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Sporobolomyces yuccicola, a new species of ballistosporous yeast equipped with ubiquinone-9

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Abstract. A hitherto undescribed ballistosporous yeast was isolated from a dead leaf of *Yucca* sp. in Canada. This yeast produces apiculate or short-ellipsoidal ballistospores, produces pale colored colonies, has Q-9 as the major ubiquinone, and does not contain xylose in the cells. This new yeast is described as *Sporobolomyces yuccicola* Nakase et Suzuki. *Sporobolomyces yuccicola* is the sixth species of the intermedius group, a group of atypical species of the genus *Sporobolomyces* equipped with Q-9.

Introduction

In the course of a survey of ballistospore-forming yeasts living on the surface of plant materials, a hitherto undescribed yeast equipped with Q-9 was found in the isolates from plant materials in Canada. This yeast is the sixth species of the intermedius group, a group of atypical species of the genus *Sporobolomyces* (Nakase & Suzuki 1987a). It is described in the present paper.

Materials and methods

The strain B-74 reported in this paper was isolated from an old dead leaf of *Yucca* sp. fallen on the ground, at the campus of The University of British Columbia, Vancouver, Canada, in April, 1971, by R.J. Bandoni.

Most of the methods employed for the examination of morphological, physiological and biochemical characteristics were those described by van der Walt & Yarrow (1984). Assimilation of nitrogen compounds was investigated on solid media with starved inoculum as described in a previous paper (Nakase & Suzuki 1986). Vitamin requirements were investigated according to Komagata & Nakase (1967). The maximum growth temperature was determined in YM broth (Difco Labs) using metal block baths. Unless otherwise stated, all of the experiments were carried out at 17° C.

Extraction and purification of ubiquinones were carried out according to Nakase & Suzuki (1986) using cells harvested in stationary growth phase. Ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC) by comparing their retention times with those of standard ubiquinones. HPLC was performed on an LC-6A liquid chromatograph (Simadzu) fitted with Zorbax ODS column (4.6 mm × 25 cm). Ubiquinone isoprenologues were eluted with a mobile phase of methanol-isopropanol (2:1, v/v) at 2.0 ml/min, and monitored at 275 nm.

DNAs were isolated and purified according to Nakase & Suzuki (1986). DNA base composition (mol% G + C) was calculated from the thermal denaturation temperature (*Tm*) measured in 0.5 X SSC solution. DNA from *Candida parapsilosis* JCM 1785 (= CBS 604) with G + C = 40.8 mol% was used as reference.

Xylose in the cells was analyzed as follows: Acetone-dried cells harvested in the logarithmic growth phase was hydrolyzed and neutralized as described by Nakase & Suzuki (1987a). Xylose in the cell hydrolyzate was analyzed by high performance liquid chromatography using Shodex SP 1010 column (Showa Denko) with water as the mobile phase at a flow rate of 0.8 ml/min at 80° C, and Shodex RS pak DC-613 column with acetonitrile-water (80 : 20, v/v) as the mobile phase at a flow rate of 0.8 ml/min at 75° C. A refractive index monitor was used for the detection of carbohydrates.

The electrophoretic enzyme patterns were examined by the method of Yamazaki & Komagata (1981).

Description

Sporobolomyces yuccicola Nakase et Suzuki, sp. nov.

In liquido 'YM', post dies 5 ad 17° C, cellulae subovoideae aut ovoideae, 2.5–8 × 4.5–10 μm, vel ellipsoidales, 2.5–5 × 5–10 μm, singulae aut binae. Sedimentum formatur. In agaro 'YM', post unum mensem, cultura subaurantiaca aut glauco-aurantiaca, glabra aut rugosa, butyracea, margine glabra. Mycelium et pseudomycelium non formantur. Ballistosporae apiculatae, brevi-ellipsoidales aut ellipsoidales, 2.5–6 × 7–11 μm. Fermentatio nulla. Glucosum, galactosum (lente), L-sorbosum (lente et exiguum), cellobiosum, trehalosum, D-xylosum (valde lente vel nullum), glycerolum, ribitolum (lente, vel lente et exiguum), D-mannitolum (lente et exiguum), D-glucitolum (lente), acidum succinicum (tardum) et acidum citricum (lente et exiguum) assimilantur at non saccharosum, maltosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, amylum solubile, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, ethanolum, erythritolum, galactitolum, α-

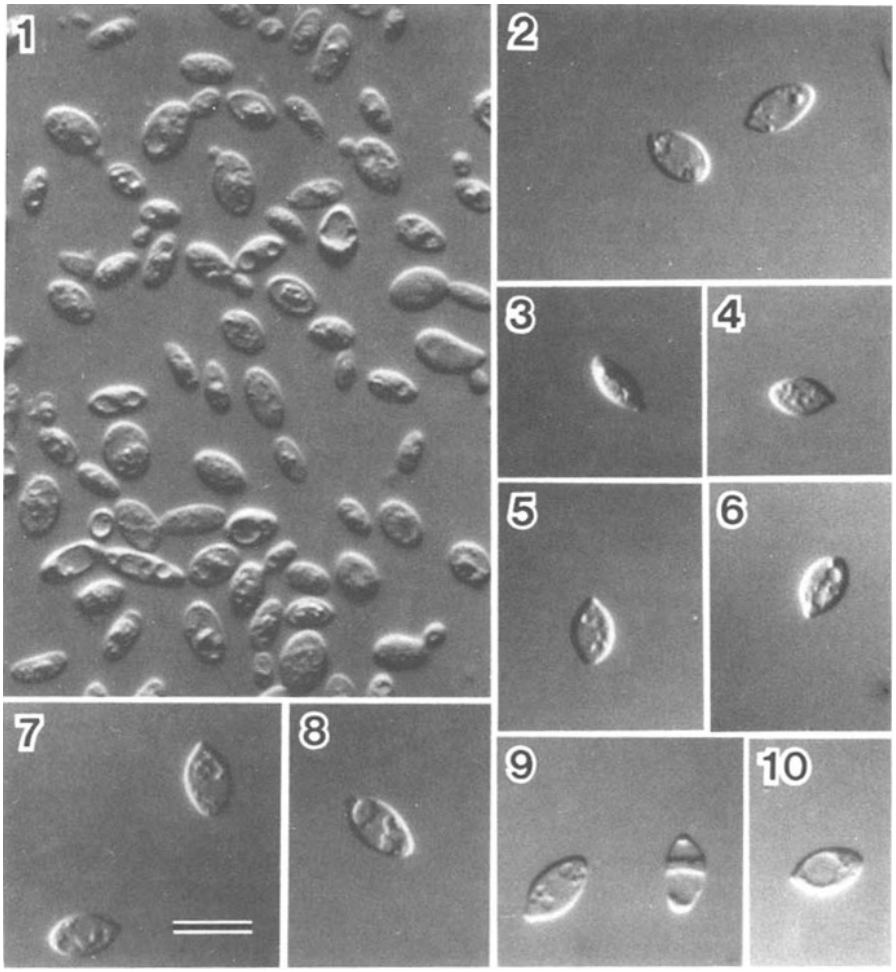

Fig. 1. Vegetative cells of *Sporobolomyces yuccicola* B-74 grown in YM broth for 5 days at 17° C.

Figs. 2–10. Ballistospores of *Sporobolomyces yuccicola* B-74 produced on corn meal agar after 11 days at 17° C. The bar represents 10 μm.

methyl-D-glucosidum, salicinum, glucono-σ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum nec inositolum. Ammonium sulfatum, kalium nitricum et natrium nitrosum assimilantur at non ethylaminum, L-lysinum nec cadaverinum. Maxima temperatura crescentiar: 24–25° C. Ad crescentiam thiaminum necessarium est. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 45.8–45.9 mol%. Ubiquinonum majus: Q-9. Xylosum in cellulis absens. Typus: Isolatus ex folio emortuo *Yuccae* sp., Vancouver, Canada, iv. 1971, R.J. Bandoni, JCM 6251 (original-

iter ut B-74) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentat.

Growth in YM broth. After 5 days at 17° C, cells are short-oval or oval, 2.5–8 × 4.5–10 μm, single or in pairs. Ellipsoidal cells measuring 2.5–5 × 5–10 μm are also present (Fig. 1). A sediment is formed. After one month at 17° C, a ring and a sediment are present.

Growth on YM agar. After one month at 17° C, the streak culture is light orange to greyish orange, dull-shining, smooth or wrinkled near the bottom of the tube, butyrous, and has an entire margin.

Dalmau plate culture on corn meal agar. Pseuodomycelium and true mycelium are not formed.

Production of ballistospores. Ballistospore production is poor. They are apiculate or short-ellipsoidal (broadly ellipsoidal) to ellipsoidal, 2.5–6 × 7–11 μm. Most of them are symmetrical but some are slightly curved and are asymmetrical (bilaterally symmetrical) (Figs. 2–10).

Fermentation. Negative.

Assimilation of carbon compounds

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

Assimilation of nitrogen compounds

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

Maximum growth temperature: 24–25° 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
Hydrolysis of fat: Negative
G + C content of nuclear DNA: 45.8–45.9 mol%
Major ubiquinone: Q-9
Xylose in the cells: Absent

Type strain. B-74, isolated by R.J. Bandoni from an old dead leaf of *Yucca* sp. fallen on the ground, the campus of The University of British Columbia, Vancouver, Canada. This strain (living and dried) is deposited in the Japan Collection of Microorganisms, Wako, Saitama, as JCM 6251.

Discussion

In 1984, we isolated a new ballistosporous yeast which produced pale colored colonies and bilaterally symmetrical ballistospores from a dead leaf of *Oryza sativa.* It was described as a new species of the genus *Bullera, B. intermedia,* because it was considered to have more close relationship to the genus *Bullera* than *Sporobolomyces* in spite of its bilaterally symmetrical ballistospores (Nakase & Suzuki 1985). However, this yeast has Q-9 as the major component of ubiquinones and differs from any other known species of ballistosporous yeasts in this respect. We supposed that this yeast might be a yeast phase of an undescribed species of *Itersonilia,* because Q-9 was reported as the major ubiquinone of strains of *Itersonilia* (Yamada & Konda 1984).

Later, we transfered this species to the genus *Sporobolomyces* as *Sporobolomyces intermedius,* based on the observation that xylose was absent in whole cell hydrolyzates (Nakase & Suzuki 1987b). Later three new species equipped with Q-9 were isolated from dead leaves of *Miscanthus sinensis* and described as *Sporobolomyces miscanthi, Sporobolomyces subroseus,* and *Sporobolomyces weijmanii* (Nakase & Suzuki 1987b). The fifth species equipped with Q-9 as the major ubiquinone was isolated from a dead leaf of *Sasa* sp. collected on a mountain in central Japan and named *Sporobolomyces naganoensis* (Nakase & Suzuki 1987c).

Table 1. Comparison of electrophoretic *R*m values of eight enzymes from the type strains of *Sporobolomyces yuccicola, Sporobolomyces miscanthi* and *sporobolomyces subroseus.*

Microorganisms	*R*m values							
	GDH	G6PDH	6PGDH	MDH	HK	LDH	Fma	Est
Sp. yuccicola B-74	0.20	ND[a]	0.41	0.30	0.34–0.40[b]	0.20 0.22 (0.24) (0.26)	0.26 0.30	0.17, (0.32),[c] 0.52, (0.58), 0.60, 0.66, (0.75)
Sp. miscanthi JCM 5733	0.15	0.27	0.44	0.35	0.53	0.20 0.23	0.31	0.39, 0.62, (0.68), 0.70
Sp. subroseus JCM 5735	0.16	0.23	0.44	0.37 0.40	0.35–0.40[b]	0.20	0.28	(0.28), 0.42, 0.56, 0.62, 0.64, 0.72, 0.74

GDH: Glutamate dehydrogenase (EC 1.4.1.4), G6PDH: Glucose-6-phosphate dehydrogenase (EC 1.1.1.49), 6PGDH: 6-Phosphogluconate dehydrogenase (EC 1.1.1.44), MDH: Malate dehydrogenase (EC 1.1.1.37), HK: Hexokinase (EC 2.7.1.1), LDH: Lactate dehydrogenase (EC 1.1.1.27), Fma: Fumarase (EC 4.2.1.2), Est: Esterase (EC 3.1.1.4).

[a] ND: Not detected; [b] Difused band; [c] ( ): Weak band.

Table 2. Salient characteristics of species of intermedius group in the genus *Sporobolomyces*.

Species	Major ubiquinone	Xylose in cells	Shape of ballistospores	Color of colonies	Mol% G + C	Vitamins required	Assimilation of nitrate	Assimilation of: Glucono-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	Sucrose	Maltose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Soluble starch	D-Ribose	Ethanol	Ribitol	Salicin
Sp. intermedius	Q-9	−	asy[a]	whitish	57.8	T[b]	−	+[c]	+	+	−	−	+	−	−	−	−	−	+	+	+	−
Sp. weijmanii	Q-9	−	asy	whitish	60.0	Nil	−	+	+	+	+	+	L	−	−	−	+	*l*	L	+	L	−
Sp. miscanthi	Q-9	−	asy	pale pink	47.2–47.5	T, PABA	+	−	−	−	+	−	+	V	−	V	−	−	−	−	L	−
Sp. subroseus	Q-9	−	asy	pale pink	46.5	T, PABA	−	−	−	−	+	+	+	L	−	−	−	+	−	−	−	−
Sp. naganoensis	Q-9	−	asy	pale brown	55.8	T, PABA	+	*l*	−	−	+	+	−	−	+	+	+	+	−	−	*l*	L
Sp. yuccicola	Q-9	−	sy-asy	greyish orange	45.8–45.9	T	+	−	−	−	−	−	+	−	−	−	−	−	−	−	L	−

[a] sy: rotationally symmetrical, asy: bilaterally symmetrical.

[b] T: thiamine, PABA: *p*-aminobenzoic acid.

[c] +: positive, −: negative, L; latent, *l*: latent and weak, V: variable.

We considered that ballistosporous yeasts equipped with Q-9 constitute a natural taxonomic group and they even be placed in a new genus to be established in the near future. Tentatively, however, we placed them in the genus *Sporobolomyces* and proposed to call them species of the 'intermedius group' (Nakase & Suzuki 1987c).

Sporobolomyces yuccicola is the sixth species of the intermedius group of the genus *Sporobolomyces*. This yeast has a G + C content of DNA of 45.8–45.9 mol%. Among the species of Q-9 equipped ballistosporous yeasts, this value is similar to that of *Sp. miscanthi* and *Sp. subroseus*.

However, the electrophoretic pattern of enzymes clearly demonstrated the difference of *Sp. yuccicola* from the other two species (Table 1). The similarity of the enzyme pattern of *Sp. yuccicola* to that of *Sp. miscanthi* is calculated at 7% and that to *Sp. subroseus* at 18%. In the taxonomic characteristics commonly employed, *Sp. yuccicola* shows the most close resemblance to *Sp. miscanthi* but it is clearly distinguished from the latter species in the lack of assimilation of sucrose and the absence of requirement of *p*-aminobenzoic acid (Table 2).

Sporobolomyces yuccicola produces apiculate or short-ellipsoidal ballistospores which resemble those of *Bullera alba* (Figs. 2, 4–7, and 10).

Sometimes, curved ballistospores are also produced by this yeast (Figs. 3, 8, and 9) but a clear-cut discrimination between these two types of ballistospores is not easy. The shape of ballistospores has been considered to be an important taxonomic criterion to define the taxa of ballistosporous yeasts at the generic level (Lodder & Kreger-van Rij 1952; Rodrigues de Miranda 1984; Fell & Tallman 1984). However, it is not a decisive characteristic to separate the genera. In biochemical and physiological characteristics, *Sp. yuccicola* shows a close resemblance to *Sporobolomyces* species but not to *Bullera* species. It cannot assimilate 2-ketogluconic acid, 5-ketogluconic acid, or inositol, and cannot produce starch-like substances.

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