Sporobolomyces fushanensis sp. nov., a new species of ballistoconidium-forming yeast in the Microbotryum lineage isolated from a plant in Taiwan

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Taiwan is located in the subtropical region of Asia and is assumed to be rich in biodiversity of yeasts, especially in its subtropical rain forests. However, only a few reports have been published on yeasts living in the phyllosphere of Taiwan (Nakase et al., 2002, 2003, 2004). In the course of a survey of yeasts in the phyllosphere of the Asian region, we found a yeast strain coinciding with the genus Sporobolomyces from a plant in Taiwan. Since this strain could not be assigned to any known species of Sporobolomyces, it is described in this paper as a new species of this genus.

Strain FK-5 used in this study was isolated from a leaf of Melastoma candidum D. Don collected in a subtropical rain forest in Fu-Shan Experimental Forest of Taiwan Forestry Research Institute, by an improved ballistoconidia-fall method (Nakase and Takashima, 1993). Most of its morphological, physiological and biochemical characteristics were examined according to Yarrow (1998). Maximum growth temperature was determined in YM broth using a metal block bath. Vitamin requirement was tested by the method of Komagata and Nakase (1967). Ubiquinones were isolated and identified according to the method of Nakase and Suzuki (1986). The DNA was isolated and purified according to Takashima and Nakase (2000). The DNA base composition (mol% G+C) was determined by nucleoside analysis using HPLC after digesting the DNA with Nuclease P1 and phosphatase as described by Tamaoka and Komagata (1984). Cellular xylose was analyzed by HPLC as described by Suzuki and Nakase (1988). The 18S rDNA and the D1/D2 region of 26S rDNA were directly sequenced using PCR products by ABI 310 sequencer with an ABI Prism BigDye Terminator Cycle Sequence kit (Applied Biosystems, Stafford, USA) as previously described (Nakase et al., 2002). The sequences determined in this study were deposited in the DDBJ database under following accession numbers: 18S rDNA, AB176530; D1/D2 domain of 26S rDNA, AB176591. The se-
quences were aligned with the computer program Clustal W or X, version 1.8 (Thompson et al., 1997). The evolutionary distances for the neighbor-joining method (Saitou and Nei, 1987) were calculated according to Kimura (1980). A bootstrap analysis was conducted with 1,000 replications (Felsenstein, 1985).

Strain FK-5 produced kidney-shaped or lunate ballistoconidia on agar media. The production of ballistoconidia is moderate on agar media but only a small number of ballistoconidia were found when they were collected on the glass slide probably due to their weak discharging ability. FK-5 lacked xylose in the whole cell hydrolysate and had Q-10 as the major component of ubiquinones. These characteristics coincide with the genus Sporobolomyces (Boekhout and Nakase, 1998).

In a phylogenetic tree constructed by the neighbor-joining method based on the 18S rDNA sequences, FK-5 was related to species of Microbotryum lineage of Scorzetti et al. (2002) and constituted a cluster with Sporobolomyces griseoflavus with high bootstrap confidence level (Fig. 1).

In a phylogenetic tree constructed by the neighbor-joining method based on the D1/D2 domain sequences of 26S rDNA, FK-5 constituted a cluster with S. griseoflavus though bootstrap confidence level was low (Fig. 2). Sporobolomyces griseoflavus is the near-

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**Fig. 1.** Phylogenetic tree for *Sporobolomyces fushanensis* FK-5 based on 18S rDNA sequences. The tree was constructed by the neighbor-joining method from the data set aligned on the 1,720 sites in 18S rDNA. The numerals represent the percentages from 1,000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated.

**Fig. 2.** Phylogenetic tree for *Sporobolomyces fushanensis* FK-5 based on D1/D2 domain of 26S rDNA sequences. The tree was constructed by the neighbor-joining method from the data set aligned on the 557 sites in the D1/D2 domain of 26S rDNA. The numerals represent the percentages from 1,000 replicate bootstrap resamplings. Sequences were retrieved from the DDBJ databases under the accession numbers indicated.
est species to FK-5 in the sequence similarity of D1/D2 domain; however, 23 nucleotides (3.9%) differ between the two yeasts. This clearly indicates the difference of the two yeasts at the species level. Therefore, FK-5 is described as *Sporobolomyces fushanensis* sp. nov.

**Description**

*Sporobolomyces fushanensis* Nakase, Lee & Takashima, sp. nov.


Holotypus: Stirps FK-5, isolatus ex folio *Melastoma candidum* D. Don, in pluvial sylva subtropica, Fu-Shan, Taiwan, cultura viva ex holotypo huius speciei conservatur in collectionibus culturarum in ‘Biore-source Collection and Research Center (BCRC), the Food Industry Research and Development Institute (FIRDI),’ Hsinchu, Formosa, ut BCRC 23025 in statu lyophilo sustentat.

Growth in YM broth: After 3 days at 25°C a ring, islets and a sediment are produced. Cells in the sediment are long ellipsoidal, cylindrical or elongate, 1–4.5×4–14 μm, single, in pairs, in chains, in clusters or in pseudomyclia (Fig. 3A). Cells in the ring and islets are bigger than those of sediment, ellipsoidal, long ellipsoidal, cylindrical or elongate, 3–5.5×5.5–12–20 μm, single, in pairs, usually in pseudomyclia (Fig. 3B). After 1 month at 20°C, an incomplete pellicle and a sediment are present.

Growth on YM agar: After 1 month at 20°C, the streak culture is brownish yellow to dark yellow, smooth to delicately wrinkled, dull, soft and has an entire margin.

Slide culture on corn meal agar: Primitive pseudomyclia are produced.

Ballistoconidium: Ballistoconidia are produced on agar media and the surface of liquid media. They are kidney-shaped or lunatae, 1.8–2.5×6.5–8 μm (Fig. 3, C–K).

Fermentation: Absent.

Assimilation of carbon compounds:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Assimilated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+ (latent)</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>–</td>
</tr>
</tbody>
</table>
Sucrose (+)  
Maltose (+) (may be latent)  
Cellulobiose (+) (latent)  
Trehalose (+)  
Lactose (−)  
Melibiose (−)  
Raffinose (−)  
Melezitose (+) (may be latent)  
Inulin (−)  
Soluble starch (−)  
D-Xylose (−)  
L-Arabinose (−)  
D-Arabinose (−)  
D-Ribose (−)  
L-Rhamnose (−)  
D-Glucosamine (−)  
N-Acetyl-D-glucosamine (−)  
Methanol (−)  
Ethanol (+) (latent)  
Glycerol (−)  
Erythritol (−)  
Ribitol (+) (latent)  
Galactitol (−)  
D-Mannitol (+) (may be latent)  
D-Glucitol (+) (latent)  
Xylitol (+) (latent & weak)  
L-Arabinose (−)  
α-Methyl-D-glucoside (−) or (+) (latent & weak)  
Salicin (−) or (+) (latent & weak)  
Glucono-δ-lactone (+) (latent) or (−)  
D-Gluconic acid (−)  
2-Ketogluconic acid (−)  
5-Ketogluconic acid (−)  
dl-Lactic acid (−)  
Succinic acid (+)  
Citric acid (+) (latent & weak)  
Saccharic acid (−)  
D-Glucuronic acid (−)  
D-Galacturonic acid (−)  
Inositol (−)  
Propane 1,2 diol (−)  
Butane 2,3 diol (−)  
Hexadecane (−)  
Vitamins required: Pyridoxine and thiamine.  
Production of starch-like substances: Negative.  
Growth on 50% (w/w) glucose-yeast extract agar: Negative.  
0.1% cycloheximide resistance: Negative.  
Maximum growth temperature: 27–28°C.  
Liquefaction of gelatin: Negative.  
Acid production on chalk agar: Negative.  
Diazonium blue B color reaction: Positive.  
Urease: Positive.  
Hydrolysis of fat: Negative.  
G+C content of nuclear DNA: 51.6 mol% (by HPLC).  
Major ubiquinone: Q-10.  
Xylose in the cells: Absent.  
Holotype: FK-5, isolated from a leaf of Melastoma candidum D. Don. collected in a subtropical rain forest in Fu-Shan Experimental Forest of Taiwan Forestry Research Institute, Taiwan, in May, 1997, is the holotype strain of this species. It was deposited at the Bioresource Collection and Research Center (BCRC), the Food Industry Research and Development Institute (FIRDI), Hsinchu 300, Taiwan, as BCRC 23025. The same strain was deposited also at the Japan Collection of Microorganisms (JCM), RIKEN, as JCM 12422.  
Etymology: The specific epithet “fushanensis” was derived from the place where this yeast was isolated.  
Sporobolomyces fushanensis is distinguished from S. griseoflavus, the phylogenetically closest species, by the ability to assimilate L-lysine, inability to assimilate nitrate and nitrite as sole sources of nitrogen, by the requirement of pyridoxine and low mol% G+C of 51.6% that is 9% lower than for S. griseoflavus. In the conventional taxonomic criteria, S. fushanensis resembles Rhodotorula auriculariae. However, this species is clearly distinguished from the latter by the inability to assimilate D-gluconic acid and saccharic acid as sole sources of carbon, the ability to assimilate L-lysine as a sole nitrogen source, and the lack of biotin requirement.

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References


