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

Makiko Hamamoto, Vu Nguyen Thanh and Takashi Nakase

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(H$_2$) 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 bischofiae 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(H$_2$) 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(H$_2$), no additional basidiomycetous yeasts with the unique ubiquinone isopre-
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(H$_{10}$), 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(H$_{10}$) 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.

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(H$_{10}$) 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(H$_{10}$). 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.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolate no.</th>
<th>Source</th>
<th>G + C content (mol%)</th>
<th>Accession no.</th>
</tr>
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<tr>
<td><em>Bannoa hahajimensis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JCM 10333</td>
<td>OK-52</td>
<td>Dead leaves of <em>Bryophyllum</em></td>
<td>47.8</td>
<td>AB035894</td>
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<td><em>Rhaphiolepis wrightiana</em></td>
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<td>AB035895</td>
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<td>AB035896</td>
</tr>
<tr>
<td>JCM 10336&lt;sup&gt;T&lt;/sup&gt;</td>
<td>OK-248&lt;sup&gt;T&lt;/sup&gt;</td>
<td><em>Bryophyllum pinnatum</em></td>
<td>48.1</td>
<td>AB035897</td>
</tr>
<tr>
<td><em>Sporobolomyces bischofiae</em></td>
<td>OK-257&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Dead leaves of <em>Bischofia</em></td>
<td>49.6</td>
<td>AB035721</td>
</tr>
<tr>
<td>JCM 10338</td>
<td>OK-1</td>
<td>Dead leaves of <em>Bischofia</em></td>
<td>50.0</td>
<td>AB035712</td>
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<tr>
<td>JCM 10325</td>
<td>OK-14&lt;sup&gt;T&lt;/sup&gt;</td>
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<td>AB035713</td>
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<tr>
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<tr>
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<td>52.0</td>
<td>AB035719</td>
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<tr>
<td><em>Sporobolomyces syzygii</em></td>
<td>OK-227&lt;sup&gt;T&lt;/sup&gt;</td>
<td><em>Syzygium buxifolium</em></td>
<td>48.9</td>
<td>AB035720</td>
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<tr>
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<td>–</td>
<td>IFO 1058&lt;sup&gt;T&lt;/sup&gt;</td>
<td>50.0</td>
<td>AB036065</td>
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<td>JCM 1545&lt;sup&gt;T&lt;/sup&gt;</td>
<td></td>
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<td><em>Sporobolomyces elongatus</em></td>
<td>–</td>
<td>CBS 8080&lt;sup&gt;T&lt;/sup&gt;</td>
<td>55.0</td>
<td>AB036066</td>
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<td>JCM 5354&lt;sup&gt;T&lt;/sup&gt;</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>Sporobolomyces yunnanensis</em></td>
<td>–</td>
<td>CH 2.141&lt;sup&gt;T&lt;/sup&gt;</td>
<td>50.0*</td>
<td>AB030353</td>
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<tr>
<td>JCM 10687&lt;sup&gt;T&lt;/sup&gt;</td>
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</table>

* Data from Bai et al. (2001).

The 18S rDNA sequences of four isolates that were representative of each of the clusters from the ITS-based dendrogram, strains OK-14<sup>T</sup>, OK-227<sup>T</sup>, OK-248<sup>T</sup> and OK-257<sup>T</sup>, 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 hasegawanum*, *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 kluyverieli*, *Sporobolomyces oryzicola*, *Sporobolomyces phyllomatis*, *Sporobolomyces syzygii*.
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\textsuperscript{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\textsuperscript{T}, OK-227\textsuperscript{T} and OK-257\textsuperscript{T} and Erythrobasidium hasegawanum JCM 1545\textsuperscript{T}. Strain OK-14\textsuperscript{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\textsuperscript{T}, OK-248\textsuperscript{T} and OK-257\textsuperscript{T} and Erythrobasidium hasegawanum JCM 1545\textsuperscript{T}. Strains OK-227\textsuperscript{T} and OK-257\textsuperscript{T} showed low degrees of relative binding (15–49%) with all the other novel isolates, Erythrobasidium hasegawanum JCM 1545\textsuperscript{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\textsuperscript{T} and strains OK-219 and OK-248\textsuperscript{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 dacyroid, 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 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 infirmominiatum (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 Sporidiiales, Agaricostilbum/Bensingtonia, Erythrobasidium and subbrunneus lineages in the Urediniozymycetes. 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 basegavianum, which contains Q-10(H\(_2\)), 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 dacyroides, which also contains Q-10, produces teliospores that germinate to give two to four-celled basidium 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 Urediniozymycetes, 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 Rhodorula species (Rhodotorula armeniaca, Rhodotorula aurantiaca, Rhodotorula laryngis, Rhodotorula pallida, Rhodotorula marina and Rhodotorula sloofiae), 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 sloofiae clustered with Rhodotorula minuta (bootstrap value of 100\%), Rhodotorula armeniaca and Rhodotorula aurantiaca clustered with Sporobolomyces kluyveriinelli, Sporobolomyces phyllo maltis 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 bischofiae sp. nov., Sporobolomyces ogasawarenensis sp. nov. and Sporobolomyces syzygii sp. nov. As shown in Table 2, the seven species containing Q-10(H\(_2\)) in the Erythrobasidium lineage can also be distinguished by their phenotypic characteristics.

**Latin diagnosis of Bannoa Hamamoto gen. nov.**

*Genus ad Urediniozymycetes pertinens. Coloniae rubroaurantiaca. Cellulae sphaericae vel ovoideae singulae aut binae. Ballistosporae ovoideae vel ellipsoideae. Pseudomyxillum absens. Mycelium secundum copula-

http://ijs.sgmjournals.org
Table 2. Physiological and biochemical characteristics that differentiate species containing Q-10(H₂)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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<th>6</th>
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<td>Assimilation of:</td>
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<td></td>
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</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>v</td>
<td>+</td>
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<tr>
<td>D-Glucitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>-</td>
</tr>
<tr>
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<td>v</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>2-Ketogluconate</td>
<td>+</td>
<td>+</td>
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<td>-</td>
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<td>+</td>
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<tr>
<td>Nitrate</td>
<td>-</td>
<td>+</td>
<td>-</td>
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</tr>
<tr>
<td>L-Lysine</td>
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<tr>
<td>G+C content (mol%)</td>
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<td>55-0</td>
<td>51-5-52-0</td>
<td>48-9</td>
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Assimilation of: L-Arabinose, L-Rhamnose, D-Glucitol, Methyl α-d-glucoside, 2-Ketogluconate, Nitrate, L-Lysine.

G+C content (mol%) range from 47.6 to 50.0.

**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), cellulara 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.


**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), cellulara 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.


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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 × 7.0–140 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, celllobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, d-xylene, 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-keto-butyric acid, 5-keto-glucaric acid, δ-lacturonic acid (variable), succinic acid, citric acid and d-glucuronic acid. Does not assimilate lactose, inulin, erithritol, inositol or d-galacturonic acid. Assimilates l-lysin. 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 thiamine 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 47.6–48.1 mol%, as determined by HPLC. The type strain is strain OK-248T (= JCM 10336T = CBS 9039T).

**Latin diagnosis of Sporobolomyces bischofiae**
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 *agaro* YM *post 1 mensem* (25 °C), aurantiaca, glabra aut rugosa, butyrosa et marginé glabra. *Pseudohyphae* *nullae*. *Ballistoosporae* ovoideae vel ellipsoidae, 4.0–5.0 × 8.0–10.0 μm. *Fermentatio* *nulla*. *Glucosum*, *galactosum*, l-sorbosum, saccharosum, *maltosum*, celllobiosum, trehalosum, melibiosum, raffinosum, melezitosum, *amylosum*, d-xyluosum, glycerolium, ribitolum, d-mannitolum, d-glucitolum, salicinum, *aciddum* 2-keto-gluconicum, *aciddum* 5-keto-glucuronicum, dl-lacticum, *acidum* sucumnicum, *acidum* citricum et *aciddum* d-glucuronicum assimilantur, at non *lactosum*, inulimum, l-arabinosum, d-arabinosum, d-ribosum, l-rhamnorum, *ethanolum*, erithritolum, *galactitolum*, methyl α-D-glucosidum, *glucuronicum*, *inositosum* *aciddum* d-galacturonicum. Lysinum assimilatur at non *kalium* *nitricum*, natrium *nitrosum*, *ethylaminum* *aciddum* *cadaverinum*. *Maxima* *temperatura* *crescentiae*: 29–30 °C. Ad *crescentiiam* thiaminium *necessarium* est. *Materia* *amyloidea* *iodophila* non *formantur*. *Ureum* *hydrolysatur*. *Diazonium* *caeruleum* B: *positivum*. *Proprio* *molaris* guanini-i-cytosini *in* *acido* *deoxyribo-nucleinico*: 49.6 mol% (per HPLC). *Ubiquinonum* *majus*: Q-10(H₂). *Xylosum* *in* *cellulis* *absens*. *Typus* *stirps* OK-257T ex *folio* *Bischofia* *javanica*, *Japonia*,

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Fig. 4. Photomicrographs of vegetative cells on YM agar (a–d) and ballistoconidia on corn meal agar (e–h). (a, e) *Bannoa* *hahajimensis* OK-248T (= JCM 10336T). (b, f) *Sporobolomyces* *bischofiae* OK-257T (= JCM 10338T). (c, g) *Sporobolomyces* *ogasawarensis* OK-14T (= JCM 10326T). (d, h) *Sporobolomyces* *syzygii* OK-227T (= JCM 10337T). Bars, 10 μm.
isolata est. In collectionibus culturarium quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10338T deposita est.

Description of Sporobolomyces bischofiae
Hamamoto, Thanh & Nakase sp. nov.

Sporobolomyces bischofiae [bi.so.cho.i.a.e. N.L. gen. n. bischofiae 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 hydrophilic 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, 40–50 × 8.0–10.0 µm (Fig. 4f). Does not ferment d-glucose. Assimilates d-glucose, galactose, l-sorbose, sucrose, maltose, cellobiose, trehalose, melibiose, rafinose, 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-rihamnose, ethanol, erythritol, galactitol, methyl α-D-glucoside, glucono-δ-lactone, inositol or D-galacturonic acid. Assimilates L-lysin. 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/v) 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(H$_2$). The G + C content of the nuclear DNA is 49.6 mol%, as determined by HPLC. The type strain is strain OK-257T (= JCM 10338T = CBS 9041T).

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


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), rafinose, soluble starch, d-xylose, L-rihamnose, D-arabinose (variable), L-rihamnose (variable), glycerol, ribitol (variable), galactitol (variable), d-mannitol, d-glucitol, salicin, glucono-δ-lactone, 2-ketogluconic acid, 5-ketogluconic acid, 2-oxoglutaric acid, α-ketoglutaric 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-lysin. 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/v) 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(H$_2$). The
Latin diagnosis of Sporobolomyces syzygii
Hamamoto, Thanh & Nakase sp. nov.


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 (4–6–9 x 6–10–10 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. 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, 3–5 x 6–9 x 8–10–10 μm (Fig. 4h). They do not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xyllose, 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-Amino-benzoic 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-227⁷ (= JCM 10337⁷ = CBS 9040⁷).

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REFERENCES


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