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Four new species of the genus *Sporobolomyces* isolated from leaves in Thailand

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Thirteen undescribed strains of ballistoconidium-forming yeasts, isolated from leaves collected in the suburbs and along the southeast seacoast of Bangkok, Thailand, were divided into four different groups in the genus *Sporobolomyces* on the basis of morphological, physiological, biochemical, and chemotaxonomical characteristics, and analyses of the sequences of 18S rDNA and internal transcribed spacer regions. DNA-DNA reassociation experiments with related species revealed that these four groups were four new distinct species. *Sporobolomyces nylandii* sp. nov., *S. poonsookiae* sp. nov., *S. blumeae* sp. nov. and *S. vermiculatus* sp. nov. are proposed for these strains.

Key Words——*Sporobolomyces blumeae* sp. nov.; *Sporobolomyces nylandii* sp. nov.; *Sporobolomyces poonsookiae* sp. nov.; *Sporobolomyces vermiculatus* sp. nov.; systematics.

In a study of ballistoconidium-forming yeasts, we isolated 60 *Sporidiobolus* Nyland / *Sporobolomyces* Kluyver et van Niel strains from leaves collected in Thailand. Of these, 40 strains were identified as three known species, *Sporidiobolus ruineniae* Holzschu, Tredick et Phaff, *Sporobolomyces salmonicolor* (Fischer et Brebeck) Kluyver et van Niel and *Sporobolomyces shibatanus* (Okunuki) Verona et Ciferri. The remaining 20 strains were divided into four groups on the basis of physiological and biochemical characteristics. A total of 13 strains were used in this study, singled out by their physiological and biochemical properties and the isolation source. These strains were characterized by Q-10 as the major ubiquinone, the absence of xylose in the cells, proliferation by the budding cells and ballistoconidia, and the lack of sexual stages. Each group was deduced to have Urediniomycetes affinity based on 18S rDNA sequences. DNA-DNA reassociation experiments revealed that these *Sporobolomyces* strains represent four undescribed species. Four new species, *Sporobolomyces nylandii* Takashima et Nakase, *Sporobolomyces poonsookiae* Takashima et Nakase, *Sporobolomyces blumeae* Takashima et Nakase, and *Sporobolomyces vermiculatus* Takashima et Nakase are described in this paper.

Materials and Methods

Yeast strains The strains used in this study are listed in Table 1 with reference strains. These strains were isolated from leaves that were collected in the suburbs and along the southeast seacoast of Bangkok, Thailand, in 1987 and 1990, respectively, using the ballistoconidium-fall method with YM agar plates (Nakase and Takashima, 1993).

Examination of morphological, physiological, and biochemical characteristics Most of the morphological, physiological, and biochemical characteristics were examined according to van der Walt and Yarrow (1984). The assimilation of nitrogen compounds was investigated on solid media using starved inoculum according to Nakase and Suzuki (1986). Vitamin requirements were determined according to the method of Komagata and Nakase (1967). The maximum growth temperature was determined in YM broth (Difco, Detroit, MI) using metal block baths.

Ubiquinone system Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in the early stationary growth phase. The cells were washed with distilled water. Extraction, purification, and identification of ubiquinones were carried out according to Nakase and Suzuki (1986).

Xylose in the cells Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in the early stationary growth phase. The cells were washed with distilled water and dried with acetone. Xylose in the cells was analyzed as described by Suzuki and Nakase (1988).

Isolation and purification of nuclear DNA Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml of YM broth on a rotary shaker at 150 rpm at 25°C and were harvested in the logarithmic growth phase. The cells were washed with distilled water and freeze-dried.

Cells were broken using a mortar and a pestle, suspended in the isolation buffer containing 200 mM Tris-

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Table 1. Yeast strains used in this study.

Scientific name	JCM No.	Strain	Source	Locality	DDBJ accession numbers	
					18S rDNA	ITS & 5.8S
Sporobolomyces sp. 1	JCM 10213	K-77	*Oryza sativa*	Suburb of Bangkok, in Dec. 1987	AB030319	AB030323
	JCM 10214	K-81	*Saccharum spontaneum*	Suburb of Bangkok, in Dec. 1987		AB030324
	JCM 10215	K-105	*Phragmites karka*	Suburb of Bangkok, in Dec. 1987		AB030325
Sporobolomyces sp. 2	JCM 10206	K-316	*Neyraudia reynaudiana*	Southeast seacoast of Bangkok, in Dec. 1990		AB030326
	JCM 10207	K-335	*Mangifera indica*	Southeast seacoast of Bangkok, in Dec. 1990	AB030320	AB030327
	JCM 10208	K-344	*Imperata cylindrica*	Southeast seacoast of Bangkok, in Dec. 1990		AB030328
	JCM 10209	K-346	*Eucalyptus* sp.	Southeast seacoast of Bangkok, in Dec. 1990		AB030329
	JCM 10211	K-362	*Ageratum conyzoides*	Southeast seacoast of Bangkok, in Dec. 1990		AB030330
Sporobolomyces sp. 3	JCM 10212	K-230	*Blumea* sp.	Southeast seacoast of Bangkok, in Dec. 1990	AB030321	AB030331
Sporobolomyces sp. 4	JCM 10221	K-17	*Imperata cylindrica*	Suburb of Bangkok, in Dec. 1987		AB030332
	JCM 10222	K-90	*Imperata cylindrica*	Suburb of Bangkok, in Dec. 1987		AB030333
	JCM 10223	K-109	*Oryza sativa*	Suburb of Bangkok, in Dec. 1987		AB030334
	JCM 10224	K-383	*Pennisetum pediocellatum*	Southeast seacoast of Bangkok, in Dec. 1990	AB030322	AB030335

Reference strains

Scientific name	JCM No.	Other designations	ITS & 5.8S
Sporidiobolus johnsonii	JCM 1840^T	=ATCC 10765=CBS 5470=IFO 6903	AB030336
Sporidiobolus microsporus	JCM 6882^T	=ATCC 26322=CBS 7041	AB030337
Sporidiobolus pararoseus	JCM 5350^T	=ATCC 24260=CBS 484=NRRL Y-5479	AB030338
Sporidiobolus ruineniae var. *coprophilus*	JCM 8097^T	=ATCC 18159=CBS 5811=IFO 14226	AB030339
Sporidiobolus ruineniae var. *ruineniae*	JCM 1839^T	=ATCC 20489=CBS 5001=IFO 1689	AB030340
Sporidiobolus salmonicolor	JCM 1841^T	=CBS 490=NRRL Y-5483=UCD 68-371	AB030341
Sporobolomyces coprosmae	JCM 8772^T	=CBS 7899	AB030343
Sporobolomyces foliicola	JCM 5355^T	=ATCC 52909=CBS 8075=IFO 10588	AB030345
Sporobolomyces holsaticus	JCM 5296^T	=ATCC 34889=CBS 1522=IFO 1034	AB030347
Sporobolomyces oryzicola	JCM 5299^T	=CBS 7228=IFO 10180	AB030349
Sporobolomyces roseus	JCM 5353^T	=ATCC 24257=CBS 486=NRRL Y-5506	AB030351

^{T}, type strain

HCl, pH 8.5, 250 mM NaCl, 25 mM EDTA, and 0.5% SDS, and incubated at 50°C for 30 min with slow shaking according to the method of Raeder and Broda (1985). Crude DNA was precipitated at 4°C for one night by addition of 2 vol. of ethanol after removing the denatured protein by chloroform-3-methyl-1-butanol (24 : 1) treatment. DNA collected by centrifugation was dissolved in TE solution (10 mM Tris-HCl, pH 7.6, 1 mM EDTA), and ultracentifuged at 15°C for 20 h in a Beckman TLA 100.4 rotor, using CsCl (1 g/1 ml of DNA solution) and 1 /10 vol. of ethidium bromide solution (5 mg/ml). Nuclear DNA fraction was obtained using a pipette under a UV lamp. After removing ethidium bromide and CsCl, nuclear DNA fraction was purified by RNase A (R5125, Sigma) and RNase T1 (R8251, Sigma), followed by Proteinase K (Merck), and spooled on a glass rod according to Nakase and Suzuki (1986).

DNA base composition The DNA base composition was determined by HPLC after enzymatic digestion of DNA to deoxyribonucleosides as described by Tamaoka and Komagata (1984). A DNA-GC Kit (Yamasa Shoyu Co., Ltd., Chiba, Japan) was used as the quantitative standard.

DNA-DNA relatedness DNA-DNA reassociation experiments were carried out using the membrane-filter method of Hamamoto and Nakase (1995).

Sequencing and phylogenetic analysis The nucleotide sequences of 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 (1999). The sequences of 18S rDNA and ITS regions including 5.8S rDNA determined in this study are listed in Table 1 with the accession numbers. Reference sequences used for the phylogenetic study, shown in Fig. 1, were obtained from the database. Generated sequences were aligned with *Sporidiobolus/ Sporobolomyces* species (Hamamoto and Nakase, 2000) and related species using the CLUSTAL W ver. 1.74 computer program (Thompson et al., 1994). The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbor-joining method (Saitou and Nei, 1987) in the PHYLIP computer program. Sites where gaps existed in any sequences were excluded. Bootstrap analyses (Felsenstein, 1985) were performed from 100 random resamplings.

Results and Discussion

Thirteen undescribed strains used in the study appear to belong to the genus *Sporobolomyces*, which possesses Q-10 as the major ubiquinone, does not contain xylose in the cells, and proliferates by budding cells and ballistoconidia. On the basis of their physiological and biochemical characteristics, these strains were divided into four groups: *Sporobolomyces* sp. 1 (K-77, K-81 and K-105), *Sporobolomyces* sp. 2 (K-316, K-335, K-344, K-346 and K-362), *Sporobolomyces* sp. 3 (K-230), and *Sporobolomyces* sp. 4 (K-17, K-90, K-109 and K-383). Each group was deduced to have Urediniomycetes affinity from the phylogenetic analysis of 18S rDNA sequences (Fig. 1). *Sporobolomyces* sp. 1 (K-77) and *Sporobolomyces* sp. 2 (K-335) showed a close relationship to *Sporidiobolus ruineniae* (group A). *Sporobolomyces* sp. 3 (K-230) was closely related to *Sporobolomyces roseus* Kluyver et van Niel, *Sporidiobolus pararoseus* Fell et Tallman and related species (group B). *Sporobolomyces* sp. 4 (K-383) clustered with *Sporobolomyces foliicola* Shivas et Rodrigues de Miranda and related species (group C).

In group A, *Sporobolomyces* sp. 1 (K-77) and *Sporobolomyces* sp. 2 (K-335) constituted a cluster with *Sporid. ruineniae* var. *ruineniae, Sporidiobolus ruineniae* var. *coprophilus* (Sugiyama et Goto) Kurtzman et Fell, and *Sporidiobolus microsporus* Higham ex Fell, Blatt et Statzell-Tallman, with 100% bootstrap value (Fig. 1). *Rhodosporidium fluviale* Fell, Kurtzman, Tallman et Buck was also located in this cluster, as indicated by Fell et al. (1998). The sequences of the ITS1 and 2 regions were then determined for members of the group other than *R. fluviale*. As shown in Table 2, the sequences of ITS1 and ITS2 were identical in two varieties of *Sporidiobolus ruineniae* and in three isolates of *Sporobolomyces* sp. 1. In five isolates of *Sporobolomyces* sp. 2, the ITS1 sequences were identical and more than 99.5% sequence similarity were detected in ITS2 sequences. The results indicated that *Sporobolomyces* sp. 1, *Sporobolomyces* sp. 2, *Sporid. ruineniae*, and *Sporid. microsporus* were different from each other (Sugita et al., 1999).

In group B, *Sporobolomyces* sp. 3 (K-230) showed a close relationship with *S. roseus, Sporid. pararoseus*, and related species based on the 18S rDNA sequences (Fig. 1). The sequence similarity of 18S rDNA between K-230 and the former two species were 99.3% in both cases. However, the mol% G+C of K-230 was 59.5 mol%, and over 10% higher than the former two species (Table 3). In the ITS region, the sequence similarities among *Sporobolomyces* sp. 3 (K-230), *S. roseus*, and *Sporid. pararoseus* were less than 90% (Table 2).

Sporidiobolus johnsonii Nyland and *Sporidiobolus salmonicolor* Fell et Tallman were located adjacent to the cluster of *S. roseus* and *Sporid. pararoseus* (Fig. 1). *Sporid. salmonicolor*, which Boekhout (1991) reported to be a synonym of *Sporid. johnsonii*, is treated as a separate species (Statzell-Tallman and Fell, 1998), and *Sporobolomyces holsaticus* Windisch ex Yarrow et Fell is regarded as an anamorphic stage of *Sporid. johnsonii* (Statzell-Tallman and Fell, 1998). The analyses of ITS sequences supported the separation of *Sporid. salmonicolor* from *Sporid. johnsonii* (sequence similarity: 90.8% for ITS1 and 89.4% for ITS2) and the close relationship between *S. holsaticus* and *Sporid. johnsonii* (sequence similarity: 100% for ITS1 and 97.1% for ITS2) (Table 2).

In group C, *Sporobolomyces* sp. 4 (K-383) clustered with *Sporobolomyces coprosmae* Hamamoto et Nakase, *S. foliicola*, and *Sporobolomyces oryzicola* Nakase et M. Suzuki in a phylogenetic tree based on 18S rDNA sequences (Fig. 1). The ITS1 sequences of all

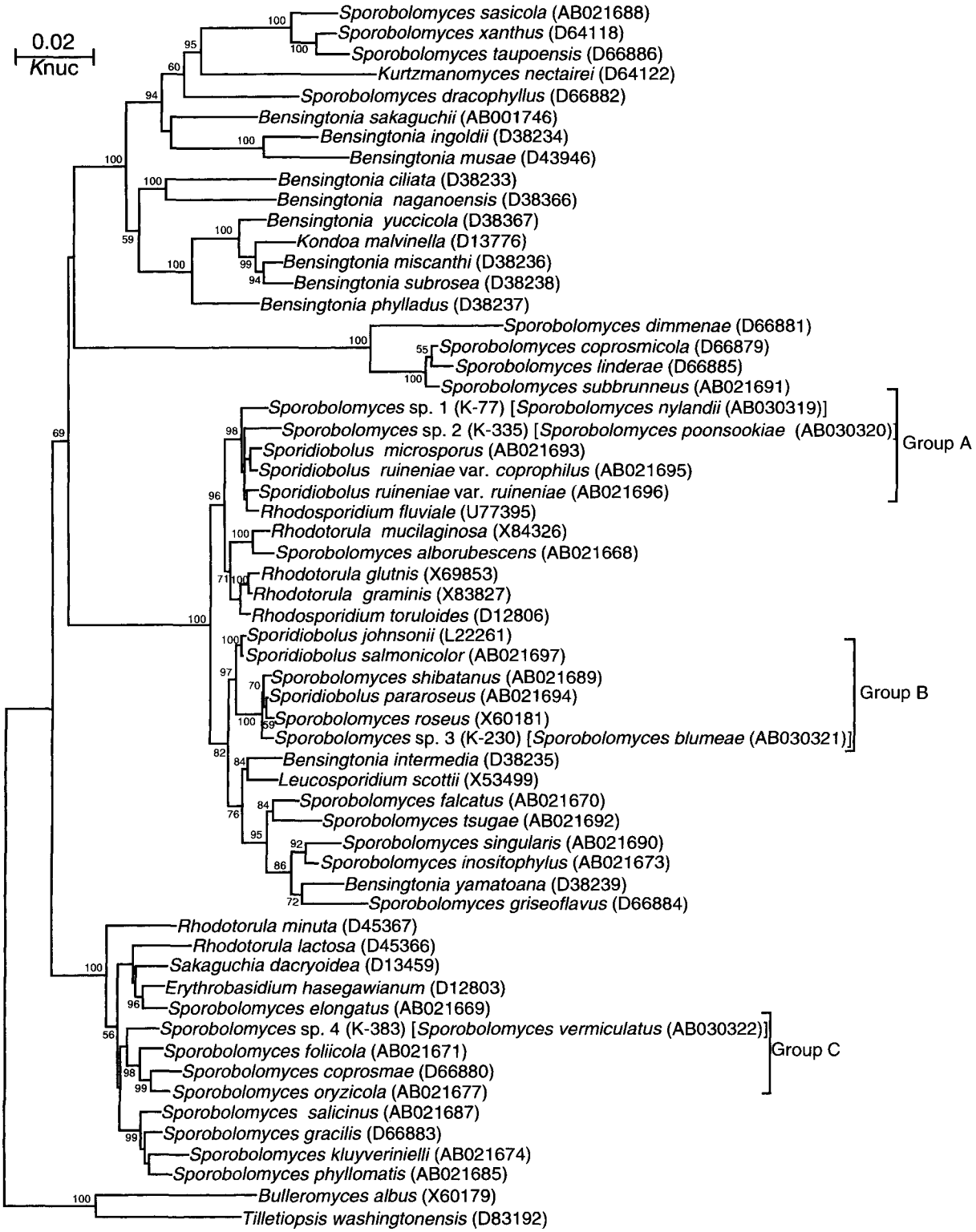

Fig. 1. Phylogenetic tree of four new species of *Sporobolomyces* and related species based on the 18S rDNA sequences. The numerals represent the percentages from 100 replicate bootstrap samplings (a frequency of less than 50% is not shown). Sequences were retrieved from the GenBank databases under the accession numbers indicated.

Sporobolomyces sp. 4 strains (K-17, K-90, K-109 and K-383) were identical, but one or two base differences between isolates were detected in the ITS2 sequences (Table 2).

Table 3 shows the DNA relatedness between *Sporobolomyces* spp. and phylogenetically related spe-

Table 2. Sequence similarity of ITS regions among *Sporobolomyces* spp. and phylogenetically closely related species[a].

Scientific name	Strain	Group A											Group B						Group C						
		K-77	K-81	K-105	K-335	K-316	K-344	K-346	K-362	JCM 1839	JCM 8097	JCM 6882	K-230	JCM 5350	JCM 5353	JCM 1841	JCM 1840	JCM 5296	K-383	K-17	K-90	K-109	JCM 5355	JCM 5299	JCM 8772
Group A																									
Sporobolomyces sp. 1	K-77	—	100	100	95.7	95.7	95.7	95.7	95.7	92.9	92.9	93.3													
	K-81	100	—	100	95.7	95.7	95.7	95.7	95.7	92.9	92.9	93.3													
	K-105	100	100	—	95.7	95.7	95.7	95.7	95.7	92.9	92.9	93.3													
Sporobolomyces sp. 2	K-335	89.3	89.3	89.3	—	99.5	100	100	99.5	95.2	95.2	96.7													
	K-316	89.3	89.3	89.3	100	—	99.5	99.5	100	94.8	94.8	96.2													
	K-344	89.3	89.3	89.3	100	100	—	100	99.5	95.2	95.2	96.2													
	K-346	89.3	89.3	89.3	100	100	100	—	99.5	95.2	95.2	96.7													
	K-362	89.3	89.3	89.3	100	100	100	100	—	94.8	94.8	96.2													
Sporid. ruineniae var. *ruineniae*	JCM 1839[T]	94.0	94.0	94.0	90.6	90.6	90.6	90.6	90.6	—	100	93.4													
Sporid. ruineniae var. *coprophilus*	JCM 8097[T]	94.0	94.0	94.0	90.6	90.6	90.6	90.6	90.6	100	—	93.4													
Sporid. microsporus	JCM 6882[T]	93.4	93.4	93.4	88.8	88.8	88.8	88.8	88.8	94.2	94.2	—													
Group B																									
Sporobolomyces sp. 3	K-230												—	85.0	90.9	84.3	81.2	81.8							
Sporid. pararoseus	JCM 5350[T]												85.8	—	88.5	79.6	81.2	80.4							
S. roseus	JCM 5353[T]												84.7	88.1	—	82.9	79.9	80.0							
Sporid. salmonicolor	JCM 1841[T]												81.7	79.1	80.6	—	89.4	89.8							
Sporid. johnsonii	JCM 1840[T]												80.8	78.0	81.2	90.8	—	97.1							
S. holsaticus	JCM 5296[T]												80.8	78.0	81.2	90.8	100	—							
Group C																									
Sporobolomyces sp. 4	K-383																		—	99.0	99.0	99.5	86.4	86.2	89.0
	K-17																		100	—	99.0	99.5	86.9	86.3	89.0
	K-90																		100	100	—	99.5	86.4	86.2	89.0
	K-109																		100	100	100	—	86.9	86.7	89.5
S. foliicola	JCM 5355[T]																		85.8	85.8	85.8	85.8	—	87.9	88.7
S. oryzicola	JCM 5299[T]																		85.4	85.4	85.4	85.4	87.5	—	97.1
S. coprosmae	JCM 8772[T]																		84.1	84.1	84.1	84.1	87.6	99.3	—

[T], type strain; *Sporid., Sporidiobolus; S., Sporobolomyces*.

[a], Lower left triangle shows ITS1 similarity, and upper right triangle shows ITS2 similarity.

Table 3. DNA relatedness among *Sporobolomyces* spp. and phylogenetically closely related species.

Scientific name	Strain	mol% G+C	% relative binding of DNA from														
			Group A							Group B					Group C		
			K-77	K-105	K-335	K-316	JCM 1839	JCM 8097	JCM 6882	K-230	JCM 5350	JCM 5353	JCM 1841	JCM 1840	K-383	K-17	JCM 5355
Group A																	
Sporobolomyces sp. 1	K-77	60.2	100	89	24		17	14	17								
	K-81	60.4	117	112	29		21	20	23								
	K-105	61.5	107	100			18	16	19								
Sporobolomyces sp. 2	K-335	62.7	41	37	100	92	20	18	16								
	K-316	61.9			83	100											
	K-344	62.1			101	98											
	K-346	62.4			62	54											
	K-362	62.1			92	100											
Sporid. ruineniae var. *ruineniae*	JCM 1839^T	60.1	41	37	22		100	50	19								
Sporid. ruineniae var. *coprophilus*	JCM 8097^T	60.3	47	45	25		76	100	27								
Sporid. microsporus	JCM 6882^T	61.8	37	38	29		22	18	100								
Group B																	
Sporobolomyces sp. 3	K-230	59.5								100	19	25	14	12			
Sporid. pararoseus	JCM 5350^T	46.9								12	100	29					
S. roseus	JCM 5353^T	48.5								8	9	100					
Sporid. salmonicolor	JCM 1841^T	53.7								16			100	19			
Sporid. johnsonii	JCM 1840^T	54.6								14			28	100			
Group C																	
Sporobolomyces sp. 4	K-383	51.5													100	72	14
	K-17	51.7													65	100	13
	K-90	52.0													72	65	12
	K-109	52.1													78	63	13
S. foliicola	JCM 5355^T	56.1													7	10	100

^{T}, type strain; *Sporid., Sporidiobolus; S. Sporobolomyces.*

Table 4. Differential characteristics among *Sporobolomyces nylandii, Sporobolomyces poonsookiae*, and *Sporidiobolus ruineniae*.

Scientific name	Assimilation of				
	Galactose	Raffinose	Melezitose	Galactitol	L-Lysine
Sporobolomyces nylnadii	−	+, w	+, w	−	−
Sporobolomyces poonsookiae	+, L	−	−	+	LW, −
*Sporidiobolus ruineniae**	+	+	−	+	n
Sporidiobolus ruineniae (JCM 10220)	L	+	−	+	+

+, positive; −, negative; w, weak; L, latent; LW, latent and weak; n, no data.
*, Statzell-Tallman and Fell (1998).

Table 5. Differential characteristics among *Sporobolomyces blumeae* and phylogenetically closely related species.

Scientific name	Assimilation of				
	Raffinose	Ribitol	α-Methyl-D-glucoside	Citiric acid	Potassium nitrate
Sporobolomyces blumeae	+	−	−	−	−
Sporobolomyces roseus[1)]	+	V	V	V	+
Sporidiobolus pararoseus[2)]	+	V	+	V	−
Sporidiobolus johnsonii[2)]	−	s	+	V	+
Sporidiobolus salmonicolor[2)]	V	+	V	−	+

+, positive; −, negative; s, slow; V, variable.
[1)], Boekhout and Nakase (1998); [2)], Statzell-Tallman and Fell (1998).

Table 6. Differential characteristics among *Sporobolomyces vermiculatus* and phylogenetically closely related species.

Scientific name	Assimilation of[a)]								Vitamin required[b)]	mol% G+C
	Cellobiose	Ethanol	Ribitol	2-Keto-gluconic acid	Citric acid	D-Glucuronic acid	Potassium nitrate	Sodium nitrite		
S. vermiculatus	L, W, +	−	LW, L, −	L/−	W, −	−	−	−	T	51.5–52.1
S. oryzicola[1)]	L	−	LW	L	L	n	−	−	T, PABA	58.8[2)]
S. coprosmae[2)]	LW	−	+	+	+	+	−	−	T, PABA	58.0[2)]
S. foliicola[1)]	−	+	+	+	−	+	+	+	n	56.1

[a)] +, positive; -, negative; w, weak; L, latent; LW, latent and weak; n, no data.
[b)] T, thiamin; PABA, *p*-aminobenzoic acid.
[1)], Boekhout and Nakase (1998); [2)], Hamamoto and Nakase (1995).

cies. The low DNA relatedness of *Sporobolomyces* sp. 1 from other members (*Sporobolomyces* sp. 2, *Sporid. ruineniae*, and *Sporidiobolus microsporus*) revealed that *Sporobolomyces* sp. 1 is a distinct species from *Sporobolomyces* sp. 2, *Sporid. ruineniae*, and *Sporid. microsporus*. In *Sporobolomyces* sp. 2, K-346 showed a intermediate value (54–62%) to K-316 and K-335 but we concluded to treat these strains one distinct species based on physiological and biochemical characteristics and ITS sequences. The result also supports the validation of *Sporid. microsporus* based on a phylogenetic analysis of the partial sequence of 26S rDNA by Fell et al. (1998). In group B, DNA relatedness values between *Sporobolomyces* sp. 3 and related species were less than 30%, indicating that *Sporobolomyces* sp. 3 is distinct from other species. The separation of *Sporid. salmonicolor* from *Sporid. johnsonii* (Statzell-Tallmann and Fell, 1998) is also supported by the results. In group C, the low level of DNA relatedness between *Sporobolomyces* sp. 4 and *S. foliicola* (7 to 14%) revealed that *Sporobolomyces* sp. 4 is a distinct species.

From the results, four new species of *Sporobolomyces*, namely, *Sporobolomyces nylandii* Takashima et Nakase, *Sporobolomyces poonsookiae* Takashima et Nakase, *Sporobolomyces blumeae* Takashima et Nakase, and *Sporobolomyces vermiculatus* Takashima et Nakase are proposed for *Sporobolomyces* sp. 1, 2, 3, and 4, respectively. In practice, *S. nylandii* can be distinguished from *Sporid. ruineniae* by its assimilation of galactose, melezitose, galactitol and L-lysine

(Table 4). *S. poonsookiae* is differentiated from *S. nylandii* by the assimilation of galactose, raffinose, melezitose and galactitol, and from *Sporid. ruineniae* by the assimilation of raffinose (Table 4). The physiological and biochemical characteristics of *S. blumeae* are similar to those of *S. roseus* and *Sporid. pararoseus*, but the former assimilates both nitrate and α-methyl-D-glucoside (Table 5). *S. vermiculatus* can be differentiated from *S. oryzicola* and *S. coprosmae* by its lower mol% G+C (51.5–52.1 mol% vs. 58.8 mol% and 58.0 mol%, respectively; Hamamoto and Nakase, 1995) and also by its phenotypic characteristics (Table 6).

Sporobolomyces nylandii Takashima et Nakase, sp. nov. Fig. 2

In liquido YM: Post dies 3 ad 25°C cellulae ovoideae, ellipsoidales, elongatae vel cylindraceae, 3.0–8.0×4.0–15.0 μm, singulae, binae vel catenulatae, sedimentum formans; post unum mensem ad 17°C pellicula fragilis et incompleta vel completa et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura aurantiaca, glabra vel rugosa, nitida, mollis, margine erosa. In cultura in ramina vitrea pseudomycelium et mycelium formantur. Ballistosporae in CMA abundanter formatae reniformes vel ellipsoidales, 3.0–5.5×6.0–12.0 μm. Fermentatio nulla. Glucosum, L-sorbosum (lente), saccharosum, maltosum, cellobiosum, trehalosum, raffinosum, melezitosum, amylum solubile (exigue), D-arabinosum (lente), D-ribosum (lente et exigue), ethanolum, glycerolum, ribitolum (lente), D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, glucono-δ-lactonum (lente et exigue), acidum D-gluconicum, acidum succinicum (exigue), xylitolum (lente vel lente et exigue) et 1,2-propanediolum assimilantur, autem galactosum, lactosum, melibiosum, inulinum, D-xylosum, L-arabinosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, erythritolum, galactitolum, salicinum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum citricum, inositolum, hexadecanum, acidum saccharicum, L-arabinitolum, 2,3-butanediolum, acidum D-glucuronicum et acidum D-galacturonicum non assimilantur. Kalium nitricum, natrium nitrosum et ethylaminum assimilantur, autem L-lysinum et cadaverinum non assimilantur. Maxima temperatura crescentiae: 33–35°C. Ad crescentiam vitaminum non necessarium est. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 60.2–61.5 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum in cellulis absens. Holotypus: Isolatio ex folio emortuo *Oryzae sativae* L., Bangkok, Thailandia, T. Nakase et B. Fungsin, Dec. 1987, JCM 10213 (originaliter ut K-77) in Collectione Culturarum Japonensium quas 'Japan Collection of Microorganisms', Wako, Saitama conservata.

In YM broth, after 3 d at 25°C, the vegetative cells are ovoidal, ellipsoidal, elongate, or cylindrical, 3.0–8.0×4.0–15.0 μm, single, in pairs or in chains (Fig. 2a). Sediment is formed. After 1 mo at 17°C, an incomplete or complete fragile pellicle and sediment are present.

On YM agar, after 1 mo at 17°C, the streak culture is deep orange to reddish orange, shiny to semishiny, smooth to delicate but wrinkled near the bottom, soft, and has an erose margin.

On slide culture, pseudomycelia and true mycelia are produced.

Ballistoconidia are abundantly produced on corn meal agar. They are kidney-shaped or ellipsoidal, 3.0–5.5×6.0–12.0 μm (Fig. 2b).

Does not ferment glucose.

Assimilates glucose, L-sorbose (slow), sucrose, mal-

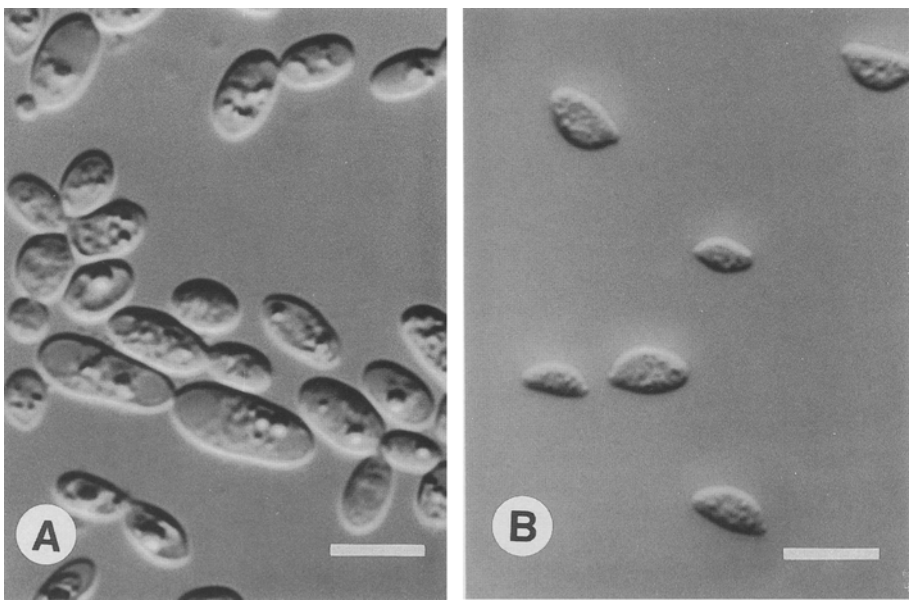

Fig. 2. *Sporobolomyces nylandii* JCM 10213 (a) Vegetative cells grown in YM broth for 3 d at 25°C. (b) Ballistoconidia produced on corn meal agar after 4 d at 17°C. Scale bars indicate 10 μm.

tose, cellobiose, trehalose, raffinose, melezitose, soluble starch (weak), D-arabinose (latent), D-ribose (latent and weak), ethanol, glycerol, ribitol (latent), D-mannitol, D-glucitol, α-methyl-D-glucoside, glucono-δ-lactone (latent and weak), D-gluconate, succinic acid (weak), xylitol (latent or latent and weak), and propane 1, 2 diol. Does not assimilate galactose, lactose, melibiose, inulin, D-xylose, L-arabinose, L-rhamnose, D-glucosamine, *N*-acetyl-D-glucosamine, methanol, erythritol, galactitol, salicin, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, citric acid, inositol, n-hexadecane, saccharic acid, D-glucuronic acid, L-arabinitol, butane 2, 3 diol, or D-galacturonic acid.

Assimilates potassium nitrate, sodium nitrite, and ethylamine hydrochloride. Does not assimilate L-lysine hydrochloride or cadaverine dihydrochloride.

Maximum growth temperature is 33–35°C.

No vitamins are required for growth.

No starch-like substances are produced.

Growth occurs weakly on 50% (w/w) glucose-yeast extract agar.

Urease positive.

Does not liquefy or only weakly liquefies gelatin.

Does not hydrolyze fat.

The diazonium blue B reaction is positive.

The G+C content of nuclear DNA is 60.2–61.5 mol% (by HPLC).

The major ubiquinone is Q-10.

Xylose is absent in the cells.

Strains examined: Three strains were examined. Strain K-77, which was isolated from a dead leaf of *Oryza sativa* L. collected by T. Nakase and B. Fungsin in Dec. 1987, Thailand, is the type strain of the species. This strain is deposited in the Japan Collection of Microorganisms (JCM), RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama, as JCM 10213 and the Thailand Institute of Scientific and Technological Research (TISTR), Bangkok, Thailand, as TISTR 5724. The other strains, K-81 and K-105, are also deposited in JCM and TISTR as JCM 10214=TISTR 5583 and JCM 10215=TISTR 5597, respectively.

Etymology: *nylandii* (*nylandii*: of Nyland, honoring G. Nyland, the first discoverer of ballistoconidiogenous teleomorphic genus *Sporidiobolus*.)

Sporobolomyces poonsookiae Takashima et Nakase, sp. nov. Fig. 3

In liquido YM: Post dies 3 ad 25°C cellulae ovoideae, ellipsoidales, elongatae vel cylindraceae, 3.0–5.0×4.0–14.0 μm, singulae, binae, catenulatae, vel ramulosae, sedimentum formans; post unum mensem ad 17°C pellicula fragilis et incompleta vel completa et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura rubro-aurantiaca, glabra vel rugosa, nitida, mollis, margine erosa. In culture in lamina vitrea pseudomycelium et mycelium formantur. Ballistosporae in CMA formatae, reniformes, 3.0–5.5×6.0–12.0 μm. Fermentatio nulla. Glucosum, galactosum (lente), L-sorbosum (lente), cellobiosum, trehalosum, amylum solubile (exigue), D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, salicinum, glucono-δ-lactonum, acidum D-gluconicum, acidum succinicum (lente), acidum citricum, xylitolum, L-arabinitolum et 1,2-propanediolum assimilantur, autem saccharosum, maltosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, erythritolum, α-methyl-D-glucosidum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, inositolum, hex-

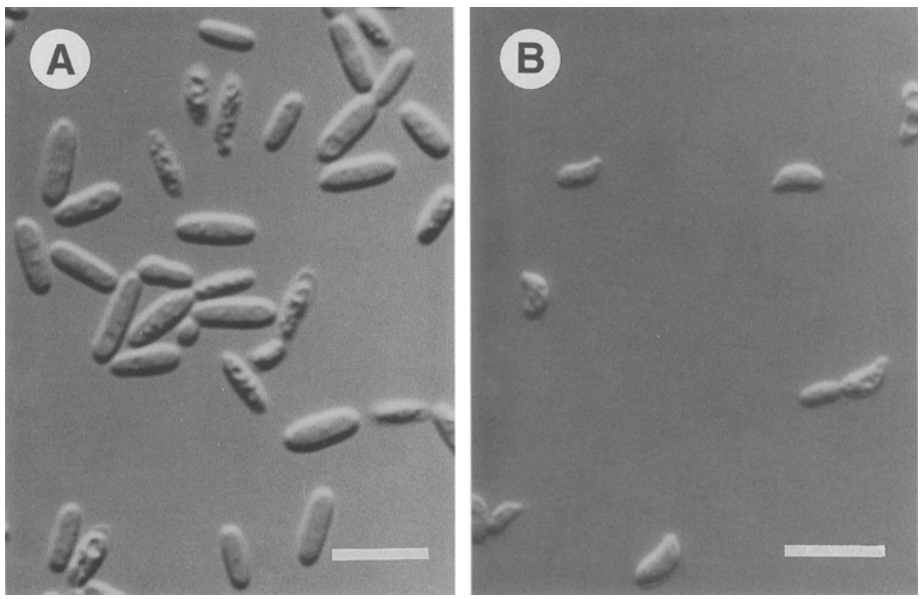

Fig. 3. *Sporobolomyces poonsookiae* JCM 10207 (a) Vegetative cells grown in YM broth for 3 d at 25°C. (b) Ballistoconidia produced on corn meal agar after 5 d at 25°C. Scale bars indicate 10 μm.

adecanum, acidum saccharicum et 2,3-butanediolum non assimilantur. Kalium nitricum, natrium nitrosum et ethylaminum assimilantur, autem L-lysinum (lente et exigue) et cadaverinum non assimilantur. Maxima temperatura crescentiae: 33–36°C. Ad crescentiam vitaminum non necessarium est. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 61.9–62.7 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum in cellulis absens. Holotypus: Isolatio ex folio emortuo *Mangiferae indicae* L., Bangkok, Thailandia, T. Nakase et B. Fungsin, Dec. 1990, JCM 10207 (originaliter ut K-335) in Collectione Culturarum Japonensium quas 'Japan Collection of Microorganisms', Wako, Saitama conservata.

In YM broth, after 3 d at 25°C, the vegetative cells are ovoidal, ellipsoidal, elongate or cylindrical, 3.0–5.0 × 4.0–14.0 μm, single, in pairs, in chains, or in branched chains (Fig. 3a). Sediment is formed. After 1 mo at 17°C, an incomplete or complete fragile pellicle and sediment are present.

On YM agar, after 1 mo at 17°C, the streak culture is reddish orange, shiny to semishiny, smooth to delicate but wrinkled near the bottom, soft, and has an erose margin.

On slide culture, pseudomycelia and true mycelia are produced.

Ballistoconidia are abundantly produced on corn meal agar. They are kidney-shaped, 3.0–5.5 × 6.0–12.0 μm (Fig. 3b).

Does not ferment glucose.

Assimilates glucose, galactose (latent), L-sorbose (latent), cellobiose, trehalose, soluble starch (weak), D-xylose, L-arabinose, D-arabinose, D-ribose, ethanol, glycerol, ribitol, galactitol, D-mannitol, D-glucitol, salicin, glucono-δ-lactone, D-gluconic acid, succinic acid (slow), citric acid (weak), xylitol, L-arabinitol, and propane 1,2 diol. Does not assimilate sucrose, maltose, lactose, melibiose, raffinose, melezitose, inulin, L-rhamnose, D-glucosamine, *N*-acetyl-D-glucosamine, methanol, erythritol, α-methyl-D-glucoside, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, inositol, n-hexadecane, saccharic acid, or butane 2, 3 diol.

Assimilates potassium nitrate, sodium nitrite, and ethylamine hydrochloride. Does not assimilate L-lysine hydrochloride (latent and weak) or cadaverine dihydrochloride.

Maximum growth temperature is 33–36°C.

No vitamins are required for growth.

No starch-like substances are produced.

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

Urease positive.

Does not liquefy gelatin.

Does not hydrolyze fat.

The diazonium blue B reaction is positive.

The G+C content of nuclear DNA is 61.9–62.7 mol% (by HPLC).

The major ubiquinone is Q-10.

Xylose is absent in the cells.

Strains examined: Five strains were examined. Strain K-335, which was isolated from a dead leaf of *Mangifera indica* L. collected by T. Nakase and B. Fungsin in Dec. 1990, Thailand, is the type strain of the species. This strain is deposited in JCM as JCM 10207 and TISTR as TISTR 5722. The other strains K-316, K-344, K-346, and K-362 are also deposited in JCM and TISTR as JCM 10206=TISTR 5743, JCM 10208=TISTR 5744, JCM 10209=TISTR 5745, and JCM 10211=TISTR 5747, respectively.

Etymology: *poonsookiae* (*poonsookiae*: of Poonsook, honoring Ms. Poonsook Attasampunna for her long contribution of more than 15 years to the collaborative partnership between JCM and TISTR.)

Sporobolomyces blumeae Takashima et Nakase, sp. nov. Fig. 4

In liquido YM: Post dies 3 ad 25°C cellulae ovoideae vel ellipsoideae, 3.0–8.0 × 4.0–15.0 μm, singulae, binae, vel aggregatae, sedimentum formans; post unum mensem ad 17°C coloniae punctato annulatae vel insulares et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura glauco-aurantiaca vel rubro-aurantiaca, glabra vel rugosa, hebetata, mollis, margine glabra. In culture in lamina vitrea pseudomycelium formantur. Ballistosporae in CMA abundanter formatae, reniformes, ellipsoidales, vel longi-ellipsoidales, 3.0–5.5 × 6.0–12.0 μm. Fermentatio nulla. Glucosum, L-sorbosum (lente), saccharosum, maltosum, cellobiosum (lente), trehalosum, raffinosum, melezitosum, amylum solubile, D-mannitolum (lente), D-glucitolum (lente), salicinum, glucono-δ-lactonum (exigue), acidum D-gluconicum (lente et exigue), acidum succinicum (lente et exigue) et 1,2-propanediolum (lente et exigue) assimilantur, autem galactosum, lactosum, melibiosum, inulinum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, α-methyl-D-glucosidum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum citricum, inositolum, hexadecanum, acidum saccharicum, xylitolum, L-arabinitolum et 2,3-butanediolum non assimilantur. L-Lysinum et cadaverinum assimilantur, autem kalium nitricum, natrium nitrosum, et ethylaminum non assimilantur. Maxima temperatura crescentiae: 32–33°C. Ad crescentiam vitaminum non necessarium est. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 59.5 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum in cellulis absens. Holotypus: Isolatio ex folio emortuo *Blumeae* sp., Bangkok, Thailandia, T. Nakase et B. Fungsin, Dec. 1990, JCM 10212 (originaliter ut K-230) in Collectione Culturarum Japonensium quas 'Japan Collection of Microorganisms', Wako, Saitama conservata.

In YM broth, after 3 d at 25°C, the vegetative cells are ovoidal or ellipsoidal, (3.0–8.0) × (4.0–15.0) μm, single, in pairs, or in groups (Fig. 4a). Sediment is formed. After 1 mo at 17°C, a dotted ring, islets,

and sediment are present.

On YM agar, after 1 mo at 17°C, the streak culture is grayish orange to reddish orange, semishiny to dull, smooth to delicate but wrinkled near the bottom, soft, and has an entire margin.

On slide culture, pseudomycelia are produced.

Ballistoconidia are abundantly produced on corn meal agar. They are kidney-shaped, ellipsoidal or long-ellipsoidal, 3.0–5.5 × 6.0–12.0 μm (Fig. 4b).

Does not ferment glucose.

Assimilates glucose, L-sorbose (latent), sucrose, maltose, cellobiose (latent), trehalose, raffinose, melezitose, soluble starch, D-mannitol (latent), D-glucitol (latent), salicin, glucono-δ-lactone (weak), D-gluconic acid (latent and weak), succinic acid (latent and weak), and propane 1, 2 diol (latent and weak). Does not assimilate galactose, lactose, melibiose, inulin, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, *N*-acetyl-D-glucosamine, methanol, ethanol, glycerol, erythritol, ribitol, galactitol, α-methyl-D-glucoside, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, citric acid, inositol, n-hexadecane, saccharic acid, xylitol, L-arabinitol, or butane 2, 3 diol.

Assimilates L-lysine hydrochloride and cadaverine dihydrochloride (weak). Does not assimilate potassium nitrate, sodium nitrite, or ethylamine hydrochloride.

Maximum growth temperature is 32–33°C.

No vitamins are required for growth.

No starch-like substances are produced.

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

Urease positive.

Does not liquefy gelatin.

Does not hydrolyze fat.

The diazonium blue B reaction is positive.

The G+C content of nuclear DNA is 59.5 mol% (by HPLC).

The major ubiquinone is Q-10.

Xylose is absent in the cells.

Strains examined: Strain K-230, which was isolated from a dead leaf of *Blumea* sp. collected by T. Nakase and B. Fungsin in Dec. 1990, Thailand, is the type strain of the species. This strain is deposited in JCM as JCM 10212 and TISTR as TISTR 5723.

Etymology: *blumeae* (*blumeae*: of *Blumea*, the plant from which the type strain was isolated.)

Sporobolomyces vermiculatus Takashima et Nakase, sp. nov. Fig. 5

In liquido YM: Post dies 3 ad 25°C cellulae ovoideae vel ellipsoidales, 2.0–5.0 × 3.0–9.0 μm, singulae vel bina, sedimentum formans; post unum mensem ad 17°C coloniae annulatae vel insulares et sedimentum formantur. In agaro YM post unum mensem ad 17°C cultura rubro-aurantiaca, glabra vel rugosa, nitida, mollis, margine glabra. In cultura in lamina vitrea pseudomycelium non formatur. Ballistosporae in CMA formatae, ellipsoidales vel longi-ellipsoidales, rectae, 2.0–4.5 × 6.0–12.0 μm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum (lente et exigue), saccharosum (lente), cellobiosum, trehalosum, melezitosum, amylum solubile, D-xylosum, L-arabinosum, D-arabinosum (lente), D-ribosum (exigue), glycerolum, ribitolum (lente et exigue), D-mannitolum (lente), D-glucitolum (lente), glucono-δ-lactonum (lente et exigue), acidum succinicum (lente et exigue), xylitolum (lente et exigue), L-arabinitolum (lente et exigue) et 1,2-propanediolum (lente et exigue) assimilantur, autem maltosum, lactosum, melibiosum, raffinosum, inulinum, L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, methanolum, ethanolum, erythritolum, galactito-

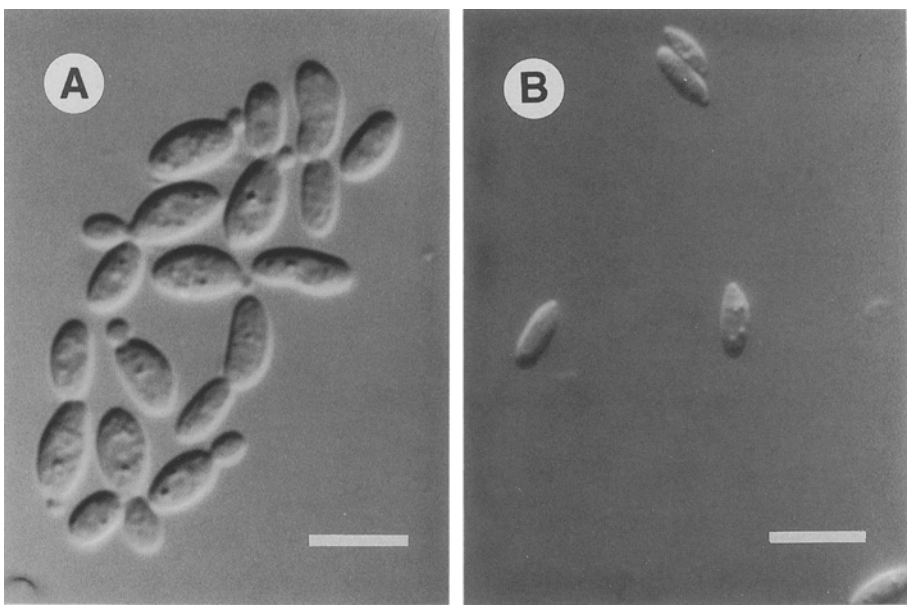

Fig. 4. *Sporobolomyces blumeae* JCM 10212 (a) Vegetative cells grown in YM broth for 3 d at 25°C. (b) Ballistoconidia produced on corn meal agar after 4 d at 25°C. Scale bars indicate 10 μm.

lum, α-methyl-D-glucosidum, salicinum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum citricum, inositolum, hexadecanum, acidum saccharicum, 2,3-butanediolum, acidum D-glucuronicum, et acidum D-galacturonicum non assimilantur. L-Lysinum assimilatur, autem kalium nitricum, natrium nitrosum, ethylaminum et cadaverinum non assimilantur. Maxima temperatura crescentiae: 29–34°C. Ad crescentiam acidum *p*- aminobenzoicum et thiaminum necessarium sunt. Commutatio colori per diazonium caeruleum B positiva. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 51.5–52.1 mol% (per HPLC). Ubiquinonum majus: Q-10. Xylosum in cellulis absens. Holotypus: Isolatio ex folio emortuo *Penniseti pediocellati*, Bangkok, Thailandia, T. Nakase et B. Fungsin, Dec. 1990, JCM 10224 (originaliter ut K-383) in Collectione Culturarum Japonesium, quas 'Japan Collection of Microorganisms', Wako, Saitama conservata.

In YM broth, after 3 d at 25°C, the vegetative cells are ovoidal or ellipsoidal, (2.0–5.0) × (3.0–9.0) μm, single or in pairs (Fig. 5a). Sediment is formed. After 1 mo at 17°C, a complete ring, islets, and sediment are present.

On YM agar, after 1 mo at 17°C, the streak culture is reddish orange to orange-red, shiny to semishiny, smooth to delicate but wrinkled near the bottom, soft, and has an entire margin.

On slide culture, pseudomycelia are not produced.

Ballistoconidia are produced on corn meal agar. They are ellipsoidal or long-ellipsoidal, and usually straight, 2.0–4.5 × 6.0–12.0 μm (Fig. 5b).

Does not ferment glucose.

Assimilates glucose, galactose, L-sorbose (latent and weak), sucrose (latent), cellobiose (latent), trehalose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose (latent), D-ribose (weak), glycerol, ribitol (latent and weak), D-mannitol (latent), D-glucitol (latent), glucono-δ-lactone (latent and weak), succinic acid (latent and weak), xylitol (latent and weak), L-arabinitol (latent and weak), and propane 1, 2 diol (latent and weak). Does not assimilate maltose, lactose, melibiose, raffinose, inulin, L-rhamnose, D-glucosamine, *N*-acetyl-D-glucosamine, methanol, ethanol, erythritol, galactitol, α-methyl-D-glucoside, salicin, D-gluconic acid, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, citric acid, inositol, n-hexadecane, butane 2, 3 diol, D-glucuronic acid, or D-galacturonic acid.

Assimilates L-lysine hydrochloride. Does not assimilate potassium nitrate, sodium nitrite, ethylamine hydrochloride, or cadaverine dihydrochloride.

Maximum growth temperature is 29–34°C.

p-Aminobenzoic acid and thiamin are required for growth.

No starch-like substances are produced.

Growth does not occur on 50% (w/w) glucose-yeast extract agar.

Urease positive.

Does not liquefy gelatin.

Does not hydrolyze fat.

The diazonium blue B reaction is positive.

The G+C content of nuclear DNA is 51.5–52.1 mol% (by HPLC).

The major ubiquinone is Q-10.

Xylose is absent in the cells.

Strains examined: Four strains were examined. Strain K-383, which was isolated from a dead leaf of *Pennisetum pediocellatum* collected by T. Nakase and B. Fungsin in Dec. 1990, Thailand, is the type strain of the species. This strain is deposited in JCM as JCM 10224 and TISTR as TISTR 5749. The other strains K-17, K-

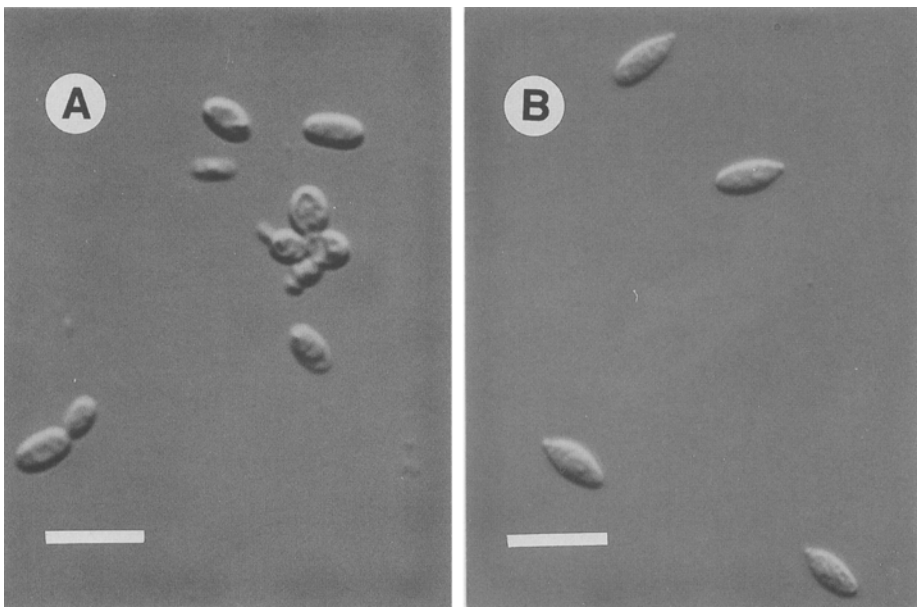

Fig. 5. *Sporobolomyces vermiculatus* (a) Vegetative cells of JCM 10224 grown in YM broth for 3 d at 25°C. (b) Ballistoconidia of JCM 10224 produced on corn meal agar after 5 d at room temperature. Scale bars indicate 10 μm.

90, and K-109 are also deposited in JCM and TISTR as JCM 10221=TISTR 5727, JCM 10222=TISTR 5748, and JCM 10223=TISTR 5641, respectively.
Etymology: *vermiculatus* (*vermiculatus*: referring to the color of the culture of this species)

Acknowledgements——We thank Miss P. Atthasampunna and Mr. B. Fungsin, Thailand Institute of Scientific and Technological Research, Bangkok, Thailand, for their help in collecting plant materials and the isolation of the strains reported in this paper. This study was supported in part by special coordination funds for promoting science and technology from the Science and Technology Agency of the Japanese Government.

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