ): *B. ciliata* (D38233), *B. ingoldii* (D38234), *B. intermedia* (D38235), *B. miscanthi* (D38236), *B. musae* (D43946), *B. naganoensis* (D38366), *B. phylladus* (D38237), *B. subrosea* (D38238), *B. yamatoana*

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(D38239), *B. yuccicola* (D38367); *Bulleromyces albus* (X60179), *Cystofilobasidium capitatum* (D12801), *Erythrobasidium hasegawianum* (D12803), *Fellomyces polyborus* (D64117), *Filobasidiella neoformans* (X60183), *Kockovaella thailandica* (D64133), *Kondoa malvinella* (D13776), *Kurtzmanomyces nectairei* (D64122), *Leucosporidium scottii* (X53499), *Mrakia frigida* (D12802), *Rhodospiridium dacryoidum* (D13459), *R. toruloides* (D12806); *Rhodotorula glutinis* (X69853), *R. graminis* (X83827), *R. mucilaginosa* (X84326); *Sporidiobolus johnsonii* (L22261), *Sporobolomyces roseus* (X60181), *S. xanthus* (D64118); *Sterigmatomyces halophilus* (D64119), *Sterigmatosporidium polymorphum* (D64120), *Tilletia caries* (U00972), *Tsuchiyaea wingfieldii* (D64121), *Udeniomyces piricola* (D31659), *Ustilago hordei* (D00973) and *U. maydis* (X62396). The ascomycetous yeasts *Saccharomyces cerevisiae* (J01353) and *Candida albicans* (X53497) and the filamentous ascomycete *Neurospora crassa* (X04971) were used as outgroups. For analysis using the neighbor-joining method (Saito and Nei, 1987), distances between the sequences were calculated using Kimura's two-parameter model (Kimura, 1980). Sites where gaps existed in any of the sequences were excluded. Bootstrap analysis (Felsenstein, 1985) was performed with 1,000 random resamples.

We determined 2,617 base sequences of SSU rDNA including the introns for two strains of *Bensingtonia sakaguchii*. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession numbers AB001746 (OK-255) and AB001747 (OK-259). The phylogenetic tree for *B. sakaguchii* and the other ten *Bensingtonia* species including related basidiomycetous yeasts was constructed by the neighbor-joining method based on 1,587 bases of the sequences (Fig. 2). Five base differences of SSU rDNA sequences were found between the two strains of *B. sakaguchii*. The sequence of the type strain was employed in Fig. 1. The designations of clusters were the same in Takashima et al. (1995). *Bensingtonia sakaguchii* was located in cluster 2a2. *Bensingtonia sakaguchii* made a branch with *B. musae*, *B. ingoldii* and the stalked-conidium forming yeast *Sterigmatomyces halophilus* with a 66% bootstrap confidence level. *S. halophilus* differs from the *Bensingtonia* species by its ability to produce stalked-conidia and its inability to produce ballistoconidia. As shown in Table 1, strain OK-255 showed a high DNA relatedness to strain OK-259. Therefore, they are classified in the same species. On the other hand, the DNAs of the two strains of *B. sakaguchii* indicated low homology values (10–20%) to those of *B. musae* and *B. ingoldii*, thus suggesting that *B. sakaguchii* repre-

Fig. 1. Vegetative cells and ballistoconidia of *Bensingtonia sakaguchii* sp. nov.

A: Vegetative cells grown in YM broth for 5 days at 25°C. B: Ballistoconidia produced on corn meal agar after 7 days at 17°C. Scale bars indicate 10 µm.

sents a new species in the genus *Bensingtonia*.

Practically, *B. sakaguchii* can be distinguished from both *B. musae* and *B. ingoldii* by its ability to assimilate maltose and its inability to assimilate erythritol, ethanol, glucuronic acid, glucono- δ -lactone, glycerol, 2-ketogluconic acid, 5-ketogluconic acid, D-xylose and L-lysine (Table 2).

The description is given below.

Bensingtonia sakaguchii Sugita, Takashima, Hamamoto et Nakase, sp. nov.

In liquido 'YM,' post dies 5 ad 25°C, cellulae ovoideae, ellipsoideae, elongatae, 3.0–4.5×4.4–7.6 µm, singulae aut binae. Sedimentum formatur. Post unum mensem ad 17°C, anulus et sedimentum formantur. In agar 'YM,' post unum mensem ad 17°C, cultura eburnea, seminitida, margine glabra aut erosa. Pseudomycelium primitivum formatur. Ballistoconidia reniformes, 3.2–3.5×6.6–7.4 µm. Fermentatio nulla. Glucosum, maltosum, cellobiosum, trehalosum, lactosum, melezitolum, amyllum solubile, ribitolium, D-mannitolium, D-glucitolium, α -methyl-D-glucosidum (lente), acidum succinicum et acidum citricum (lente ex exiguum) assimilantur at non galactosum, L-sorbosum, saccharosum, melibiosum, raffinolum, inulinum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolium, glycerolum, erythritolum, galactitolium, salicinum (vel lente et exiguum), glucono- δ -lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum DL-lacticum nec inositolum. Ammonium sulfatum, ethylaminum et cadaverinum assimilantur at non kalium nitricum, natrium nitrosum nec L-lysinum. Maxima tem-

Fig. 2. Neighbor-joining tree resulting from analysis of SSU rDNA sequences of *Bensingtonia sakaguchii* sp. nov. and related taxa.

The numerals represent the confidence level (%) from 1,000 replicate bootstrap sampling (the frequency less than 50% was not indicated). The distance corresponding to one base change per hundred nucleotide positions is indicated by a bar.

peratura 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: 52.6–52.8 mol% (per HPLC). Ubiquinum majus: Q-9. Xylosum in cellulis absens. Holotypus: Isolatus ex folio *Bischofia javanicae*, JCM 10047 (originaliter ut OK-255) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms,' Wako, Saitama sustentat.

Growth in YM broth: After 5 days at 25°C, cells are ovoidal, ellipsoidal, elongated, 3.0–4.5 × 4.4–7.6 µm, single or in pairs (Fig. 1A). A sediment is formed. After 1 month at 17°C, a ring and sediment are present.

Growth on YM agar: After 1 month at 17°C, the streak culture is ivory, semi-shining and has an entire to eroded margin.

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

Production of ballistoconidia: Ballistoconidia are

Table 1. DNA relatedness among *Bensingtonia* sp. OK-255, OK-259 and phylogenetically closely related species.

Species	Strain	mol% G+C	% relative binding of DNA with			
			OK-255	OK-259	JCM 7445	JCM 8801
<i>Bensingtonia</i> sp.	OK-255	52.6	100	86	20	13
<i>Bensingtonia</i> sp.	OK-259	52.8	92	100	19	19
<i>Bensingtonia ingoldii</i>	JCM 7445	55.7 ^a	12	10	100	ND
<i>Bensingtonia musae</i>	JCM 8801	52.3 ^b	17	15	ND	100

^a Nakase et al. (1989).^b Takashima et al. (1995).

ND, Not determined.

Table 2. Differential characteristics of *Bensingtonia sakaguchii*, *B. musae* and *B. ingoldii*.

Species	Carbon assimilation														Nitrogen assimilation	
	Sucrose	Maltose	Cellobiose	Lactose	Melezitose	Soluble starch	D-Xylose	Ethanol	Glycerol	Erythritol	Glucono- δ -lactone	2-Ketogluconic acid	5-Ketogluconic acid	Glucuronic acid	Nitrate	L-Lysine
<i>B. sakaguchii</i>	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
<i>B. musae</i>	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–	+
<i>B. ingoldii</i>	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+, positive; –, negative.

produced on corn meal agar. They are kidney-shaped, 3.2–3.5×6.6–7.4 μ m (Fig. 1B).

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Ethanol	–
Galactose	–	Glycerol	–
L-Sorbose	–	Erythritol	–
Sucrose	–	Ribitol	+
Maltose	+	Galactitol	–
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
Lactose	+	α -Methyl-D-glucoside	+(latent)
Melezitose	–	Salicin	– or + (latent and weak)
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	–	Succinic acid	+
D-Ribose	–	Citric acid	+(latent or weak)
L-Rhamnose	–	Inositol	–

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+
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Potassium

nitrate

Sodium nitrite

L-Lysine

hydrochloride

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: 52.6–52.8 mol% (by HPLC).

Major ubiquinone: Q-9.

Xylose in the cells: Absent.

Type strain: OK-255, isolated by T. Nakase from a leaf of *Bischofia javanica* collected by T. Sato in Nov. 1994, Ogasawara Islands, Japan. This strain has been deposited in the Japan Collection of Microorganisms, Wako, Saitama, as JCM 10047.

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