Phylogenetic analysis of the ballistoconidium-forming yeast genus *Sporobolomyces* based on 18S rDNA sequences

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The 18S rDNA nucleotide sequences of 25 *Sporobolomyces* species and five *Sporidiobolus* species were determined. Those of *Sporobolomyces dimmenae* JCM 8762^T, *Sporobolomyces ruber* JCM 6884^T, *Sporobolomyces sasicola* JCM 5979^T and *Sporobolomyces taupoensis* JCM 8770^T showed the presence of intron-like regions with lengths of 1586, 324, 322 and 293 nucleotides, respectively, which were presumed to be group I introns. A total of 63 18S rDNA nucleotide sequences was analysed, including 33 published reference sequences. *Sporobolomyces* species and the other basidiomycetes species were distributed throughout the phylogenetic tree. The resulting phylogeny indicated that *Sporobolomyces* is polyphyletic. *Sporobolomyces* species were mainly divided into four groups within the Urediniomycetes. The groups are designated as the Sporidiales, *Agaricostilbum/Bensingtonia*, *Erythrobasidium* and subbrunneus clusters. The last group, comprising four species, *Sporobolomyces coprosmicola*, *Sporobolomyces dimmenae*, *Sporobolomyces linderae* and *Sporobolomyces subbrunneus*, forms a new and distinct cluster in the phylogenetic tree in this study.

Keywords: ballistoconidium-forming yeasts, phylogeny, rDNA, *Sporobolomyces*

INTRODUCTION

Members of the genus *Sporobolomyces* (Kluyver & van Niel, 1924) are anamorphic basidiomycetous yeasts, which are characterized by the formation of ballistoconidia, the absence of xylose in whole-cell hydrolysates, the presence of Q-10 or Q-10(H₂) as the major ubiquinone isoprenologue, the inability to ferment sugars, and positive diazonium blue B (DBB) and urease reactions (Boekhout & Nakase, 1998b). The genus consists of 21 recognized species that are physiologically and biochemically distinguished (Boekhout, 1991; Boekhout & Nakase, 1998b). The teleomorphic state of this genus is known as *Sporidiobolus*, in which four species are accepted (Statzell-Tallman & Fell, 1998b). This genus is characterized by the formation of teliospores in addition to the formation of ballistoconidia (Boekhout, 1991; Statzell-Tallman & Fell, 1998b).

Nakase *et al.* (1993, 1995) determined partial 18S rRNA sequences (positions 1451–1618 in *Saccharo-*

myces cerevisiae) of 49 ballistoconidium-forming yeasts and related non-ballistoconidium-forming yeasts. This work indicated that *Sporobolomyces* species were widely dispersed on the phylogenetic tree. Fell *et al.* (1992, 1995) analysed partial 26S rDNA sequences of basidiomycetous yeasts and similarly showed *Sporobolomyces* species to be polyphyletic.

In this paper, we report the phylogenetic analysis of the genus *Sporobolomyces* and related taxa based on 18S rDNA sequences.

METHODS

Strains investigated. The strains used in this study shown in Table 1 were obtained from JCM (Japan Collection of Microorganisms). All strains were grown at 17 or 25 °C in YM agar (Difco Laboratories), prior to extraction of nuclear DNA.

Nucleotide sequence analyses. The DNA extraction procedure was as follows. One loop of cells was suspended in an equal volume of extraction buffer (200 mM Tris/HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5%, w/v, SDS) and 1 spoonful aluminium oxide (50–70 mg). The suspended cells were disrupted with a pestle (Kontes) on ice for 1–2 min. An equal volume of phenol saturated with TE buffer (10 mM

The DDBJ accession numbers for the 18S rDNA sequences reported in this paper are given in Table 1.

Table 1. Strains used in this study

Species	Strain*	DDBJ/EMBL/GenBank accession no.
<i>Agaricostilbum hyphaenes</i>	RJB 75-661-A9	U40809
<i>Bensingtonia ciliata</i>	JCM 6865 ^T	D38233
<i>Bensingtonia ingoldii</i>	JCM 7445 ^T	D38234
<i>Bensingtonia intermedia</i>	JCM 5291 ^T	D38235
<i>Bensingtonia miscanthi</i>	JCM 5733 ^T	D38236
<i>Bensingtonia musae</i>	JCM 8801 ^T	D43946
<i>Bensingtonia naganoensis</i>	JCM 5978 ^T	D38366
<i>Bensingtonia phyllada</i>	JCM 7476 ^T	D38237
<i>Bensingtonia subrosea</i>	JCM 5735 ^T	D38238
<i>Bensingtonia yamatoana</i>	JCM 2896 ^T	D38239
<i>Bensingtonia yuccicola</i>	JCM 6251 ^T	D38367
<i>Bulleromyces albus</i>	MUCL 30301 ^T	X60179
<i>Chionosphaera apobasidialis</i>	ATCC 52639	U77662
<i>Cronartium ribicola</i>	Unknown	M94338
<i>Erythrobasidium hasegawianum</i>	IAM 12911 ^T	D12803
<i>Gymnoconia nitens</i>	Unknown	U41565
<i>Heterogastridium pycnidioideum</i>	RJB 75-845L	U41567
<i>Kondoa malvinella</i>	IAM 13523 ^T	D13776
<i>Kurtzmanomyces nectairei</i>	JCM 6906 ^T	D64122
<i>Leucosporidium scottii</i>	MUCL 28629 ^T	X53499
<i>Mixia osmundae</i>	IFO 32408 ^T	D14163
<i>Nyssopsora echinata</i>	Unknown	U77061
<i>Rhodosporeidium dacryoideum</i>	IAM 13522 ^T	D13459
<i>Rhodosporeidium toruloides</i>	IAM 13469 ^T	D12806
<i>Rhodotorula glutinis</i>	MUCL 30253	X69853
<i>Rhodotorula graminis</i>	NCYC 502 ^T	X83827
<i>Rhodotorula lactosa</i>	JCM 1546 ^T	D45366
<i>Rhodotorula minuta</i>	JCM 3777 ^T	D45367
<i>Rhodotorula mucilaginosa</i>	NCYC 63 ^T	X84326
<i>Sporidiobolus johnsonii</i>	KW41.1 ^T	L22261
<i>Sporidiobolus microsporus</i>	JCM 6882 ^T	AB021693†
<i>Sporidiobolus pararoseus</i>	JCM 5350	AB021694†
<i>Sporidiobolus ruineniae</i> var. <i>coprophilus</i>	JCM 8097	AB021695†
<i>Sporidiobolus ruineniae</i> var. <i>ruineniae</i>	JCM 1839 ^T	AB021696†
<i>Sporidiobolus salmonicolor</i>	JCM 1841 ^T	AB021697†
<i>Sporobolomyces alborubescens</i>	JCM 5352 ^T	AB021668†
<i>Sporobolomyces coprosmae</i>	JCM 8772 ^T	D66880†
<i>Sporobolomyces coprosmicola</i>	JCM 8767 ^T	D66879†
<i>Sporobolomyces dimmenae</i>	JCM 8762 ^T	D66881†
<i>Sporobolomyces dracophyllus</i>	JCM 8773 ^T	D66882†
<i>Sporobolomyces elongatus</i>	JCM 5354 ^T	AB021669†
<i>Sporobolomyces falcatus</i>	JCM 6838 ^T	AB021670†
<i>Sporobolomyces foliicola</i>	JCM 5355 ^T	AB021671†
<i>Sporobolomyces gracilis</i>	JCM 2963 ^T	D66883†
<i>Sporobolomyces griseoflavus</i>	JCM 5653 ^T	D66884†
<i>Sporobolomyces holsaticus</i>	JCM 5296	AB021672†
<i>Sporobolomyces inositophilus</i>	JCM 5654 ^T	AB021673†
<i>Sporobolomyces kluyveri</i>	JCM 6356 ^T	AB021674†
<i>Sporobolomyces lactophilus</i>	JCM 7595 ^T	AB021675†
<i>Sporobolomyces linderiae</i>	JCM 8856 ^T	D66885†
<i>Sporobolomyces oryzae</i>	JCM 5299 ^T	AB021677†
<i>Sporobolomyces phyllomatis</i>	JCM 7549 ^T	AB021685†
<i>Sporobolomyces roseus</i>	MUCL 30251 ^T	X60181
<i>Sporobolomyces ruber</i>	JCM 6884 ^T	AB021686†

Table 1 (cont.)

Species	Strain*	DDBJ/EMBL/GenBank accession no.
<i>Sporobolomyces salicinus</i>	JCM 2959 ^T	AB021687†
<i>Sporobolomyces sasicola</i>	JCM 5979 ^T	AB021688†
<i>Sporobolomyces shibatanus</i>	JCM 5732	AB021689†
<i>Sporobolomyces singularis</i>	JCM 5356 ^T	AB021690†
<i>Sporobolomyces subbrunneus</i>	JCM 5278 ^T	AB021691†
<i>Sporobolomyces taupoensis</i>	JCM 8770 ^T	D66886†
<i>Sporobolomyces tsugae</i>	JCM 2960 ^T	AB021692†
<i>Sporobolomyces xanthus</i>	JCM 6885 ^T	D64118
<i>Sterigmatomyces halophilus</i>	JCM 6905 ^T	D64119

* IAM, IAM Culture Collection, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; KW, Dr K. Wells; MUCL, Mycotheque de l'Université Catholique, Louvain-la Neuve, Belgium; RJB, Dr R. J. Bandoni, University of British Columbia, Canada.

† Sequence determined in this study.

Tris/HCl pH 7.6, 1 mM EDTA pH 8.0)/chloroform (1:1, v/v) was added to the broken-cell suspension and mixed well. Following centrifugation, the aqueous upper layer was transferred to a new microtube. Nucleic acids were precipitated from the aqueous phase with 0.1 vol. 3 M sodium acetate pH 5.2 and 2 vols 2-propanol kept at -80°C for 10 min. Nucleic acids were recovered by centrifugation.

The primers used for the amplification and sequencing of 18S-rRNA-encoding genes were those described by Suh & Nakase (1995) and Suh *et al.* (1996b). 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.

The 30 18S rDNA sequences which we determined were aligned with the sequences of 33 other basidiomycetes retrieved from the GenBank and DDBJ libraries by using CLUSTAL W 1.75 (Thompson *et al.*, 1994) and were manually adjusted. *Bulleromyces albus* was the designated outgroup. Evolutionary distances were calculated using the PHYLIP 3.57c program DNADIST (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 AND DISCUSSION

Long insertions in the 18S rDNA were found in *Sporobolomyces dimmenae* JCM 8762^T (1586 nucleotides), *Sporobolomyces ruber* JCM 6884^T (324 nucleotides), *Sporobolomyces sasicola* JCM 5979^T (322 nucleotides) and *Sporobolomyces taupoensis* JCM 8770^T (293 nucleotides), and these were presumed to

be introns. The long insertions in *Sporobolomyces dimmenae* exist as seven regions whose lengths were 221, 212, 269, 217, 209, 219 and 239 nucleotides, respectively. The inserted regions of *Sporobolomyces ruber* and *Sporobolomyces sasicola* are considered to be group I introns, because they had conserved sequence elements P, Q, R and S (Cech, 1988). Those of *Sporobolomyces dimmenae* and *Sporobolomyces taupoensis* were also presumed to be group I introns because they start next to thymine (T) and finish at guanine (G) (Cech, 1988). However, the conserved regions of group I introns, P, Q, R and S could not be found in the intron-like regions of *Sporobolomyces dimmenae* and *Sporobolomyces taupoensis*. Further studies are needed on the secondary structure to evaluate these regions.

Almost complete 18S rDNA sequences (1749–1807 nucleotides; including introns, 2045–3377 nucleotides) were determined for 25 *Sporobolomyces* species and 5 *Sporidiobolus* species. The sequence data for these 30 fungi were aligned, together with 33 sets of published data. A total of 1424 nucleotides present in all species between position 48 and position 1682 (in *Saccharomyces cerevisiae*) excluding introns, was used for analysis. *Sporobolomyces* species and the other basidiomycetes species were distributed throughout the phylogenetic tree (Fig. 1). Twenty-seven species of the genus *Sporobolomyces* were distributed into four major clusters in the Urediniomycetes. These results indicate that *Sporobolomyces* is a polyphyletic genus and do not conflict with those of Fell *et al.* (1992, 1995) and Nakase *et al.* (1993, 1995).

The Urediniomycetes is composed of four major lineages, the Sporidiales, *Agaricostilbum*, *Erythrobasidium*/*Naohidea* and Uredinales clades, based on 18S rRNA gene sequences (Swann & Taylor, 1995a,

Fig. 1. Phylogenetic relationships of *Sporobolomyces* species and related taxa based on 18S rDNA or 18S rRNA sequence comparison. The branching pattern was generated by the neighbour-joining method as described in the text. The numbers on the tree indicate bootstrap values greater than 50%.

b). In our analysis, 23 out of 27 *Sporobolomyces* species used were placed in the Sporidiales, *Agaricostilbum*/*Bensingtonia* and *Erythrobasidium* clusters, which corresponded to those of Swann & Taylor (1995a, b). The other four *Sporobolomyces* species formed a new lineage in our phylogenetic tree. This is designated here as the subbrunneus cluster for convenience. These results accordingly show that the Urediniomycetes is composed of five rather than four major lineages (Fig. 1).

Species within the Sporidiales cluster

Nine *Sporobolomyces* species, *Sporobolomyces alborubescens*, *Sporobolomyces falcatus*, *Sporobolomyces griseoflavus*, *Sporobolomyces holsaticus*, *Sporobolomyces inositophilus*, *Sporobolomyces roseus* (the type species of the genus *Sporobolomyces*), *Sporobolomyces shibatanus*, *Sporobolomyces singularis* and *Sporobolomyces tsugae*, were included in the Sporidiales cluster, together with six *Sporidiobolus* species, *Bensingtonia yamatoana*, *Leucosporidium scottii*, *Mastigobasidium intermedium*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Rhodotorula graminis*, *Rhodotorula mucilaginosa* and *Heterogastridium pycnidioideum* (Fig. 1).

Sporobolomyces holsaticus and *Sporidiobolus salmonicolor* are suggested to be synonyms of *Sporidiobolus johnsonii* based on the high DNA complementarity values (Boekhout, 1991). In *The Yeasts* (Statzell-Tallman & Fell, 1998b), *Sporobolomyces holsaticus* is recognized as a synonym of *Sporidiobolus johnsonii* based on phenotypic properties and partial 26S rRNA sequences (Boekhout, 1991; Yamazaki & Komagata, 1983; Fell *et al.*, 1992). However, *Sporidiobolus salmonicolor* is not recognized as a synonym of *Sporidiobolus johnsonii* because of the presence of five nucleotide differences in partial 26S rRNA sequences (Fell *et al.*, 1992). In our phylogenetic tree, *Sporidiobolus johnsonii* and *Sporobolomyces holsaticus* formed a coherent cluster with *Sporidiobolus salmonicolor* supported by a high bootstrap value (100%) (Fig. 1), indicating that these three species have a close genealogical relationship.

Sporobolomyces roseus is suggested to be closely related to *Sporobolomyces shibatanus*, which is recognized as a synonym of *Sporidiobolus pararoseus* (Statzell-Tallman & Fell, 1998b) based on phenotypic characteristics and partial 18S rRNA sequences (Boekhout, 1991). Our phylogenetic tree showed that these three species formed a coherent cluster statistically well-supported (bootstrap = 90%) (Fig. 1), confirming that *Sporobolomyces roseus* is closely related to *Sporobolomyces shibatanus* and *Sporidiobolus pararoseus*.

Sporobolomyces falcatus, *Sporobolomyces griseoflavus*, *Sporobolomyces inositophilus*, *Sporobolomyces singularis* and *Sporobolomyces tsugae* are named the 'singularis' group in the genus *Sporobolomyces*

(Nakase, 1989), characterized by pale-coloured colonies and assimilation of 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucuronate and D-galacturonate as a sole source of carbon. These five species formed a cluster with *B. yamatoana*, *L. scottii*, *M. intermedium* and *H. pycnidioideum* (Fig. 1), again not conflicting with the results of Fell *et al.* (1992, 1995). *B. yamatoana*, *L. scottii* and *M. intermedium* are similar to the *Sporobolomyces* species in the 'singularis' group with regard to their colony colour and physiological properties but not to the major ubiquinone isoprenologue (Boekhout & Nakase, 1998a; Statzell-Tallman & Fell, 1998a; Golubev, 1999). *L. scottii*, *M. intermedium* and *H. pycnidioideum* are morphologically divergent in their teleomorphic forms in that *L. scottii* (Statzell-Tallman & Fell, 1998a) and *M. intermedium* (Golubev, 1999) produce teliospores, whereas *H. pycnidioideum* produces a pycnidoid basidiocarp, gasteroid-auricularioid basidia, single-celled conidia on conidiophores and endospores in hyphae (Oberwinkler *et al.*, 1990). Because the relationship between the *Sporobolomyces* species in the 'singularis' group and related taxa are not statistically well-supported, further studies using other gene sequences are needed.

Sporobolomyces alborubescens clustered with *Rhodotorula mucilaginosa*, *Rhodotorula glutinis*, *Rhodotorula graminis* and *Rhodospiridium toruloides* in our phylogenetic tree (Fig. 1). A close relationship between *Sporobolomyces alborubescens* and *Rhodotorula mucilaginosa* is suggested based on phenotypic characteristics (Boekhout, 1991; Statzell-Tallman & Fell, 1998b) and partial 26S rRNA sequences (Fell *et al.*, 1992). Our phylogenetic analysis supported such a relationship by a high bootstrap value (99%).

Sporidiobolus ruineniae var. *coprophilus* and *Sporidiobolus microsporus* have been considered synonyms of *Sporidiobolus ruineniae* var. *ruineniae* based on similar physiological and morphological properties (Boekhout, 1991). The placement of *Sporidiobolus ruineniae* var. *coprophilus* is recognized to be reasonable based on a similar DNA base composition, intermediate levels of DNA complementarity (Kurtzman & Fell, 1991) and an identical 26S rRNA sequence (Fell *et al.*, 1992; Statzell-Tallman & Fell, 1998b). Recently, Fell *et al.* (1998) reported the validation of *Sporidiobolus microsporus* based on a partial 26S rDNA sequence. In our phylogenetic tree (Fig. 1), these three species formed a distinct cluster supported by a high bootstrap value (100%), confirming that they had a phylogenetically close relationship.

A close taxonomic relationship has been suggested for *Sporobolomyces* and *Rhodotorula* by various studies (Janke, 1954; Crook & Johnson, 1962; Tsuchiya *et al.*, 1969; Weijman & Rodrigues de Miranda, 1988; Golubev, 1989a, b; Dörfler, 1990; Fell *et al.*, 1992). The present study also confirmed the close relationship between these genera. In practice, it is

impossible to distinguish *Sporobolomyces* from *Rhodotorula* morphologically if the former loses the ability to form ballistoconidia. The ability to form ballistoconidia is, however, not adequate as a generic criterion from an evolutionary point of view, although this property is still used for generic separation for practical reasons (Boekhout & Nakase, 1998b). As our analysis was limited by the number of available strains of *Rhodotorula* species, we suggest further studies to define the generic concept for basidiomycetous yeasts based on molecular biological criteria, including *B. yamatoana*, *H. pycnidioideum*, *L. scottii*, *M. intermedium*, *Rhodospiridium toruloides*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa* and *Rhodotorula graminis*.

Species within the *Agaricostilbum/Bensingtonia* cluster

Six *Sporobolomyces* species, *Sporobolomyces lactophilus*, *Sporobolomyces ruber*, *Sporobolomyces sasicola*, *Sporobolomyces taupoensis* and *Sporobolomyces xanthus*, were placed in the *Agaricostilbum/Bensingtonia* cluster (Fig. 1). This cluster was composed of morphologically and chemotaxonomically divergent taxa. *Agaricostilbum* is characterized by basidia which do not arise from teliospores and occur predominantly in synnemata-like basidiomata (Bandoni & Boekhout, 1998), *Bensingtonia* by ballistoconidia and Q-9 as the major ubiquinone isoprenologue (Boekhout & Nakase, 1998a), *Chionosphaera* by long nonseptate basidia and Q-10 (Kwon-Chung, 1998), *Kurtzmanomyces* by stalked conidia and Q-9 (Yamada & Banno, 1998), *Kondoa* by teliospores and Q-9 (Yamada *et al.*, 1989), *Sterigmatomyces* by stalked conidia and Q-10 (Banno & Yamada, 1998), and *Sporobolomyces* by ballistoconidia and Q-10 (Boekhout & Nakase, 1998b).

Sporobolomyces lactophilus was basal to all species in the *Agaricostilbum/Bensingtonia* cluster. *Sporobolomyces dracophyllus*, *Sporobolomyces ruber*, *Sporobolomyces sasicola*, *Sporobolomyces taupoensis* and *Sporobolomyces xanthus* clustered together with *Agaricostilbum hyphaenes*, *Bensingtonia ingoldii*, *Bensingtonia musae*, *Chionosphaera apobasidialis*, *Kurtzmanomyces nectairei* and *Sterigmatomyces halophilus* and are supported by a high bootstrap value (97%). There is considerable morphological divergence among these species. The six *Sporobolomyces* species in this cluster are phylogenetically divergent from the type species of *Sporobolomyces*, despite being phenotypically similar. At this stage, we cannot conclude whether their taxonomic position at the generic level is proper or not. The relationship of these six species to *Agaricostilbum*, *Bensingtonia*, *Chionosphaera*, *Kondoa*, *Kurtzmanomyces* and *Sterigmatomyces* is also not clear. A distinct *Agaricostilbum/Bensingtonia* lineage was not strongly supported (bootstrap = 70%). Phylogenetic analyses of other gene sequences are clearly needed to define the relationship among species in this cluster.

Species within the subbrunneus cluster

Four *Sporobolomyces* species, *Sporobolomyces coprosmicola*, *Sporobolomyces dimmenae*, *Sporobolomyces linderiae* and *Sporobolomyces subbrunneus* grouped together and were well supported by bootstrapping (100%). The species in the subbrunneus cluster were completely separated from those of the presently recognized yeasts in the Urediniomycetes (Fig. 1).

Sporobolomyces subbrunneus occupied a unique position on the phylogenetic tree inferred from partial 18S rRNA sequences and had a unique fingerprint segment which had not been found in any other basidiomycetous yeasts (Nakase *et al.*, 1993). However, the phylogenetic distinctness of *Sporobolomyces subbrunneus* and relatives was not clear until the recently described *Sporobolomyces coprosmicola*, *Sporobolomyces dimmenae* and *Sporobolomyces linderiae* (Nakase *et al.*, 1994; Hamamoto & Nakase, 1995) were used in the present analysis. It seems that the isolation of new yeasts from natural sources is important not only from the viewpoint of biodiversity but also for the establishment of reasonable systematics for basidiomycetous yeasts.

The rather low similarities of 18S rDNA sequences between the subbrunneus cluster and the other three clusters (84.7–88.2%) suggest that *Sporobolomyces coprosmicola*, *Sporobolomyces dimmenae*, *Sporobolomyces linderiae* and *Sporobolomyces subbrunneus* should be transferred to a new genus.

Species within the *Erythrobasidium* cluster

Eight species, *Sporobolomyces coprosmae*, *Sporobolomyces elongatus*, *Sporobolomyces foliicola*, *Sporobolomyces gracilis*, *Sporobolomyces kluyverinii*, *Sporobolomyces oryzicola*, *Sporobolomyces phyllomatis* and *Sporobolomyces salicinus* were present in the *Erythrobasidium* cluster, together with *Erythrobasidium hasegawianum*, *Rhodospiridium dacryoideum*, *Rhodotorula lactosa* and *Rhodotorula minuta* (Fig. 1). These are morphologically and chemotaxonomically divergent. *E. hasegawianum* is characterized by one-celled basidia which do not arise from teliospores and Q-10(H₂) as the major ubiquinone isoprenologue (Hamamoto *et al.*, 1988a, b; Sugiyama & Hamamoto, 1998), *Rhodospiridium dacryoideum* by teliospores and Q-10 (Fell & Statzell-Tallman, 1998a), *Rhodotorula lactosa* by Q-9 (Fell & Statzell-Tallman, 1998b), *Rhodotorula minuta* by Q-10 (Fell & Statzell-Tallman, 1998b), *Sporobolomyces elongatus* by ballistoconidia and Q-10(H₂) (Nakase & Suzuki, 1986) and the other *Sporobolomyces* by ballistoconidia and Q-10 (Boekhout & Nakase, 1998b).

Suh *et al.* (1996a) reported that *E. hasegawianum*, *Rhodospiridium dacryoideum*, *Rhodotorula lactosa* and *Rhodotorula minuta*, which were characterized as requiring *p*-aminobenzoic acid (PABA) for growth, were closely related based on 18S rDNA sequences. The *Erythrobasidium* cluster in the present study apparently corresponded to the lineage consisting of

PABA-requiring yeasts in the study of Suh *et al.* (1996a). However, for the eight *Sporobolomyces* species belonging to the *Erythrobasidium* cluster, differences in PABA requirements were observed by Barnett *et al.* (1990) and Boekhout (1991). In addition, Barnett *et al.* (1990) and Suh *et al.* (1996a) observed different PABA requirements for *E. hasegawianum* and *Rhodotorula minuta*. Therefore, it seems that a PABA requirement may not be a reproducible characteristic nor a necessarily representative character for species belonging to the *Erythrobasidium* cluster.

Sporobolomyces elongatus is considered to be a divergent taxon from other *Sporobolomyces* because it is the only species that has a dehydrogenated Q-10 as the major ubiquinone isoprenologue. Phylogenetically, *Sporobolomyces elongatus* is divergent from the type species of *Sporobolomyces*, whilst *Sporobolomyces elongatus* has a close relationship to the other seven *Sporobolomyces* species in the *Erythrobasidium* cluster. The sequences among these eight "*Sporobolomyces* species in this cluster showed high similarity ranging from 96.3 to 99.2%. *Sporobolomyces elongatus* was grouped with *E. hasegawianum* and *Rhodotorula lactosa* (Fig. 1). This result does not conflict with that of Swann & Taylor (1995a) which suggested a relationship between *Sporobolomyces elongatus* and *E. hasegawianum* based on partial 18S rDNA sequences, and that of Suh *et al.* (1996a) which suggested a relationship between *Rhodotorula lactosa* and *E. hasegawianum* based on 18S rDNA sequences. However, the relationship of these three species is still not clear because the lineage was not strongly supported (bootstrap < 50%) (Fig. 1).

Seven *Sporobolomyces* species (the exception being *Sporobolomyces elongatus*) in the *Erythrobasidium* cluster are phylogenetically divergent from the type species of *Sporobolomyces*, despite being phenotypically similar. They formed two groups statistically well-supported: *Sporobolomyces gracilis*, *Sporobolomyces kluyveri*, *Sporobolomyces phyllomatis* and *Sporobolomyces salicinus* (bootstrap = 99%); and *Sporobolomyces coprosmae*, *Sporobolomyces foliicola* and *Sporobolomyces oryzicola* (bootstrap = 100%) (Fig. 1). There is a genealogical relationship between these *Sporobolomyces* species and the other members in the *Erythrobasidium* cluster in spite of the morphological and chemotaxonomic divergence. At this stage, we cannot confirm their taxonomic status at the generic level. Further studies using other gene sequences are needed.

In the present study, we have demonstrated that the genus *Sporobolomyces* is polyphyletic in the phylogenetic tree inferred from the 18S rDNA nucleotide sequences. It further suggests that some species of the currently recognized *Sporobolomyces* species should be transferred to other genera. We consider that phylogenetic analyses of other gene sequences are needed to corroborate the present work and that the present data are not enough for discussion of the

relationship between *Sporobolomyces* species and related genera. Therefore, we do not propose a formal taxonomic rearrangement of *Sporobolomyces* species in this paper.

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