Phylogeny among the basidiomycetous yeasts inferred from small subunit ribosomal DNA sequence

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The sequence of the small subunit nuclear ribosomal DNA (18S rDNA) was determined for seven selected species of the teliospore-forming yeasts and Filobasidiaceae in the basidiomycetous yeasts. A phylogenetic tree, including published reference sequences, was inferred from 1623 sites which could be unambiguously aligned. The molecular phylogeny, using a chytridiomycete as an outgroup, divided the eight basidiomycetous yeasts into two groups which correlated well with both septal ultrastructure (simple pore or dolipore) and cellular xylose (present or absent). The first group included the teliospore-forming yeasts *Rhodosporeidium toruloides* and *Leucosporidium scottii*, and *Erythrobasidium hasegawianum*, which is currently a member of the Filobasidiaceae. The second group was formed by the filobasidiaceous yeasts, comprising a *Cystofilobasidium capitatum*/*Leucosporidium lari-marini*/*Mrakia frigida* branch, and a *Filobasidium floriforme*/*Filobasidiella neoformans* branch. Our molecular data support the principal chemotaxonomic and ultrastructural evidence, which indicates a very close affinity between *C. capitatum* and *L. lari-marini*.

Introduction

Basidiomycetous yeasts have been classified into three taxonomic categories: the teliospore-forming yeasts, and the yeasts of the Filobasidiaceae, and the Tremellales (Kreger-van Rij, 1984a). Teliospore formation and basidial morphology were considered as primary taxonomic characters for the classification of the Filobasidiaceae and teliospore-forming yeasts. *Rhodosporeidium*, *Leucosporidium* and *Sporidiobolus* were included in the teliospore-forming yeasts.

Since the publication of 'The Yeasts, a Taxonomic Study' (Kreger-van Rij, 1984b), several new basidiomycetous yeast genera have been proposed on the basis of chemotaxonomic and ultrastructural studies. Among these, the genera *Cystofilobasidium* (Oberwinkler *et al.*, 1983), *Mrakia* (Yamada & Komagata, 1987), and *Kondoa* (Yamada *et al.*, 1989) have been separated from

the genera *Leucosporidium* and *Rhodosporeidium* in the teliospore-forming yeasts, and included within the Filobasidiaceae. Hamamoto *et al.* (1988, 1991) proposed the genus *Erythrobasidium* in the Filobasidiaceae for the teleomorph of *Rhodotorula hasegawae*. However, phylogenetic relationships among these basidiomycetous yeast taxa are still controversial. For example, recent chemotaxonomic and ultrastructural studies (Suh & Sugiyama, 1993) have suggested that *Leucosporidium lari-marini* is related to the Filobasidiaceae.

The phylogenetic value of rRNA sequences has been discussed in recent reviews (Bruns *et al.*, 1991; Kurtzman, 1992; Kohn, 1992; Hibbett, 1992). 18S rDNA (and rRNA) sequences have been especially useful in elucidating the evolution and phylogeny of fungi (Hendriks *et al.*, 1991a, b, 1992; Berbee & Taylor, 1992a, b; Van de Peer *et al.*, 1992; Nishida & Sugiyama, 1993). We report the 18S rDNA sequence and phylogenetic relationships of seven species of basidiomycetous yeasts. We compare our results with information on other taxonomic characters.

Methods

Cultures. The following yeast strains were used for sequencing: *Rhodosporeidium toruloides* IAM 13469^T (= IFO 0559 = CBS 14, mating type A); *Leucosporidium lari-marini* IAM 14213^T (= CBS 7420,

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The nucleotide sequence data reported in this paper have been submitted to DDBJ and have been assigned the accession numbers D12801 (*Cystofilobasidium capitatum*), D12802 (*Mrakia frigida*), D12803 (*Erythrobasidium hasegawianum*), D12804 (*Filobasidiella neoformans*), D12805 (*Leucosporidium lari-marini*), D12806 (*Rhodosporeidium toruloides*) and D13460 (*Filobasidium floriforme*).

self-sporulating); *Cystofilobasidium capitatum* IAM 13521^T (= ATCC 24507, self-sporulating); *Mrakia frigida* IAM 14136^T (= CBS 5270, self-sporulating); *Erythrobasidium hasegawianum* IAM 12911^T (= IFO 1058 = ATCC 9536, self-sporulating); *Filobasidium floriforme* IAM 14212^T (= CBS 6241, mating type A); *Filobasidiella neoformans* IAM 14211^T (= CBS 6886 = ATCC 28958, mating type a). Strains with a superscript T are derived from the type. The yeasts were all grown in YM broth at 25 °C for DNA extraction.

PCR and sequencing. DNA was obtained from cells broken by sonication. The PCR and sequencing of 18S rDNA were done according to Nishida & Sugiyama (1993).

Phylogenetic analysis. We sequenced the 18S rDNA of the seven basidiomycetous yeasts mentioned above and added known sequence data from the chytridiomycete *Blastocladiella emersonii* (Förster *et al.*, 1990), the ascomycetous yeasts *Candida albicans* (Hendriks *et al.* 1989), *Kluyveromyces lactis* (Maleszka & Clark-Walker, 1990), and *Saccharomyces cerevisiae* (Mankin *et al.*, 1986), the filamentous ascomycete *Neurospora crassa* (Sogin *et al.*, 1986), the basidiomycetous yeast *Leucosporidium scottii* (Hendriks *et al.*, 1991b), the basidiomycetous anamorphic yeasts *Sporobolomyces roseus*, *Bullera alba*, and *Trichosporon cutaneum* (Van de Peer *et al.*, 1992), and the ustilaginalean basidiomycete *Ustilago maydis* (De Wachter *et al.*, 1992). Recently, the 18S rRNA sequences of *R. toruloides* and *Filobasidiella neoformans* were published by Van de Peer *et al.* (1992). Comparing their sequence data with our own, however, revealed three and eleven base differences in *R. toruloides* and *Filobasidiella neoformans*, respectively. All sequences were aligned using the multialignment program CLUSTAL V (Higgins *et al.*, 1992). Distances between the sequences were calculated using the two-parameter model of Kimura (1980). Sites where gaps existed in any of the sequences were excluded. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987). Bootstrap analysis (Felsenstein, 1985) used 1000 random samples from the multiple alignment. The tree was rooted using *Blastocladiella emersonii* as an outgroup.

Results and Discussion

For each strain, we sequenced about 1750 bases of PCR amplification product of the gene encoding 18S rRNA. The sequence data were well aligned for all 17 fungi, and

Fig. 1. Evolutionary tree of 17 species based on 1623 sites among about 1750 bases of 18S rDNA. The tree was constructed from the evolutionary distance data according to Kimura (1980) using the neighbour-joining method (Saitou & Nei, 1987). Each number indicates the percentage of bootstrap samplings, derived from 1000 samples, supporting the internal branches. The distance corresponding to one base change per hundred nucleotide positions is indicated by a bar.

a phylogenetic tree (Fig. 1) was constructed from these sequences using the neighbour-joining method (Saitou & Nei, 1987). Four ascomycete and twelve basidiomycete genera were well-separated, based on the 1623 sites compared. The phylogenetic tree divided the basidiomycetous yeasts into two groups, which correlated well

Table 1. *Basidiomycetous yeast species and selected taxonomic characteristics*

Species	Septal pore structure*	Xylose in the cells†	Major ubiquinone system‡	Teliospore§	Basidial form§
<i>Leucosporidium scottii</i>	Simple	—	9,10	+	Phragmo
<i>Leucosporidium lari-marini</i>	Dolipore	+	8	+	Holo
<i>Rhodospiridium toruloides</i>	Simple	—	9	+	Phragmo
<i>Erythrobasidium hasegawianum</i>	Simple	—	10(H ₂)	—	Holo
<i>Cystofilobasidium capitatum</i>	Dolipore	+	8	+	Holo
<i>Mrakia frigida</i>	Dolipore	+	8	+	Holo
<i>Filobasidium floriforme</i>	Dolipore	+	10	—	Holo
<i>Filobasidiella neoformans</i>	Dolipore	+	10	—	Holo

* Data from Johnson-Reid & Moore (1972), Kwon-Chung & Popkin (1976), Nakagiri & Tubaki (1983), Moore (1972), Moore & Kreger-van Rij (1972), Obwerwinkler *et al.* (1983), Suh & Sugiyama (1993) and Suh *et al.* (1993).

† Data from Sugiyama *et al.* (1985), Suh & Sugiyama (1993).

‡ Data from Sugiyama *et al.* (1985), Suh & Sugiyama (1993), Yamada & Kondo (1972a, b, 1973).

§ Data from Fell & Statzell-Tallman (1992), Hamamoto *et al.* (1988), Kreger van Rij (1984b) and Saez & Nguyen (1989).

with the septal pore type and cellular carbohydrate composition profile (Table 1).

From 5S rRNA comparisons, Walker & Doolittle (1982, 1983) and Blanz & Unsel (1987) concluded that septal pore structures were of great value as a taxonomic tool in basidiomycetes. This is confirmed by the trees produced from complete 18S rDNA sequences, as shown in Fig. 1 and in the paper by Van de Peer *et al.* (1992).

Hamamoto *et al.* (1988) discovered the teleomorphic state of *Rhodotorula hasegawae*, which is characterized by unicellular basidia, the lack of teliospores, and the type of ubiquinone (Q-10/H₂). They proposed the new genus *Erythrobasidium* for the teleomorph and tentatively placed it in the Filobasidiaceae. However, Suh *et al.* (1993) suggested from data on hyphal septal pore ultrastructure that *Erythrobasidium* has an affinity with the teliospore-forming yeasts rather than with the filobasidiaceous taxa. *Erythrobasidium hasegawianum* (Hamamoto *et al.*, 1991), the type species of this genus, is of the simple septal pore type, which is very similar to that of *Rhodospiridium toruloides* and related taxa (Johnson-Reid & Moore, 1972; Kreger-van Rij & Veenhuis, 1971; Moore, 1972; Suh *et al.*, 1993; cf. Table 1). This association is supported by the molecular phylogeny based on 18S rDNA sequence (Fig. 1), which relates *E. hasegawianum* to the teliospore-forming yeast species *R. toruloides* and *L. scottii*. The bootstrap analysis supported the topologies of this group including *L. scottii*, *R. toruloides* and *E. hasegawianum*, at the 100% confidence level.

Cystofilobasidium and *Mrakia* have been distinguished from *Rhodospiridium* and *Leucosporidium* mainly by ultrastructural and chemotaxonomic data and were assigned to the Filobasidiaceae (Oberwinkler *et al.*, 1983; Yamada & Komagata, 1987). In *C. capitatum* and *M. frigida*, the septal pores are dolipores similar to other genera of the Filobasidiaceae (Nakagiri & Tubaki, 1983; Oberwinkler *et al.*, 1983; Suh *et al.*, 1993; cf. Table 1). *Cystofilobasidium capitatum* and *M. frigida* were well separated from *R. toruloides* and *L. scottii*, and showed a relationship with *Filobasidium floriforme*, the type genus and type species of the Filobasidiaceae, and *Filobasidiella neoformans* (Fig. 1).

Buff-coloured *Leucosporidium lari-marini* showed a very close relationship with the reddish-coloured *C. capitatum* originally assigned to the genus *Rhodospiridium*. No base differences were found in the 1749 bases compared between *C. capitatum* and *L. lari-marini*. The two show a close relationship in many taxonomic characteristics: i.e. both species are characterized by the Q-8 ubiquinone system, xylose in their cellular carbohydrates and dolipore-like septa without typical parthenosomes (Suh & Sugiyama, 1993; cf. Table 1). The close relationships between *L. lari-marini*, *C. capitatum*

and *M. frigida* were supported at the 99.9% confidence level in a bootstrap test. Recently, Fell & Statzell-Tallman (1992) transferred *L. lari-marini* to *Cystofilobasidium* as *C. lari-marini* on the basis of a comparison of 26S rRNA partial sequences, and the use of basidial morphology. Our 18S rDNA data supported this transfer but more information, including DNA base composition and DNA-DNA hybridization, is needed to assign *L. lari-marini* precisely at the species level.

Basidiomycetous anamorphic yeasts were also divided into two phylogenetic groups, which correlated with their teleomorphs. *Sporobolomyces roseus*, an anamorph of *Sporidiobolus* in teliospore-forming yeasts, was related to *L. scottii*. On the other hand, *Bullera alba* and *Trichosporon cutaneum* were related to *F. neoformans* and *F. floriforme*. The phylogenetic tree (Fig. 1) correlated well with the cellular carbohydrate composition profiles. Unlike *B. alba* (von Arx & Weijman, 1979), *S. roseus* has no xylose (Crook & Johnston, 1962).

In conclusion, the molecular phylogeny based on 18S rRNA gene sequences divided twelve representatives of the basidiomycetous yeasts into two lineages (the teliospore-forming yeasts, including *Erythrobasidium hasegawianum*, and the Filobasidiaceae, including *Leucosporidium lari-marini*) and showed a good correlation with the septal pore type and cellular carbohydrate composition. The value of these properties as taxonomic characters has thus been supported by the molecular data.

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