***Bannoa hahajimensis* gen. nov., sp. nov., and three related anamorphs, *Sporobolomyces bischofia* sp. nov., *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov., yeasts isolated from plants in Japan**

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Fourteen ballistoconidium-forming yeast strains were isolated from leaves of plants collected in the Ogasawara Islands, which are isolated islands in the Pacific Ocean, about 1000 km south of the Japanese mainland, in the subtropical zone. The 14 isolates were characterized by the absence of xylose in whole-cell hydrolysates, the presence of Q-10(H₂) as the major ubiquinone isoprenologue, G+C contents of 47.6–52.0 mol%, the inability to ferment sugars or to assimilate nitrate and positive Diazonium blue B and urease reactions. They formed a phylogenetically coherent cluster within the *Erythrobasidium* lineage in the Urediniomycetes of the Basidiomycota based on 18S rDNA sequences. Analyses of the nucleotide sequences of internal transcribed spacer regions and DNA complementarity showed that four genospecies were recognized among the 14 isolates. A mating reaction was observed in one of the four genospecies, which produced one-celled basidia on dikaryotic hyphae with clamp connections. On the basis of the morphological, physiological, chemotaxonomic and phylogenetic analyses, a new teleomorphic genus, *Bannoa*, is proposed, in which one novel species is described, *Bannoa hahajimensis* gen. nov., sp. nov. (type strain OK-248^T = JCM 10336^T = CBS 9039^T). The other three anamorphic genospecies are described as *Sporobolomyces bischofia* sp. nov. (type strain OK-257^T = JCM 10338^T = CBS 9041^T), *Sporobolomyces ogasawarensis* sp. nov. (type strain OK-14^T = JCM 10326^T = CBS 9038^T) and *Sporobolomyces syzygii* sp. nov. (type strain OK-227^T = JCM 10337^T = CBS 9040^T).

Keywords: *Bannoa hahajimensis* gen. nov., sp. nov., *Sporobolomyces*, ballistoconidium-forming yeast, 18S rDNA, ITS

INTRODUCTION

The two species *Erythrobasidium hasegawianum* Hamamoto, Sugiyama & Komagata 1991 and *Sporobolomyces elongatus* Shivas & Rodrigues de Miranda 1983 are the only known basidiomycetous yeasts with the hydrogenated Q-10 Q-10(H₂) as the major ubiquinone

isoprenologue (Yamada & Kondo, 1973; Yamada *et al.*, 1973; Nakase & Suzuki, 1986). So far, only one strain has been assigned to each species (Boekhout & Nakase, 1998; Sugiyama & Hamamoto, 1998). *Erythrobasidium hasegawianum* was isolated from an old culture of beer yeast (Robbins & Ma, 1944) and *Sporobolomyces elongatus* was isolated from the surface of a leaf of *Callistemon viminalis* (Soland ex Gaertn.) G. Don ex Loud. in Australia (Shivas & Rodrigues de Miranda, 1983). Since the isolation of these two strains with Q-10(H₂), no additional basidiomycetous yeasts with the unique ubiquinone isopre-

Abbreviation: ITS, internal transcribed spacer.

The DDBJ accession numbers for the 18S rDNA and the internal transcribed spacer sequences obtained in this study are given in Table 1.

nologue have been found, in spite of our intensive survey of basidiomycetous yeasts from plant samples in various regions.

Recently, we found a third yeast strain containing Q-10(H₂), from a leaf collected in China, and described a novel species of the genus *Sporobolomyces*, *Sporobolomyces yunnanensis* (Bai *et al.*, 2001). Subsequently, we isolated 14 strains of undescribed ballistoconidium-forming yeasts containing Q-10(H₂) from leaves in the Ogasawara Islands, which are isolated islands in the Pacific Ocean, about 1000 km south of the Japanese mainland, in the subtropical zone. The strains were characterized by their inability to assimilate nitrate and formed a phylogenetically coherent cluster within the *Erythrobasidium* lineage in the Urediniomycetes of the Basidiomycota. Of these 14 isolates, four had the ability to mate between compatible mating types and produced one-celled basidia on dikaryotic hyphae with clamp connections. In this paper, we report the taxonomic characterization and classification of the 14 isolates and propose the new teleomorphic genus *Bannoa*, with *Bannoa hahajimensis* gen. nov., sp. nov., and three novel *Sporobolomyces* species, *Sporobolomyces bischofia* sp. nov., *Sporobolomyces ogasawarenensis* sp. nov. and *Sporobolomyces syzygii* sp. nov.

METHODS

Yeast isolation and cultivation. Samples of dead leaves of six plant species were collected on Chichi-jima and Haha-jima Islands of the Ogasawara Islands, Japan. The procedure used for yeast isolation and purification has been described previously (Nakase & Suzuki, 1985; Nakase & Takashima, 1993). All strains were grown at 17 or 25 °C in yeast extract/malt extract broth or yeast extract/malt extract agar (Difco) for purification and cultivation. The strains used in this study are listed in Table 1.

Morphological, physiological and biochemical characteristics. The methods for morphological, physiological, biochemical and chemotaxonomic characterization and DNA–DNA reassociation experiments were described previously (Hamamoto & Nakase, 1995). The mating experiment was performed according to the method described by Yarrow (1998). The mating phenomenon was observed with a light microscope and Hitachi S-2400 scanning electron microscope. Specimens for scanning electron microscopy were prepared as described by Itoh *et al.* (1989). The staining of nuclei was carried out as described previously (Hamamoto *et al.*, 1988).

Nucleotide sequence analyses. The method for genomic DNA extraction was described previously (Hamamoto *et al.*, 2000). The primers used for the amplification and sequencing of 18S rDNA and the internal transcribed spacer (ITS) region were those described by White *et al.* (1990). The PCR products were sequenced using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems). Analyses of DNA sequence reactions were performed with an Applied Biosystems model 310 sequencer. Sequences were aligned using CLUSTAL W version 1.81 (Thompson *et al.*, 1994) and were adjusted manually. Evolutionary distances were calculated using the program DNADIST of PHYLIP version 3.57c (Felsenstein, 1995) with Kimura's two-parameter model and trees were constructed

in NEIGHBOR by the neighbour-joining method (Saitou & Nei, 1987). The confidence values of branches were determined by performing a bootstrap analysis (Felsenstein, 1985) with 1000 replicates. The sequences determined were deposited in the DDBJ database under the accession numbers shown in Table 1.

RESULTS

Morphological, physiological and chemotaxonomic characteristics

The 14 isolates formed ballistoconidia whose shape was asymmetric. They did not form hyphae or pseudo-hyphae on yeast extract/malt extract agar, potato dextrose agar or corn meal agar (Difco) and the colonies were orange to salmon-red in colour. Sugars were not fermented, starch-like substances were not produced and nitrate was not assimilated. The assimilation patterns are given below in the descriptions of the four novel species. Diazonium blue B and urease reactions were positive. All the isolates contained Q-10(H₂) as the major ubiquinone isoprenologue and xylose was absent from whole-cell hydrolysates. The G + C contents of the DNA of the isolates ranged from 47.6 to 52.0 mol %, as shown in Table 1. The values for the type strains of *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus* were respectively 50.0 and 55.0 mol %.

Nucleotide sequence analyses

The nucleotide sequences of ITS regions of the 17 strains shown in Table 1 were determined to clarify the relationships between them. The unrooted dendrogram in Fig. 1 was constructed from datasets aligned by CLUSTAL W version 1.81 on the basis of 148 (ITS1) and 211 (ITS2) sites. Our 14 isolates were separated clearly from the three known species that contain Q-10(H₂). These isolates were divided into four major clusters in the ITS-based dendrogram. Strains OK-227^T and OK-257^T were placed independently in the ITS-based dendrogram. The levels of sequence similarity of the ITS regions of strains OK-52, OK-173, OK-219 and OK-248^T were 100 %, and these isolates formed a tight phylogenetic cluster in the ITS-based dendrogram with a high bootstrap value (100 %). Strains OK-1, OK-14^T, OK-19, OK-21, OK-38, OK-39, OK-166 and OK-198 formed another tight phylogenetic cluster in the ITS-based dendrogram, with a high bootstrap value (99 %). The levels of sequence similarity of the ITS region of the eight strains ranged from 97.2 to 100.0 %.

Analysis of 18S rDNA sequences revealed that 13 of our 14 isolates had introns, which were considered to be group I introns with conserved sequence elements P, Q, R and S (Cech, 1988). Single introns were found in strains OK-1, OK-14^T, OK-19, OK-21, OK-38, OK-39, OK-166, OK-198 (all 382 nt), OK-52, OK-173, OK-219, OK-248^T (all 328 nt) and OK-257^T (total of 687 nt). Strain OK-257^T contained introns of 297 and 390 nt, in separate locations.

Table 1. Strains used in this study and their DNA base composition

Haha-jima and Chichi-jima are islands in the Ogasawara Islands, Japan.

Strain	Isolate no.	Source	G + C content (mol %)	Accession no.
<i>Bannoa hahajimensis</i>				
JCM 10333	OK-52	Dead leaves of <i>Bryophyllum pinnatum</i> , Haha-jima	47.8	AB035894
JCM 10334	OK-173	Dead leaves of <i>Rhaphiolepis wrightiana</i> , Haha-jima	48.0	AB035895
JCM 10335	OK-219	Dead leaves of <i>Syzygium buxifolium</i> , Haha-jima	47.6	AB035896
JCM 10336 ^T	OK-248 ^T	Dead leaves of <i>Bryophyllum pinnatum</i> , Haha-jima	48.1	AB035897
<i>Sporobolomyces bischofia</i>	OK-257 ^T	Dead leaves of <i>Bischofia javanica</i> , Chichi-jima	49.6	AB035721
JCM 10338 ^T				
<i>Sporobolomyces ogasawarensis</i>				
JCM 10325	OK-1	Dead leaves of <i>Osmanthus insularis</i> , Chichi-jima	51.6	AB035712
JCM 10326 ^T	OK-14 ^T	Dead leaves of <i>Schima mertensiana</i> , Chichi-jima	51.5	AB035713
JCM 10327	OK-19	Dead leaves of <i>Schima mertensiana</i> , Chichi-jima	51.6	AB035714
JCM 10328	OK-21	Dead leaves of <i>Schima mertensiana</i> , Chichi-jima	51.5	AB035715
JCM 10329	OK-38	Dead leaves of <i>Schima mertensiana</i> , Chichi-jima	51.9	AB035716
JCM 10330	OK-39	Dead leaves of <i>Schima mertensiana</i> , Chichi-jima	52.0	AB035717
JCM 10331	OK-166	Dead leaves of <i>Syzygium buxifolium</i> , Chichi-jima	51.6	AB035718
JCM 10332	OK-198	Dead leaves of <i>Syzygium buxifolium</i> , Haha-jima	52.0	AB035719
<i>Sporobolomyces syzygii</i>	OK-227 ^T	Dead leaves of <i>Syzygium buxifolium</i> , Haha-jima	48.9	AB035720
JCM 10337 ^T				
<i>Erythrobasidium hasegawianum</i>	—	IFO 1058 ^T	50.0	AB036065
JCM 1545 ^T				
<i>Sporobolomyces elongatus</i>	—	CBS 8080 ^T	55.0	AB036066
JCM 5354 ^T				
<i>Sporobolomyces yunnanensis</i>	—	CH 2.141 ^T	50.0*	AB030353
JCM 10687 ^T				

* Data from Bai *et al.* (2001).

Fig. 1. Unrooted dendrogram showing relationships among strains of *Bannoa* and three related novel *Sporobolomyces* species based on ITS1 and ITS2 sequences. The dendrogram was constructed using the neighbour-joining method, as described in the text. Numbers by nodes are bootstrap percentages (values < 50 % not shown) derived from 1000 replicates.

The 18S rDNA sequences of four isolates that were representative of each of the clusters from the ITS-based dendrogram, strains OK-14^T, OK-227^T, OK-248^T and OK-257^T, were aligned with 14 published sequences to define their phylogenetic positions within the basidiomycetes. Fig. 2 shows a phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987) on the basis of the 1684 positions alignable in all the species examined here. All our isolates

were placed in the *Erythrobasidium* lineage in the Urediniomycetes, together with *Erythrobasidium hasegawianum*, *Rhodotorula lactosa*, *Rhodotorula minuta*, *Sakaguchia dacryoides* and nine species that are currently classified in the genus *Sporobolomyces*, namely *Sporobolomyces coprosmae*, *Sporobolomyces elongatus*, *Sporobolomyces foliicola*, *Sporobolomyces gracilis*, *Sporobolomyces kluyverinii*, *Sporobolomyces oryzicola*, *Sporobolomyces phyllomatis*, *Sporo-*

Fig. 2. Phylogenetic positions of the type strains of *Bannoa hahajimensis* and three related novel *Sporobolomyces* species based on 18S rDNA. The branching pattern was generated by the neighbour-joining method, as described in the text. Numbers by nodes are bootstrap percentages (values < 50 % not shown) derived from 1000 replicates.

bolomyces salicinus and *Sporobolomyces yunnanensis* (Fig. 2). Our isolates formed a phylogenetically coherent cluster within the *Erythrobasidium* lineage, with a high bootstrap value (99 %).

DNA–DNA reassociation

Strain OK-248^T showed high degrees of relative binding (94–100 %) with strains OK-52, OK-173 and OK-219, but low degrees of binding (11–49 %) with strains OK-14^T, OK-227^T and OK-257^T and *Erythrobasidium hasegawianum* JCM 1545^T. Strain OK-14^T showed high degrees of relative binding (76–100 %) with strains OK-1, OK-19, OK-21, OK-38, OK-39, OK-166 and OK-198, but low degrees of binding (9–40 %) with strains OK-227^T, OK-248^T and OK-257^T and *Erythrobasidium hasegawianum* JCM 1545^T. Strains OK-227^T and OK-257^T showed low degrees of relative binding (15–49 %) with all the other novel isolates, *Erythrobasidium hasegawianum* JCM 1545^T and each other.

Sexual reproduction

After 2–3 days on yeast carbon base agar [11.7 g yeast carbon base (Difco), 20 g agar, 1000 ml distilled water] or corn meal agar (Difco), conjugation was observed between strains OK-52 and OK-173 (Fig. 3a, b), strains OK-173 and OK-219, strains OK-173 and OK-248^T and strains OK-219 and OK-248^T. Subsequently, dikaryotic mycelium with clamp connections developed (Fig. 3c). After 10 days, basidia developed laterally, were clamped at their base (Fig. 3d, e) and sometimes grew terminally. The basidia were obovate to dacryoid, 8.0–10.0 × 15.0–20.0 µm. They appeared to be diploid, as the size of their nuclei was twice that of nuclei in the dikaryotic mycelium (Fig. 3f, g). Germination of the basidia occurred by the formation of hyphae. These terminated with a slender apex, on which yeast cells originated laterally. The yeast cells were monokaryotic and the size of the nuclei was

Fig. 3. Mating phenomenon in *Bannoa hahajimensis*. (a, b) A conjugated cell with a copulation tube (OK-52 × OK-173). (c, e) Basidia developed laterally, clamped at their base (c, OK-219 × OK-248^T; e, OK-173 × OK-248^T). (d) Dikaryotic hyphae (OK-173 × OK-219). (f) Monokaryotic basidium (OK-173 × OK-248^T). (g) Basidium developing hyphae for germination (OK-52 × OK-173). (h) Yeast cells originating laterally on a hypha (OK-52 × OK-173). Bars, 5 µm.

comparable to that of the nuclei in the dikaryophase or the solitary yeast phase (Fig. 3h).

DISCUSSION

The ITS region has been found to be useful in resolving relationships among closely related taxa, because of its high substitution rate compared with that of 18S and

26S rDNA (Berbee *et al.*, 1995; Waalwijk *et al.*, 1996; James *et al.*, 1996; Oda *et al.*, 1997; Nagahama *et al.*, 1999; Hamamoto *et al.*, 2000). To clarify the relationships among our isolates at the species level, the sequences of the ITS regions were determined and analysed. Strains OK-227^T and OK-257^T, which were clearly separated from any other isolates in the ITS-based dendrogram, were considered to represent single species. Strains OK-52, OK-173, OK-219 and OK-248^T were considered to be members of a single species because their ITS sequences were identical. Some substitutions were found in the ITS sequences of strains OK-1, OK-14^T, OK-19, OK-21, OK-38, OK-39, OK-166 and OK-198. However, the strains formed a coherent cluster in the ITS-based dendrogram, supported by a high bootstrap value (99%), and they showed rather high similarities in ITS sequences (97.2–100.0%). This suggests that these eight strains are members of a single species.

The levels of DNA complementarity confirmed that our 14 isolates belonged to four genospecies. The four species based on DNA–DNA reassociation experiments corresponded well with those suggested by the ITS-based dendrogram. The ITS sequence would be one of the important criteria for the discrimination of these yeast species.

As a result of the mating experiments, the genospecies comprising strains OK-52, OK-173, OK-219 and OK-248^T was considered to be teleomorphic. Based on mating compatibility, three distinct mating types (A1, A2 and A3) have been detected in this genospecies: strain OK-248^T (mating type A1), strain OK-173 (mating type A2) and strains OK-52 and OK-219 (mating type A3). When intermated (A1 × A2, A2 × A3 and A3 × A1), all combinations gave complete life cycles, except the combination between strains OK-52 and OK-248^T. In this conjugated pair, conjugated cells with a copulation tube and hyphal development were detected, but the formation of diploid basidia was not observed. This may be due to some incompatibility. The multiallelic bipolar mating system observed in strains OK-52, OK-173, OK-219 and OK-248^T is similar to that described in *Cystofilobasidium infirmominium* (Fell *et al.*, 1973) in the Hymenomycetes. Conjugation was not observed when any of the mating types was mixed with the other isolates.

The morphological and biochemical characters of our 14 isolates are consistent with the currently recognized description of the genus *Sporobolomyces* (Boekhout & Nakase, 1998). However, the genus *Sporobolomyces* has been revealed to be polyphyletic, based on analysis of 18S rDNA sequences (Hamamoto & Nakase, 2000). Specifically, *Sporobolomyces* species are placed in the Sporidiales, *Agaricostilbum*/*Bensingtonia*, *Erythrobasidium* and subbrunneus lineages in the Urediniomycetes. Our four genospecies formed a monophyletic cluster in the *Erythrobasidium* lineage, based on 18S rDNA sequences (Fig. 2). There are three species with different sexual life cycles in this lineage (Fell *et al.*,

2000). *Erythrobasidium hasegawianum*, which contains Q-10(H₂), produces unicellular basidia without mating (Hamamoto *et al.*, 1988). *Occultifur externus*, which contains Q-10 (determined in this study), produces four-celled auricularioid basidia after conjugation between two yeast cells (Sampaio *et al.*, 1999). *Sakaguchia dacryoides*, which also contains Q-10, produces teliospores that germinate to give two to four-celled basidia with repetitively budding basidiospores after mating between the different mating pairs (bifactorial mating system) (Fell & Statzell-Tallman, 1998). One of our four species has a mating system (the multiallelic bipolar mating system) that is unique in the Urediniomycetes, and it produces unicellular basidia on a clamp. This sexual cycle is different from those of the other three known teleomorphic species in the *Erythrobasidium* lineage.

Occultifur externus and six *Rhodotorula* species (*Rhodotorula armeniaca*, *Rhodotorula aurantiaca*, *Rhodotorula laryngis*, *Rhodotorula pallida*, *Rhodotorula marina* and *Rhodotorula slooffiae*), which were placed in the *Erythrobasidium* lineage in the large subunit (LSU) rDNA-based tree (Fell *et al.*, 2000), were not included in the analysis of 18S rDNA sequences in this study. However, in the LSU rDNA-based tree (Fell *et al.*, 2000), *Occultifur externus*, *Rhodotorula laryngis*, *Rhodotorula pallida* and *Rhodotorula slooffiae* clustered with *Rhodotorula minuta* (bootstrap value of 100%), *Rhodotorula armeniaca* and *Rhodotorula aurantiaca* clustered with *Sporobolomyces kluyveri*, *Sporobolomyces phyllomatis* and *Sporobolomyces salicinus* (bootstrap value of 99%) and *Rhodotorula marina* clustered with *Sporobolomyces gracilis* (bootstrap value of 95%). As mentioned above, our four species formed a monophyletic cluster in the *Erythrobasidium* lineage based on 18S rDNA sequences. Therefore, our four species were considered to be phylogenetically separated from *Occultifur externus* and the six *Rhodotorula* species.

All the data presented here, therefore, support the conclusion that the teleomorphic species belongs to a new teleomorphic genus, for which we propose the name *Bannoa* gen. nov., as *Bannoa hahajimensis* sp. nov., with a multiallelic bipolar mating system. Moreover, we propose three novel *Sporobolomyces* species for our anamorphs, which are phylogenetically closely related to the genus *Bannoa*: *Sporobolomyces bischoffiae* sp. nov., *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov. As shown in Table 2, the seven species containing Q-10(H₂) in the *Erythrobasidium* lineage can also be distinguished by their phenotypic characteristics.

Latin diagnosis of *Bannoa* Hamamoto gen. nov.

Genus ad Urediniomycetes pertinet. Coloniae rubro-aurantiaca. Cellulae sphaericae vel ovoideae singulae aut binae. Ballistosporae ovoideae vel ellipsoideae. Pseudomycelium absens. Mycelium secundum copula-

Table 2. Physiological and biochemical characteristics that differentiate species containing Q-10(H₂)

Species are listed as: 1, *Bannoa hahajimensis*; 2, *Erythrobasidium hasegawianum*; 3, *Sporobolomyces bischoffiae*; 4, *Sporobolomyces elongatus*; 5, *Sporobolomyces ogasawarensis*; 6, *Sporobolomyces syzygii*; 7, *Sporobolomyces yunnanensis*. Characters are scored as: +, positive; –, negative; v, variable.

Characteristic	1	2	3	4	5	6	7
Assimilation of:							
L-Arabinose	+	+	–	+	+	+	+
L-Rhamnose	+	–	–	+	v	+	–
D-Glucitol	+	+	+	+	+	–	–
Methyl α -D-glucoside	v	–	–	–	–	+	–
2-Ketogluconate	+	+	+	–	+	+	+
Nitrate	–	+	–	–	–	–	+
L-Lysine	+	+	+	–	+	+	–
G + C content (mol%)	47.6–48.1	50.0	49.6	55.0	51.5–52.0	48.9	50.0

tionen cellularum productur, cum septis nodosis. Probasidia (teliosporae) non formantur. Basidia unicellulosa basi cum fibulae. Fermentatio nulla. Ureum hydrolysat. Deoxyribonucleasum extracellulosum positivum. Diazonium caeruleum B positivum. Xylosum in cellulis absens. Systema ubiquinoni majus: Q-10(H₂). Species typica: Bannoa hahajimensis Hamamoto, Thanh & Nakase.

Description of *Bannoa* Hamamoto gen. nov.

Bannoa (Ban'no.a. N.L. fem. n. *Bannoa* of Banno, in honour of I. Banno, for his pioneering work on the teleomorphic life cycle of basidiomycetous yeasts).

The genus is placed in the class Urediniomycetes. Colonies are orange to salmon-red. Cells are spherical to ovoid, single or in pairs. Ballistoconidia are ovoidal and ellipsoidal. Pseudomycelium is absent. True mycelium with clamp connections at septa is produced after cell conjugation. Teliospores are not formed. Unicellular basidia arise laterally on a clamp or terminally. Cells of basidia germinate with hyphae, from which yeast cells originate. Sugars are not fermented. Urease and Diazonium blue B reactions are positive. Xylose is absent from whole-cell hydrolysates. The major ubiquinone is Q-10(H₂).

Type species: *Bannoa hahajimensis* Hamamoto, Thanh & Nakase.

Latin diagnosis of *Bannoa hahajimensis* Hamamoto, Thanh & Nakase sp. nov.

In liquido YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (5.0–7.0 × 8.0–12.0 µm), singulae aut binae. Post 1 mensem sedimentum et pelliculum formantur. Cultura in agarō YM post 1 mensem (25 °C), aurantiaca, glabra aut rugosa, butyracea et margine glabra.

Pseudohyphae nullae. Ballistosporeae ovoideae vel ellipsoideae, 3.0–6.0 × 7.0–14.0 µm. Mycelium secundum copulationem cellularum (A1 et A2, A2 et A3, A3 et A1) productur, cum septis nodosis. Probasidia (teliosporae) non formantur. Basidia unicellulosa basi cum fibulae. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinose, melezitose, amyllum, D-xylosum, L-arabinosum, D-arabinosum (varium), D-ribosum (varium), L-rhamnosum, ethanolum (varium), glycerolum, ribitolum (varium), galactitolum (varium), D-mannitolum, D-glucitolum, methyl α -D-glucosidum (varium), salicinum, glucono- δ -lactonum (varium), acidum 2-ketogluconicum, acidum 5-ketogluconicum, DL-lacticum (varium), acidum succinicum, acidum citricum et acidum D-glucuronicum assimilantur, at non lactosum, inulinum, erythritolum, inositolum nec acidum D-galacturonicum. Lysinum assimilatur at non kalium nitricum, natrium nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 28–29 °C. Ad crescentiam acidum p-aminobenzoicum (varium) et thiaminum necessarium est. Materia amyloidea iodophila non formatur. Ureum hydrolysat. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 47.6–48.1 mol% (per HPLC). Ubiquinonum majus: Q-10(H₂). Xylosum in cellulis absens. Typus stirps OK-248^T ex folio Bryophyllum pinnatum, Japonia, isolata est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10336^T deposita est.

Description of *Bannoa hahajimensis* Hamamoto, Thanh & Nakase sp. nov.

Bannoa hahajimensis (ha.ha.ji.men'sis. N.L. adj. *hahajimensis* referring to Haha-jima, one of the Japanese Ogasawara Islands in the Pacific Ocean, where the type strain was isolated).

Fig. 4. Photomicrographs of vegetative cells on YM agar (a–d) and ballistoconidia on corn meal agar (e–h). (a, e) *Bannoa hahajimensis* OK-248^T (= JCM 10336^T). (b, f) *Sporobolomyces bischofia* OK-257^T (= JCM 10338^T). (c, g) *Sporobolomyces ogasawarensis* OK-14^T (= JCM 10326^T). (d, h) *Sporobolomyces syzygii* OK-227^T (= JCM 10337^T). Bars, 10 µm.

After growth in YM broth (Difco) for 3 days at 25 °C, cells are spherical to ovoid (5.0–7.0 × 8.0–12.0 µm), single or in pairs (Fig. 4a). A sediment and a hydrophobic pellicle are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 days, the streak culture is orange. After 1 month, the streak culture is red-orange, smooth, shining, butyrous and soft and has an entire margin. No pseudomycelium develops on Dalmau plate cultures on corn meal agar after 10 days at 25 °C. Ballistoconidia are formed on corn meal agar and YM agar. They are ovoidal and ellipsoidal, 3.0–6.0 × 7.0–14.0 µm (Fig. 4e). True mycelium with clamp connections at septa is produced after cell conjugation (A1 × A2, A2 × A3 and A3 × A1). Teliospores are not formed. Unicellular basidia arise laterally on a clamp, sometimes terminally. Germination of basidia occurs by the formation of hyphae. Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose (variable), D-ribose (variable), L-rhamnose, ethanol (variable), glycerol, ribitol (variable), galactitol (variable), D-mannitol, D-glucitol, methyl α-D-glucoside (variable), salicin, glucono-δ-lactone (variable), 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid (variable), succinic acid, citric acid and D-glucuronic acid. Does not assimilate lactose, inulin, erythritol, inositol or D-galacturonic acid. Assimilates L-lysine. Does not assimilate nitrate, nitrite, ethylamine or cadaverine. Maximum growth temperature is 28–29 °C. Growth does not occur in vitamin-free medium. *p*-Amino-benzoic acid (variable) and thiamin are required for growth. Growth does not occur on 50% (w/w) glucose/yeast extract agar. No starch-like substrate is produced. Xylose is absent from whole-cell hydroly-

sates. Urease and Diazonium blue B reactions are positive. The major ubiquinone is Q-10(H₂). The G + C content of the nuclear DNA is 47.6–48.1 mol%, as determined by HPLC. The type strain is strain OK-248^T (= JCM 10336^T = CBS 9039^T).

Latin diagnosis of *Sporobolomyces bischofia* Hamamoto, Thanh & Nakase sp. nov.

In liquido YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (3.0–4.0 × 9.0–12.0 µm), singulae aut binae. Post 1 mensem sedimentum et pelliculum formantur. Cultura in agarō YM post 1 mensem (25 °C), aurantiaca, glabra aut rugosa, butyracea et margine glabra. Pseudohyphae nullae. Ballistosporeae ovoideae vel ellipsoideae, 4.0–5.0 × 8.0–10.0 µm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinsum, melezitosum, amyllum, D-xylosum, glycerolum, ribitolium, D-mannitolium, D-glucitolium, salicinum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, DL-lacticum, acidum succinicum, acidum citricum et acidum D-glucuronicum assimilantur, at non lactosum, inulinum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, ethanolum, erythritolum, galactitolium, methyl α-D-glucosidum, glucono-δ-lactonum, inositolum nec acidum D-galacturonicum. Lysinum assimilatur at non kalium nitricum, natrium nitrosolum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 29–30 °C. Ad crescentiam thiaminum necessarium est. Materia amyloidea iodophila non formantur. Ureum hydrolysatur. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 49.6 mol% (per HPLC). Ubiquinonum majus: Q-10(H₂). Xylosum in cellulis absens. Typus stirps OK-257^T ex folio Bischofia javanica, Japonia,

isolata est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10338^T deposita est.

Description of *Sporobolomyces bischofia* Hamamoto, Thanh & Nakase sp. nov.

Sporobolomyces bischofia [bi.scho'fi.a.e. N.L. gen. n. *bischofia* of *Bischofia* (*Bischofia javanica*), the plant from which the type strain was isolated].

After growth in YM broth (Difco) for 3 days at 25 °C, cells are spherical to ovoid (3.0–4.0 × 9.0–12.0 µm), single or in pairs (Fig. 4b). A sediment and a hydrophobic pellicle are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 days, the streak culture is orange to salmon-red. After 1 month, the streak culture is brownish orange, smooth, shining, butyrous and soft and has an entire margin. No true mycelium or pseudomycelium develops on Dalmau plate cultures on corn meal agar after 10 days at 25 °C. Ballistoconidia are formed on corn meal agar and YM agar. They are ovoidal and ellipsoidal, 4.0–5.0 × 8.0–10.0 µm (Fig. 4f). Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, glycerol, ribitol, D-mannitol, D-glucitol, salicin, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, succinic acid, citric acid and D-glucuronic acid. Does not assimilate lactose, inulin, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, erythritol, galactitol, methyl α-D-glucoside, glucono-δ-lactone, inositol or D-galacturonic acid. Assimilates L-lysine. Does not assimilate nitrate, nitrite, ethylamine or cadaverine. Maximum growth temperature is 29–30 °C. Growth does not occur in vitamin-free medium. Thiamin is required for growth. Growth does not occur on 50 % (w/w) glucose/yeast extract agar. No starch-like substrate is produced. Xylose is absent from whole-cell hydrolysates. Urease and Diazonium blue B reactions are positive. The major ubiquinone is Q-10(H₂). The G + C content of the nuclear DNA is 49.6 mol%, as determined by HPLC. The type strain is strain OK-257^T (= JCM 10338^T = CBS 9041^T).

Latin diagnosis of *Sporobolomyces ogasawarensis* Hamamoto, Thanh & Nakase sp. nov.

In liquido YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (4.0–6.0 × 8.0–10.0 µm), singulae aut binae. Post 1 mensem pellicula imperfecta et sedimentum formantur. Cultura in agaro YM post 1 mensem (25 °C), rubro-aurantiaca, glabra aut rugosa, butyracea et margine glabra. Pseudohyphae nullae. Ballistosporae ovoideae vel ellipsoideae, 3.0–6.0 × 6.0–14.0 µm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum, saccharosum, maltosum, cellobiosum (varium), trehalosum, lactosum (varium), melibiosum (varium), raffinsum, amyllum, D-xylosum, L-arabiosum, D-arabiosum (varium), L-rhamnosum (varium), glycerolum, ribitolum (varium), galactitolum (varium), D-mannitolum, D-glu-

citolum, salicinum, glucono-δ-lactonum, acidum 2-keto-gluconicum, acidum 5-ketogluconicum, DL-lacticum (varium), acidum succinicum, acidum citricum (varium), inositolum (varium) et acidum D-glucuronicum assimilantur, at non, inulinum, D-ribosum, ethanolum, erythritolum, methyl α-D-glucosidum, nec acidum D-galacturonicum. Lysinum assimilatur at non kalium nitricum, natrium nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 29–32 °C. Ad crescentiam acidum p-aminobenzoicum necessarium (varium) et thiaminum (varium) est. Materia amyloidea iodophila non formantur. Ureum hydrolysat. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 51.5–52.0 mol% (per HPLC). Ubiquinonum majus: Q-10(H₂). Xylosum in cellulis absens. Typus stirps OK-14^T ex folio Schima mertensiana, Japonia, isolata est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10326^T deposita est.

Description of *Sporobolomyces ogasawarensis* Hamamoto, Thanh & Nakase sp. nov.

Sporobolomyces ogasawarensis (o.ga.sa.war.en'sis. N.L. adj. *ogasawarensis* referring to Ogasawara, the isolated Japanese islands in the Pacific Ocean where the type strain was isolated).

After growth in YM broth (Difco) for 3 days at 25 °C, cells are spherical to ovoid (4.0–6.0 × 8.0–10.0 µm), single or in pairs (Fig. 4c). A sediment and an incomplete ring are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 days, streak culture is orange to salmon-red. After 1 month, streak culture is brownish orange, smooth, shining, butyrous and soft and has an entire margin. No true mycelium or pseudomycelium develops on Dalmau plate cultures on corn meal agar after 10 days at 25 °C. Ballistoconidia are formed on corn meal agar and YM agar. They are ovoidal and ellipsoidal, 3.0–6.0 × 6.0–14.0 µm (Fig. 4g). Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose (variable), trehalose, lactose (variable), melibiose (variable), raffinose, soluble starch, D-xylose, L-arabinose, D-arabinose (variable), L-rhamnose (variable), glycerol, ribitol (variable), galactitol (variable), D-mannitol, D-glucitol, salicin, glucono-δ-lactone, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid (variable), succinic acid, citric acid (variable), inositol (variable) and D-glucuronic acid. Does not assimilate inulin, D-ribose, ethanol, erythritol, methyl α-D-glucoside or D-galacturonic acid. Assimilates L-lysine. Does not assimilate nitrate, nitrite, ethylamine or cadaverine. Maximum growth temperature is 29–32 °C. Growth does not occur in vitamin-free medium. p-Aminobenzoic acid (variable) and thiamin (variable) are required for growth. Growth does not occur on 50 % (w/w) glucose/yeast extract agar. No starch-like substrate is produced. Xylose is absent from whole-cell hydrolysates. Urease and Diazonium blue B reactions are positive. The major ubiquinone is Q-10(H₂). The

G + C content of the nuclear DNA is 51.5–52.0 mol %, as determined by HPLC. The type strain is strain OK-14^T (= JCM 10326^T = CBS 9038^T).

Latin diagnosis of *Sporobolomyces syzygii* Hamamoto, Thanh & Nakase sp. nov.

In liquido YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (4.0–6.0 × 6.0–10.0 µm), singulae aut binae. Post 1 mensem pellicula imperfecta et sedimentum formantur. Cultura in agar YM post 1 mensem (25 °C), aurantiaca, glabra aut rugosa, butyracea et margine glabra. Pseudohyphae nullae. Ballistosporeae ovoideae vel ellipsoideae, 3.0–5.0 × 8.0–10.0 µm. Fermentatio nulla. Glucosum, galactosum, L-sorbose, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinose, melezitose, amylosum, D-xylosum, L-arabinosum, L-rhamnosum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, methyl α-D-glucosidum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, DL-lacticum, acidum succinicum, acidum citricum, et acidum D-glucuronicum assimilantur, at non lactosum, inulinum, D-arabinosum, D-ribosum, ethanolum, erythritolum, galactitolum, inositolum nec D-galacturonicum. Lysinum assimilatur at non kalium nitricum, natrium nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 29–30 °C. Ad crescentiam acidum p-aminobenzoicum et thiaminum necessarium est. Materia amyloidea iodophila non formantur. Ureum hydrolysat. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 48.9 mol % (per HPLC). Ubiquinonum majus: Q-10(H₂). Xylosum in cellulis absens. Typus stirps OK-227^T ex folio Syzygium buxifolium, Japonia, isolata est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10337^T deposita est.

Description of *Sporobolomyces syzygii* Hamamoto, Thanh & Nakase sp. nov.

Sporobolomyces syzygii [sy.zy'gi.i. N.L. gen. n. *syzygii* of *Syzygium* (*Syzygium buxifolium*), the plant from which the type strain was isolated].

After growth in YM broth (Difco) for 3 days at 25 °C, the cells are spherical to ovoid (4.0–6.0 × 6.0–10.0 µm), single or in pairs (Fig. 4d). A sediment and an imperfect ring are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 days, the streak culture is orange. After 1 month, the streak culture is brownish orange, smooth, shining, butyrous and soft and has an entire margin. No true mycelium or pseudomycelium develops on Dalmau plate cultures on corn meal agar after 10 days at 25 °C. Ballistoconidia are formed on corn meal agar and YM agar. They are ovoidal and ellipsoidal, 3.0–5.0 × 8.0–10.0 µm (Fig. 4h). Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, L-rhamnose, glycerol, ribitol, D-mannitol,

D-glucitol, methyl α-D-glucoside, salicin, glucono-δ-lactone, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, succinic acid, citric acid and D-glucuronic acid. Does not assimilate lactose, inulin, D-arabinose, D-ribose, ethanol, erythritol, galactitol, inositol or D-galacturonic acid. Assimilates L-lysine. Does not assimilate nitrate, nitrite, ethylamine or cadaverine. Maximum growth temperature is 29–30 °C. Growth does not occur in vitamin-free medium. *p*-Aminobenzoic acid and thiamin are required for growth. Growth does not occur on 50 % (w/w) glucose/yeast extract agar. No starch-like substrate is produced. Xylose is absent from whole-cell hydrolysates. Urease and Diazonium blue B reactions are positive. The major ubiquinone is Q-10(H₂). The G + C content of the nuclear DNA is 48.9 mol %, as determined by HPLC. The type strain is strain OK-227^T (= JCM 10337^T = CBS 9040^T).

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