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

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A ballistoconidia-forming yeast strain, CH 2.141^T, 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 2.141^T 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 2.141^T, 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 2.141^T 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 2.141^T 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 2.141^T forms pink-coloured colonies on YM agar and ellipsoidal or ovoid ballistoconidia on corn meal agar (Fig. 1b). Chemotaxonomic study of

Abbreviation: ITS, internal transcribed spacer.

The GenBank accession number for the 18S rDNA sequence of strain CH 2.141^T is AF229176, and the accession numbers for the ITS region sequences (including 5-8S rDNA) of strains JCM 5354^T, JCM 1545^T and CH 2.141^T are AB030344, AB030352 and AB030353, respectively.

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 2.141^T with those of the two Q-10(H₂)-containing species

+, Positive; –, negative; L, latent; w, weakly positive.

Species	Strain	Assimilation of:													
		Soluble starch	D-Ribose	Rhamnose	Ethanol	Ribitol	Glucitol	2-Ketogluconate	Lactate	Citrate	Nitrate	Nitrite	Ethylamine	L-Lysine	Cadaverine
<i>S. yunnanensis</i>	CH 2.141 ^T	w	–	–	L	w	–	+	–	w	+	+	–	–	–
<i>E. hasegawianum</i>	JCM 1545 ^T	–	L	–	–	+	+	+	L	+	+	+	+	+	+
<i>S. elongatus</i>	JCM 5354 ^T	–	–	+	–	–	+	–	–	–	–	–	–	–	–

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

Fig. 2. Phylogenetic tree depicting the relationships of strain CH 2.141^T 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 2. DNA–DNA relatedness of strain CH 2.141^T with closely related species

Species	Strain	G + C (mol %)	Relative binding of DNA (%) from:	
			CH 2.141 ^T	JCM 1545 ^T
<i>Sporobolomyces yunnanensis</i>	CH 2.141 ^T	50.0	100	34
<i>Erythrobasidium hasegawianum</i>	JCM 1545 ^T	50.0	41	100
<i>Sporobolomyces elongatus</i>	JCM 5354 ^T	55.0	12	12

hasegawianum in only 6 nt (3 in the ITS 1 region and 3 in ITS 2), but differs from that of *Sporobolomyces elongatus* in as many as 69 nt (20 in ITS 1, 1 in the 5-8S region and 48 in ITS 2).

Taxonomic relationships between strain CH 2.141^T with *Erythrobasidium hasegawianum* and *Sporobolomyces elongatus* were further investigated by DNA–DNA reassociation. The complementarity values of CH 2.141^T with the type strains of the two Q-10(H₂)-containing species are shown in Table 2. In agreement with the results of phylogenetic analysis based on the 18S rDNA and ITS region sequences, the level of DNA complementarity between CH 2.141^T and *Erythrobasidium hasegawianum* is higher than that between CH 2.141^T and *Sporobolomyces elongatus*. However, the low degree of DNA homology (34–41 %) between CH 2.141^T and *Erythrobasidium hasegawianum* indicates that CH 2.141^T represents a distinct species from *Erythrobasidium hasegawianum*. A new species, *Sporobolomyces yunnanensis*, is therefore proposed.

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 hasegawae* Yamada & Komagata (1983) based on its Q-10(H₂) 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 with clamp connection within 2 weeks on corn meal agar 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% glucitol. Therefore, an anamorphic species name in the genus *Sporobolomyces* was proposed for CH 2.141^T, according to the current taxonomic system of basidiomycetous yeasts (Boekhout & Nakase, 1998; Boekhout *et al.*, 1998).

Latin diagnosis of *Sporobolomyces yunnanensis* sp. nov.

In YM (Difco) liquido post dies 3 ad 25 °C, cellulae vegetativae ovoideae vel ellipsoideae (2.0–4.0 × 4.0–4.8 µm), singulae aut binae. Annulus et sedimentum formatur. Post unum mensem ad 17 °C, anulus, pelliculum et sedimentum formantur. In agar YM post unum mensem ad 17 °C, cultura rubro-aurantiaca, glabra, nitida et margine glabra. Mycelium et pseudomycelium non formantur. Ballistospores ovoideae vel ellipsoideae (2.0–2.2 × 8.0–9.0 µm). Fermentatio nulla. Glucosum, galactosum (exigue), L-sorbose (exigue), saccharosum, maltosum (lente), cellobiosum (tarde), trehalosum, melezitose, amyllum solubile (tarde), D-xylosum, L-arabiosum, D-arabiosum (exigue), ethanolum (lente), glycerolum, D-mannitolum, ribitolum (exigue), salicinum (tarde), glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum (exigue), acidum succinicum et acidum citricum (exigue) assimilantur at non lactosum, melibiosum, raffinose, inulin, D-ribosum, L-rhamnosum, erythritolum, galactitolum, glucitolum, methyl α-D-glucosidum, acidum DL-lacticum, inositolum, acidum D-glucuronicum nec acidum D-galacturonicum. Ammonium sulfatum, kalium nitricum et natrum nitrosum assmilantur at non ethylaminum, cadaverinum nec L-lysinum. Ad crescentiam thiaminum necessarium sunt. Maxima temperatura crescentiae: 28–29 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 50.0 mol% (per HPLC). Ubiquinonum majus: Q-10(H₂). Xylosum in cellulis absens. Typus: isolatus ex folio *Sapindus delavayi* JCM 10687^T

(originaliter ut CH 2.141^T) conservatur in collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama, sustentat.

Description of *Sporobolomyces yunnanensis* sp. nov.

Sporobolomyces yunnanensis (yun.nan.en'sis. N.L. adj. *yunnanensis* referring to the geographic origin of the species).

In YM broth, after 3 d at 25 °C, the cells are ovoid to ellipsoidal (2.0–4.0 × 4.0–8.0 µm; Fig. 1a). A ring and sediment is formed. After 1 month at 17 °C, a ring, pellicle and sediment are present. On YM agar, after 3 d at 25 °C, the streak culture is smooth and glistening with an orange to orange-red colour. After 1 month at 17 °C, the culture is orange-red with an entire margin. Mycelia and pseudomycelia are not formed on Dalmau plate culture on corn meal agar. On corn meal agar, ballistoconidia are formed on short sterigmata, ellipsoidal or ovoid, (2.0–2.2 × 8.0–9.0 µm; Fig. 1b).

Glucose is not fermented. The following carbon compounds are assimilated: glucose, galactose (weak), L-sorbose (weak), sucrose, maltose (latent), cellobiose (slow), trehalose, melezitose, soluble starch (slow), D-xylose, L-arabinose, D-arabinose (weak), ethanol (latent), glycerol, ribitol (weak), D-mannitol, salicin (slow), glucono-δ-lactone, 2-ketogluconic acid, 5-ketogluconic acid (weak), succinic acid, citric acid (weak). The following are not assimilated: lactose, melibiose, raffinose, inulin, D-ribose, L-rhamnose, erythritol, galactitol, glucitol, methyl α-D-glucoside, DL-lactic acid, inositol, D-glucuronic acid and D-galacturonic acid. KNO₃ and NaNO₂ are utilized as sole sources of nitrogen; ethylamine, cadaverine and L-lysine are not utilized. Thiamine is required for growth. Maximum growth temperature is 28–29 °C. Growth on 50% (w/w) glucose yeast extract agar is negative. Starch-like compounds are not produced. Urease activity is positive. 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(H₂). 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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