Sporobolomyces yunnanensis sp. nov., a Q-10(H2)-containing yeast species with a close phylogenetic relationship to Erythrobasidium hasegawianum

Feng-Yan Bai,1,2 Masako Takashima,2 Makiko Hamamoto2 and Takashi Nakase2

Author for correspondence: Feng-Yan Bai. Tel: +86 10 6255 5692. Fax: +86 10 6256 0912. e-mail: baify@sun.im.ac.cn

1 Systematic Mycology and Lichenology Laboratory, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China
2 Japan Collection of Microorganisms, RIKEN (Institute of Physical and Chemical Research), Wako, Saitama, 351-0198, Japan

A ballistoconidia-forming yeast strain, CH 2.141T, isolated from a semi-dried leaf sample collected in Yunnan, China, was found to have Q-10(H2) as its major ubiquinone. Molecular phylogenetic analysis based on the nucleotide sequences of small subunit (18S) rDNA and the internal transcribed spacer region (including 5-8S rDNA) indicated that the strain was closely related to the two described Q-10(H2)-containing yeast species, Erythrobasidium hasegawianum and Sporobolomyces elongatus, with a closer relationship to the former. A DNA–DNA reassociation experiment showed that strain CH 2.141T represents a new yeast species, for which the name Sporobolomyces yunnanensis sp. nov. is proposed.

Keywords: Sporobolomyces yunnanensis sp. nov., Erythrobasidium hasegawianum, Sporobolomyces elongatus, phylogeny

INTRODUCTION

In a survey focusing on ballistoconidia-forming yeasts living on the surface of plant materials collected from Yunnan, China, a Q-10(H2)-containing ballistoconidia-forming strain, CH 2.141T, was isolated. The strain was found to be physiologically and chemotaxonomically similar to the only two described Q-10(H2)-containing yeast species Erythrobasidium hasegawianum Hamamoto, Sugiyama & Komagata and Sporobolomyces elongatus Shivas & Rodrigues de Miranda. While molecular phylogenetic analysis revealed a close relationship of strain CH 2.141T with non-ballistoconidia-forming species, a DNA–DNA reassociation experiment with Erythrobasidium hasegawianum indicated that the Yunnan strain represents a distinct, previously undescribed yeast species.

METHODS

Strain. Strain CH 2.141T was isolated from a semi-dried leaf sample of Sapindus delavayi (Franch.) Radlk. collected in Yunnan Province, China, in October 1996 by using the improved ballistoconidia-fall method as described by Nakase & Takashima (1993).

Characteristics. Most of the morphological, physiological and biochemical characteristics were examined according to the standard methods commonly employed in yeast taxonomy (van der Walt & Yarrow, 1984). Assimilation of nitrogen compounds was investigated on solid medium with starved inoculum as described by Nakase & Suzuki (1986). Vitamin requirement tests were performed according to Komagata & Nakase (1967). Extraction, purification and identification of ubiquinones were carried out according to Nakase & Suzuki (1986). Xylose in the cell hydrolysate was analysed by HPLC as described by Suzuki & Nakase (1988).

Molecular methods. DNA isolation and purification, DNA base composition determination and DNA–DNA reassociation were performed according to Hamamoto & Nakase (1995). Sequencing and phylogenetic analysis of the small subunit rRNA gene (18S rDNA) and internal transcribed spacer (ITS) region (including 5-8S rDNA) were performed as described previously (Sugita & Nakase, 1999).

RESULTS

Conventional and chemotaxonomic investigation

Strain CH 2.141T forms pink-coloured colonies on YM agar and ellipsoidal or ovoid ballistoconidia on corn meal agar (Fig. 1b). Chemotaxonomic study of
Fig. 1. Sporobolomyces yunnanensis sp. nov. (a) Vegetative cells grown on YM agar for 3 d at 25 °C. (b) Ballistoconidia produced on corn meal agar after 7 d at 17 °C. Bars, 10 µm.

Table 1. Comparison of salient physiological characteristics of strain CH 2.141T with those of the two Q-10(H2)-containing species

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Assimilation of:</th>
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<tr>
<td></td>
<td></td>
<td>Soluble starch</td>
<td>R-Ribose</td>
</tr>
<tr>
<td>S. yunnanensis</td>
<td>CH 2.141T</td>
<td>W</td>
<td>–</td>
</tr>
<tr>
<td>E. hasegawianum</td>
<td>JCM 1545T</td>
<td>–</td>
<td>L</td>
</tr>
<tr>
<td>S. elongatus</td>
<td>JCM 5354T</td>
<td>–</td>
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+, Positive; –, negative; l, latent; w, weakly positive.

The strain showed that xylose is absent in the whole-cell hydrolysate and the major ubiquinone is Q-10(H2). Among the described basidiomycetous yeasts, only two species, *Sporobolomyces elongatus* and *Erythrobasidium hasegawianum*, have been reported to contain Q-10(H2) as the major ubiquinone. Strain CH 2.141T differed from each of the two described Q-10(H2)-containing species, however, in carbon and nitrogen compound assimilation patterns (Table 1).

**Molecular phylogenetic analysis and DNA–DNA relatedness**

Molecular phylogenetic relationships of strain CH 2.141T with the two Q-10(H2)-containing species mentioned above and with other related basidiomycetous yeast species were investigated based on 18S rDNA and ITS region (including 5.8S rDNA) sequences. Almost complete 18S rDNA of CH 2.141T was sequenced and 1777 bases were determined. The 18S rDNA sequences of *Erythrobasidium hasegawianum*, *Sporobolomyces elongatus* and the other reference xylose-lacking basidiomycetous species were obtained from nucleotide sequence databases. The selection of reference sequences was based on the recent phylogenetic analysis of *Sporobolomyces* and related taxa (Hamamoto & Nakase, 2000). Xylose-containing species, *Bulleromyces albus* and *Filobasidiella neoformans*, were used as an outgroup.

A phylogenetic tree was constructed by using the neighbour-joining method based on the alignment of the 18S rDNA sequences compared. The close relationships of the three Q-10(H2)-containing strains are shown in Fig. 2. Though *Erythrobasidium hasegawianum* is a teleomorphic and non-ballistoconidia-forming species, strain CH 2.141T was located close to this species, indicating that they are phylogenetically more closely related to each other than to the other Q-10(H2)-containing ballistoconidia-forming species, *Sporobolomyces elongatus*, which was clustered near to them on a sub-branch.

The ITS region sequence comparison between strain CH 2.141T and the type strains of *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus* also indicated that CH 2.141T is more closely related to the former than the latter. The ITS region sequence of CH 2.141T differs from that of *Erythrobasidium*...
Sporobolomyces yunnanensis sp. nov.

Fig. 2. Phylogenetic tree depicting the relationships of strain CH 2.141T with described Q-10(H2)-containing basidiomycetous yeast species and related taxa based on 18S rDNA sequences. The phylogram was constructed by neighbour-joining analysis. The numbers given on branches indicate the percentage of 1000 bootstrap replicates. Sequences were retrieved from GenBank under the accession numbers indicated.

<table>
<thead>
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<th>Table 2. DNA–DNA relatedness of strain CH 2.141T with closely related species</th>
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<tr>
<td><strong>Species</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Sporobolomyces yunnanensis</td>
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<tr>
<td>Erythrobasidium hasegawianum</td>
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<td>Sporobolomyces elongatus</td>
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</table>

DISCUSSION

In agreement with the close phylogenetic relationship of *Sporobolomyces yunnanensis* sp. nov. with *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus*, the former is phenotypically similar to the latter two Q-10(H2)-containing species. In practice, however, the new species can be easily distinguished. As shown in Table 1, *Sporobolomyces yunnanensis* differs from *Erythrobasidium hasegawianum* in the assimilation reactions of soluble starch, D-ribose, ethanol, glucitol, lactate, ethylamine, L-lysine and cadaverine. The new species also differs from *Sporobolomyces elongatus* in even more physiological characters (Table 1). The G + C content of *Sporobolomyces yunnanensis* is similar to that of *Erythrobasidium hasegawianum* (50.0 mol%), but significantly different from that of *Sporobolomyces elongatus* (55.0 mol%).

The teleomorphic and non-ballistoconidia-forming basidiomycetous yeast genus *Erythrobasidium* includes
a single species, *Erythrobasidium hasegawianum*. This species was established by Hamamoto et al. (1988, 1991) for the type strain of *Rhodotorula hasegawaiae* Yamada & Komagata (1983) based on its Q-10(H$_2$) ubiquinone system and the discovery of its sexual state. Only one strain (IFO 1058$^T$ = JCM 1545$^T$) originally isolated from an old culture of beer yeast has been assigned to this species so far.

Since strain CH 2.141$^T$ was shown to be closely related to *Erythrobasidium hasegawianum* by molecular phylogenetic analysis, we have tried to induce the sexual state of this strain. The type strain of *Erythrobasidium hasegawianum* is homothallic and forms mycelia within 2 months. However, neither mycelia nor basidium-like structures were observed to occur in strain CH 2.141$^T$ after 3 months at 20–25°C and, subsequently, basidia and basidiospores within 2 months. However, neither mycelia nor basidium-like structures were observed to occur in strain CH 2.141$^T$ after 3 months at 20–25°C on corn meal agar and other media, including yeast carbon base agar and yeast nitrogen base agar with 1% malt extract-agar and other media, including yeast carbon base agar and yeast nitrogen base agar with 1% malt extract.

**Latin diagnosis of *Sporobolomyces yunnanensis* sp. nov.**

In YM (Difco) liquido post dies 3 ad 25 °C, cellulae vegetativae ovoidae vel ellipsoidae (2–0×4.0–4.8 μm), singulae aut binae. Annulus et sedimento formatur. Post unum mensem ad 17 °C, annulus, pellliculum et sedimento formatur. In agaro YM post unum mensem ad 17 °C, cultura rubro-aurantia, glabra, nitida et marginis glabra. Mycelium et pseudomycelium non formatur. Ballistospores ovoidae vel ellipsoidae (2–0×2.2×8.0–9.0 μm). Fermentatio nulla. Glucosum, galactosum (exigne), l-sorbosum (exigne), saccharosum, maltosum (lente), cellobiosum (tarde), trehalosum, melezitosum, amyllum solubile (tarde), d-xylsum, l-arabinosum, d-arabinosum (exigne), ethanolum (lente), glycerolum, d-mannitolum, ribitolum (exigne), salicium (tarde), glucono-δ-lactonum, acidum 2-ketoglucuronicum, acidum 5-ketoglucuronicum (exigne), acidum succinium et acidum citricum (exigne) assimilantur at non lactosum, melibiosum, raaffosum, inulin, d-ribosum, l-rhamnosum, erithritolum, galactitolum, glucitolum, methyl δ-g-glucosidum, acidum δl-lacticum, inositolum, acidum δ-glucuronicum nec acidum δ-galacturonicum. Ammonium sulfatulm, kalium nitricum et natrium nitrosum assimilantur at non ethylaminum, cadaverinum nec l-lysinum. Ad crescentiam thiaminum necessarium sunt. Maxima temperatura crescienae: 28–29 °C. Materia amyloidea iodophila non formatur. Urea finditur. Diazonium blue B reaction is positive. The G+C content of nuclear DNA is 50.0 mol% as determined by HPLC. The major ubiquinone is Q-10(H$_2$). Xylose is absent in the whole-cell hydrolysate.

The type strain of *Sporobolomyces yunnanensis*, CH 2.141$^T$, was isolated in 1996 from a semi-dried leaf of *Sapindus delavayi* (Franch.) Radlk. collected in Yunnan, China. This strain has been deposited in the China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China, as AS 2.2090$^T$ and in the Japan Collection of Microorganisms, Wako, Saitama, Japan, as JCM 10687$^T$.

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