Molecular phylogenetics of the genus *Rhodotorula* and related basidiomycetous yeasts inferred from the mitochondrial cytochrome *b* gene

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Phylogenetic relationships of basidiomycetous yeasts, especially of the genus *Rhodotorula*, were studied using partial sequences of the mitochondrial cytochrome *b* gene. The results demonstrated that the basidiomycetous yeasts under investigation distributed into two main clusters: one containing Tremellales, Filobasidiales and their anamorphs and the other containing Ustilaginales, Sporidiales and their anamorphs. This clustering in turn correlates with cell wall biochemistry, presence or absence of xylose, and septal ultrastructure, dolipore or simple pore. *Bullera*, *Bulleromyces*, *Filobasidiella*, *Cryptococcus* and *Trichosporon*, yeasts of the former cluster, contain xylose in the cell wall and have dolipore septa. In contrast yeasts of the latter cluster, which included *Bensingtonia*, *Erythrobasidium*, *Leucosporidium*, *Malassezia*, *Rhodospiridium*, *Rhodotorula*, *Sporidiobolus*, *Sporobolomyces* and *Ustilago*, have no xylose in the cell wall and have a simple pore septum. Yeasts of the latter group could be further divided into four clades (A–D). Species of *Rhodotorula* were distributed in all of these clades, indicating the polyphyletic nature of the genus. A limited number of *Rhodotorula* species demonstrated identical sequences, for example *Rhodotorula bacarum* and *Rhodotorula foliorum*, *Rhodotorula fujisanensis* and *Rhodotorula futronensis*, *Rhodotorula glutinis* var. *dairenensis* and *Rhodotorula mucilaginosa*. However, all the other test species of the genus *Rhodotorula* were well separated based on their 396 bp nucleotide sequences. These results demonstrate the effectiveness of the use of cytochrome *b* sequences for both species identification and the study of phylogenetic relationships among basidiomycetous yeasts.

Keywords: molecular phylogenetics, *Rhodotorula*, basidiomycetous yeasts, cytochrome *b* gene

INTRODUCTION

The genus *Rhodotorula* Harrison is a member of the basidiomycetous yeasts, occurring naturally in air and soil from which it can readily be isolated. In addition, this yeast can be isolated from human skin, stool and

food. Although most species of the genus *Rhodotorula* are non-pathogenic, some species have been regarded as emerging yeast pathogens (Hazen, 1995) amongst which *Rhodotorula mucilaginosa* is the most frequently isolated from human infections. In addition, *Rhodotorula minuta* (Goldani *et al.*, 1995) and *Rhodotorula glutinis* (Guerra *et al.*, 1992; Casolari *et al.*, 1992) are also isolated from human infections. In general, such *Rhodotorula* species are involved in fungaemia and endocarditis of immunocompromised hosts; however, *Rhodotorula* species are also isolated from corneal

Abbreviation: UPGMA, unweighted pair group method with arithmetic average.

The DDBJ/EMBL/GenBank accession numbers for the cytochrome *b* partial sequences are AB040614–AB040666 and AB041047–AB041051.

Fig. 1. Phylogenetic tree based on nucleotide sequences of the cytochrome *b* gene. The tree was constructed by using UPGMA. Sequences of *Saccharomyces douglasii* and *Candida glabrata* were obtained from GenBank. IFM, Institute for Food Microbiology (now Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan); T, type strain; NT, neotype strain; *, strain which contains intron and exon is used. Bar, 0.03 substitutions per nucleotide.

lamellar graft infection, meningitis and systemic infections (Gyaurgieva *et al.*, 1996; Lui *et al.*, 1998; Panda *et al.*, 1999; Papadogeorgakis *et al.*, 1999; Polo, 1993; Rusthoven *et al.*, 1984).

The genus *Rhodotorula* has recently expanded markedly, from only eight species (Kreger-Van Rij, 1984) to 34 (Kurtzman & Fell, 1998). This increase in number is the result of several factors. The major one is redefinition of the genus *Candida* and segregation of basidiomycetous species into *Rhodotorula* and *Cryptococcus*, based on the composition of cell wall hydrolysates, particularly xylose which has only been found in the latter genus (Weijman & Rodrigues de Miranda, 1988; Weijman *et al.*, 1988). Use of improved methods, such as the application of molecular techniques to species identification, has also contributed significantly. A recent study by Fell *et al.* (2000) described seven more new species of the genus *Rhodotorula*, according to differences in the D1/D2 region of the large-subunit rDNA. They are *Rhodotorula creatinivora*, *Rhodotorula cresolica*, *Rhodotorula laryngis*, *Rhodotorula pallida*, *Rhodotorula slooffiae*, *Rhodotorula vanillica* and *Rhodotorula yarrowii*.

Differentiation of all species of the genus *Rhodotorula* based on morphological characters and physiological tests is potentially time-consuming, expensive and requires considerable expertise. Hence, a more efficacious method may be found through the presentation of an integrated view of the genus based on molecular data and this, though not an easy task, has been done by Fell *et al.* (2000). They have also shown that species of *Rhodotorula* are distributed in the *Microbotryum*, *Sporidiobolus* and *Erythrobasidium* clades, but not in the *Agaricostilbum* clade of the class Urediniomycetes. However, *Rhodotorula acheniorum*, *Rhodotorula bacarum*, *Rhodotorula himmulea* and *Rhodotorula phylloplana* are members of the Ustilaginomycetes (Fell *et al.*, 1995, 2000).

Basidiomycetous yeasts have been divided into different lineages based on the analysis of different regions of rDNA: two main lines based on 5S rDNA (Walker & Doolittle, 1982); four main clusters according to 26S rDNA (Sporidiales, Tremellales, *Cystofilobasidium* and related taxa and the Ustilaginales; Fell *et al.*, 1995); and three main lineages according to 18S rDNA or the D1/D2 region of large-subunit rDNA (Ustilaginomycetes, Urediniomycetes and Hymenomycetes; Swann & Taylor, 1995a, b, c; Fell *et al.*, 2000). Further evidence based on different DNA sequences should be evaluated to establish phylogenetic relationships (Kurtzman, 1994). It was therefore of interest to find the relationships between the basidiomycetous yeasts by analysing mitochondrial (mt) DNA other than rDNA.

The rapid evolution of the mitochondrial genome, as well as its lack of recombination and its strict maternal inheritance, makes it an attractive marker for inferring phylogeny of closely related species (Manceau *et al.*, 1999). RFLPs of mtDNA have been shown to be

useful genetic markers for estimating relationships among fungi (Hamari *et al.*, 1997; Kozłowski & Stepień, 1982; Varga *et al.*, 1994). However, studies that investigate phylogenetic relationships based on the sequences of mtDNA (Paquin *et al.*, 1995; Walker *et al.*, 1998) are in their infancy. The use of the sequence of the mitochondrial cytochrome *b* gene to identify pathogenic *Aspergillus* species has been shown by Wang *et al.* (1998, 2000). However, similar techniques have not focused on yeasts.

This is the first study presenting a molecular phylogeny of the basidiomycetous yeasts based on sequences of the mitochondrial cytochrome *b* gene. The study included all species of the genus *Rhodotorula* described in Kurtzman & Fell (1998) and examined their relationships to closely related basidiomycetous yeasts.

METHODS

Yeast strains and isolation of DNA. Yeast strains used in this study (Figs 1 and 2) were collected from CBS (Centraalbureau voor Schimmelcultures, Baarn, The Netherlands), IFO (Institute for Fermentation, Osaka, Japan) and JCM (Japan Collection of Microorganisms, Saitama, Japan). Pure culture cells were grown on YPD slants. One-third of a loop of cells from the slant were added into 1 ml distilled water, vortexed well and centrifuged. The supernatant was discarded and the pellet was used to extract total cellular DNA using the Gen Toru Kun Kit (Takara Shuzo) according to the standard protocol. One microlitre of the extracted DNA was used to amplify the mitochondrial cytochrome *b* gene as described below.

PCR primers and amplification of the cytochrome *b* gene. PCR primers E1M4 (5'-TGRGGWGCWACWGTTATTA-CTA-3'), E1mr2 (5'-TGRGGWGCWACGWTWATTAC-TAAYYT-3'), E2M4 (5'-GGWATAGMWSKTAAWAY-AGCATA-3'), E2mr3 (5'-GGWATAGCACGTARAAY-WGCRTA-3') and E2mr4 (5'-AGCACGTARWAYWGC-RTARWAHGG-3') were designed comparing previously published amino acid sequences of mitochondrial cytochrome *b* of several organisms as described by Wang *et al.* (1998). E1M4 or E1mr2 and E2M4, E2mr3 or E2mr4 were used as forward and reverse primers, respectively. PCR was carried out using the TaKaRa Ex Taq PCR Amplification Kit (Takara Shuzo). The reactions were performed in a final reaction mixture (50 µl) containing 10 pmol of each primer, 4 µl 2.5 mM dNTP mixture (dATP, dCTP, dGTP and dTTP), 2.0 U TaKaRa Ex Taq polymerase and 5 µl 10 × reaction buffer (Takara Shuzo). The amplification reactions were performed with the following cycling parameters: 94 °C for 2 min, followed by 30 cycles consisting of denaturation for 1 min at 94 °C, annealing for 1 min at 50 °C and extension for 2 min at 72 °C, with a final extension at 72 °C for 10 min.

Sequencing and accession numbers. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen). Then both strands of the PCR products were sequenced directly on an ABI Prism 377 or 310 DNA sequencer using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) as recommended by the manufacturer. From the cytochrome *b* gene sequences, amino acid sequences were estimated using the yeast mitochondrial genetic code. The partial sequences of the mitochondrial

Fig. 2. Phylogenetic tree based on amino acid sequences of the cytochrome *b* gene. The tree was constructed by using UPGMA. The standard errors of main branching points are as follows and strains used to calculate standard errors are shown in parentheses: a, ± 0.0136 (IFM 49223, 48541 and 49224); b, ± 0.0114 (IFM 48506, 48585 and 48505); c, ± 0.0088 (IFM 49220, 48596 and 48504); d, ± 0.0120 (IFM 48506, 49220 and 48585); e, ± 0.0144 (IFM 49220, 49223 and 48585); f,

Fig. 3. Positions of introns in cytochrome *b* of *Rhodotorula* and other fungi. The arrows indicate the positions of introns. The sequences of *Aspergillus nidulans* (AAA31737), *Neurospora crassa* (0912196A), *Schizosaccharomyces pombe* (CAA38287) and *Saccharomyces cerevisiae* (X84042) were obtained from GenBank.

cytochrome *b* gene determined in this study have been deposited in the DDBJ/EMBL/GenBank database under the accession numbers shown in Figs 1 and 2.

Molecular phylogenetic analysis. DNA sequences and amino acid sequences were aligned using the program of GENETYX-MAC Genetic Information Processing Software (Software Development). Sequences were analysed by maximum-parsimony and maximum-likelihood of the Phylogenetic Analysis Using Parsimony (PAUP) package, version 4.0b4a for Macintosh (Swofford, 1998) or by unweighted pair group method with arithmetic average (UPGMA) and neighbour-joining of the GENETYX-MAC Genetic Information Processing Software. Standard errors of main branching points in the UPGMA tree were calculated adopting the method of Nei *et al.* (1985).

RESULTS

The mitochondrial cytochrome *b* gene of the 34 species of the genus *Rhodotorula* described in Kurtzman & Fell (1998) and 23 species of the related 18 genera of basidiomycetous yeasts were partially sequenced. Only 5 out of 58 different species had introns within the region we sequenced. The nucleotide sequence of a strain without any introns was 396 bp long. The species with introns were *Rhodotorula acheniorum*, *Rhodotorula araucariae*, *Rhodotorula ferulica*, *Rhodotorula himmulea* and *Rhodotorula phylloplana*. *Rhodotorula acheniorum* contained two introns of 281 and 350 bp, but the others contained single introns of 1182, 1162, 924 and 924 bp, respectively. Intron 1 of *Rhodotorula acheniorum* and the intron of *Rhodotorula ferulica* were located at the same position as *Neurospora crassa* intron 2, but the intron of *Rhodotorula araucariae* was

located at a position 2 aa away from *Neurospora crassa* intron 2. Intron 2 of *Rhodotorula acheniorum* and the introns of *Rhodotorula himmulea* and *Rhodotorula phylloplana* were located at the same position, 4 aa away from the position of *Saccharomyces cerevisiae* intron 5 (Fig. 3).

The 396 bp segment corresponding to positions 445–840 in the *Candida glabrata* cytochrome *b* coding sequence (GenBank accession no. X53862) was analysed in this study. When only species of *Rhodotorula* were used in an alignment, 239 variable nucleotide sites out of 396 (60.3%) were observed, of which 209 were phylogenetically informative (52.7%). But when all of the species listed in Fig. 1, including the outgroup species *Saccharomyces douglasii* and *Candida glabrata*, were taken into consideration, the number of variable nucleotide positions was 271 (68.4%), of which 248 were phylogenetically informative (62.6%). The inferred amino acid sequences were also very variable. There were 55 variable amino acid positions out of 132 (41.6%) when only species of *Rhodotorula* were taken into account and there were 78 (59%) informative amino acid positions when all species were considered.

Fig. 1 shows the UPGMA tree based on mitochondrial cytochrome *b* nucleotide sequences. With the ascomycetes *Saccharomyces douglasii* and *Candida glabrata* taken as outgroup species, the basidiomycetous yeasts were divided into two major clusters showing, respectively, the presence or absence of xylose in their cell wall composition. The tree described heterogeneity of the genus *Rhodotorula* that correlates with the

±0.0153 (IFM 49220, 49223 and 48191); g, ±0.0164 (IFM 48391, 48512 and 48521); h, ±0.0153 (IFM 49220, 48391 and 48191); i, ±0.0198 (IFM 5760, 49223 and 48521); j, ±0.0203 (IFM 48391, 5760 and 48513); k, ±0.0178 (IFM 48637, 48667 and 48611); l, ±0.0218 (IFM 48391, 48513 and 48611); m, ±0.0236 (IFM 48391, 48611 and X59280). Sequences of *Saccharomyces douglasii* and *Candida glabrata* were obtained from GenBank. Abbreviations and symbols are as defined in the legend to Fig. 1. Bar, 0.03 substitutions per amino acid.

phylogeny based on ribosomal gene sequences. The sequences of *Rhodotorula bacarum* and *Rhodotorula foliorum*, *Rhodotorula fujisanensis* and *Rhodotorula futronensis*, and *Rhodotorula glutinis* var. *dairenensis* and *Rhodotorula mucilaginosus* were identical, respectively. The other species of the genus *Rhodotorula* were well separated based on this 396 bp nucleotide sequence (Fig. 1).

The phylogenetic tree based on cytochrome *b* amino acid sequences constructed by UPGMA is shown in Fig. 2. In this tree, basidiomycetous yeasts segregated in a similar manner to the nucleotide-based tree, consistent with the differences in xylose content of the cells as observed by Weijman & Rodrigues de Miranda (1983). Using neighbour-joining, maximum-parsimony or maximum-likelihood, the trees did not show good topology unlike the trees derived by UPGMA (not shown). The genus *Rhodotorula* was polyphyletic and clustered with yeasts that do not contain xylose in the cell wall, such as Sporidiales, *Ustilago maydis* and members of the family Sporobolomycetaceae included in the study, *Bensingtonia* and *Sporobolomyces*. These yeasts were further divided into four clades, A–D (Fig. 2). *Rhodotorula* species were distributed in all of these clades. Clade A contains mainly anamorphic basidiomycetes: *Rhodotorula* (8 species), *Bensingtonia*, *Sporobolomyces* and *Malassezia*, except for the teleomorphic *Ustilago maydis* and *Kondoa* (*Rhodospodidium*) *malvinella*. Clade B is the largest cluster consisting of 19 species of *Rhodotorula*, including the type species of the genus *Rhodotorula glutinis* var. *glutinis* and its variety *dairenensis*, and the Sporidiales *Leucosporidium scottii*, *Rhodospodidium paludigenum* and *Sporidiobolus pararoseus*. Clade C consists of *Erythrobasidium hasegawianum*, *Sakaguchia* (*Rhodospodidium*) *dacryoides* and five species of *Rhodotorula*, *Rhodotorula armeniaca*, *Rhodotorula aurantiaca*, *Rhodotorula lactosa*, *Rhodotorula minuta* and *Rhodotorula marina*. Clade D is the smallest containing *Rhodotorula diffuens* and *Rhodotorula hylophila*.

From both trees (Figs 1 and 2), it is also clear that the genus *Cryptococcus* is polyphyletic. Species of the genus *Cryptococcus* clustered with yeasts containing xylose in the cell wall. The cluster comprises the teleomorphic yeasts *Filobasidiella neoformans*, *Filobasidium capsuligenum*, *Bulleromyces albus* and *Tremella foliacea*, and the anamorphic yeasts *Bullera crocea*, species of *Cryptococcus*, species of *Trichosporon* and *Tsuchiyaea wingfieldii*.

DISCUSSION

Generally, most fungi, e.g. *Aspergillus nidulans*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, contain introns in their cytochrome *b* genes (Lang *et al.*, 1985). Cytochrome *b* sequences of *Pneumocystis carinii* and *Candida glabrata* do not contain introns (Clark-Walker, 1991; Walker *et al.*, 1998). Among the 58 species we sequenced, only *Rhodotorula acheniorum*, *Rhodotorula*

araucariae, *Rhodotorula ferulica*, *Rhodotorula hinnulea* and *Rhodotorula phylloplana* contained introns in the region sequenced. In general, a strong conservation of amino acid sequences within cytochrome *b* occurs between different organisms (Burke *et al.*, 1984). Mitochondrial exons usually have a thymidine residue at their 3' ends and introns end with a 3'-terminal guanosine residue (Burke & RajBhandary, 1982; Burke *et al.*, 1984). To maximize amino acid identities, we compared closely related strains with and without introns, and in those with introns we determined intron positions and sizes. Introns of *Rhodotorula hinnulea* and *Rhodotorula phylloplana*, which had the same size and location, differed negligibly (by only 1 nt) in sequence, indicating that the intron in these species appeared before the separation of the two species. The size, location and homology of the introns of the other species studied indicate that they originated individually or diverged over time.

When we used UPGMA to construct phylogenetic trees from nucleotide (Fig. 1) and amino acid (Fig. 2) sequences, the two resultant trees were not fully congruent. Different nucleotide codons may give rise to the same amino acid, which may produce differences in topology of the nucleotide- and amino-acid-based phylogenetic trees. Cytochrome *b* phylogenetic analysis using UPGMA resulted in the division of basidiomycetous yeasts into two main clusters: one containing Ustilaginales, Sporidiales and their anamorphs, and the other containing Tremellales, Filobasidiales and their anamorphs. Although this clustering correlates with that determined by cell wall biochemistry, i.e. the presence or absence of xylose (Weijman & Rodrigues de Miranda, 1983; Weijman *et al.*, 1988) and septal ultrastructure (Boekhout *et al.*, 1998; Moore, 1998), it does not support the present molecular phylogenetic classification based on 18S or the D1/D2 region of large-subunit rDNA which divides basidiomycetous yeasts into three classes, Hymenomycetes, Urediniomycetes and Ustilaginomycetes (Swann & Taylor, 1995a, b, c; Fell *et al.*, 2000). In addition, cytochrome *b* phylogenetic trees constructed with the neighbour-joining or maximum-parsimony and maximum-likelihood (PAUP) methods (not shown) support neither a basidiomycetous yeasts classification system based on cell wall xylose or septal ultrastructure, nor the present molecular phylogenetic system based on 18S or the D1/D2 region of large-subunit rDNA. Use of the UPGMA method results in the correct topology when the distance measure used is exactly linear with evolutionary time, and the substitution rate of an amino acid in cytochrome *b* presumably correlates well with evolutionary time (Nei, 1985). Wang *et al.* (1998, 2000) have also shown that UPGMA is the best method to construct phylogenetic trees based on cytochrome *b* sequences of fungi.

In this study species of the genus *Rhodotorula* clustered separately from those of the genus *Cryptococcus* in the phylogenetic trees based on cytochrome *b* sequences

(Figs 1 and 2). This correlates with the differentiation of the genus *Rhodotorula* from *Cryptococcus* based on xylose in the cell wall (Weijman & Rodrigues de Miranda, 1988; Weijman *et al.*, 1988). Yeast species that do not contain xylose in the cell wall were further divided into four clades, A–D (Fig. 2). Species of *Rhodotorula* were found in all of these clades, which confirms its polyphyletic nature. Clade A contains species of the *Agaricostilbum* clade and species of the lineage Ustilaginomycetes. Clade B consists of species of the *Sporidiobolus* and *Microbotryum* clade. Species of *Erythrobasidium* clade (reported by Fell *et al.*, 2000) are found in clade C. The standard errors of two close branching points (e.g. points i and j in Fig. 2) are so large that the difference between them is not statistically significant. Therefore, which organisms diverged first cannot be ascertained. This means that clades C and D can be combined. A similar conclusion can be drawn from other closely related branching points except for l and m. The difference between these two branching points is statistically significant, indicating that basidiomycetous yeasts apparently diverged from ascomycetous yeasts.

Four species of *Rhodotorula*, *Rhodotorula acheniorum*, *Rhodotorula bacarum*, *Rhodotorula hinnulea* and *Rhodotorula phylloplana*, as well as *Malassezia furfur* and *Malassezia pachydermatis*, are in the Ustilaginomycetes class (Fell *et al.*, 1995, 2000). According to our data, all of these yeasts were clustered in clade A (Fig. 2) of the cytochrome *b* phylogenetic tree. Clade A also includes species of *Bensingtonia*, *Kondoa malvinella*, *Sporobolomyces xanthus* and five other species of *Rhodotorula*, *Rhodotorula acuta*, *Rhodotorula bogoriensis*, *Rhodotorula buffoni*, *Rhodotorula foliorum* and *Rhodotorula pustula*. Some features are common to all of these yeasts. They do not contain xylose in the cell wall (Weijman & Rodrigues de Miranda, 1988; Weijman *et al.*, 1988; Prillinger *et al.*, 1993) and they have a simple septal pore (Boekhout *et al.*, 1998; Moore, 1998). The low level of mannose content in the cell wall hydrolysates of *Rhodotorula acheniorum*, *Rhodotorula bacarum*, *Rhodotorula hinnulea* and *Rhodotorula phylloplana* (Weijman & Rodrigues de Miranda, 1988) also suggests that these yeasts are closely related to smut-like fungi.

Classification systems of the basidiomycetous yeasts vary. Prillinger *et al.* (1993) differentiated yeasts and yeasts-like fungi into seven groups, according to cell wall types, and basidiomycetous yeasts were placed into four of these groups: *Microbotryum* type, *Ustilago* type, *Dacrymyces* type and *Tremella* type. Boekhout *et al.* (1998) divided basidiomycetous yeasts into teleomorphic and anamorphic taxa with the former further divided into two groups: those containing simple septa and those containing dolipore septa. Differentiation of basidiomycetous yeasts based on sequences of rRNA/rDNA is also variable and depends on the regions used in analysis. Basidiomycetous yeasts have been divided into two main lines determined by 5S rDNA (Walker & Doolittle, 1982); into four main clusters according

to 26S rDNA – Sporidiales, Tremellales, *Cystofilobasidium* and related taxa, and the Ustilaginales (Fell *et al.*, 1995); or into three main lineages according to 18S or the D1/D2 region of large-subunit rDNA – Ustilaginomycetes, Urediniomycetes and Hymenomycetes (Swann & Taylor, 1995a, b, c; Fell *et al.*, 2000). In the present study, cytochrome *b* sequencing led to the division of basidiomycetous yeasts into two lineages as discussed above.

Rhodotorula glutinis is the type species of the genus *Rhodotorula* and contains two varieties, *Rhodotorula glutinis* var. *glutinis* and *Rhodotorula glutinis* var. *dairenensis*. *Rhodotorula glutinis* var. *glutinis*, the type culture, is closely related to *Rhodotorula graminis*. Fell & Statzell-Tallman (1998) found that the two species differed by only one base position. Cytochrome *b* sequence similarities between them also support a close relationship between the two species. Although their DNA sequences differed by two base positions, there was no difference between their amino acid sequences. In contrast, *Rhodotorula glutinis* var. *dairenensis* is more closely related to *Rhodotorula mucilaginosa* than to *Rhodotorula glutinis* var. *glutinis* and consequently *Rhodotorula glutinis* var. *dairenensis* would be elevated to the rank of species (Fell & Statzell-Tallman, 1998). According to the partial cytochrome *b* gene sequences, the *Rhodotorula glutinis* var. *dairenensis* sequence displayed no differences from that of *Rhodotorula mucilaginosa*. *Rhodotorula glutinis* var. *dairenensis* might therefore be a synonym of *Rhodotorula mucilaginosa*.

Sequence analysis of the cytochrome *b* gene could not be used to differentiate *Rhodotorula fujisanensis* from *Rhodotorula futronensis* nor *Rhodotorula bacarum* from *Rhodotorula foliorum*. The former two species have the same D1/D2 sequences (Fell *et al.*, 2000). However, *Rhodotorula fujisanensis* forms teliospores on potato dextrose agar when mixed with an opposite mating type (Fell & Statzell-Tallman, 1998) and thus will be reclassified into a teleomorphic group in the near future. *Rhodotorula hinnulea* and *Rhodotorula phylloplana* were closely related. They differed by 1 nt but had identical cytochrome *b* amino acid sequences. Their D1/D2 sequences are also identical (Fell *et al.*, 2000). For these species further studies are required to determine their relationship, for example to sequence the complete cytochrome *b* gene and/or other genes.

Standard taxonomic test results previously indicated that *Rhodotorula marina* is a synonym of *Rhodotorula minuta* (Fell *et al.*, 1984). However, in another study, the analysis of large-subunit rRNA sequences revealed that the two organisms differed by 53 base positions (Fell *et al.*, 1992) and *Rhodotorula marina* was re-established as a separate species (Fell & Statzell-Tallman, 1998). Our data on the cytochrome *b* gene sequences also resulted in the classification of *Rhodotorula marina* as a distinct species.

Yamada *et al.* (1989, 1994) proposed the creation of new genera for *Rhodospodium* (*Kondoa*) *malvinella*

and *Rhodotorula* (*Sakaguchia*) *dacryoides* due to the difference in their rRNA sequences from other species of *Rhodospiridium*. Sequence analyses indicate that *Rhodospiridium malvinella* and *Rhodotorula dacryoides* are not related to the other species of the genus *Rhodospiridium*; instead *Rhodotorula malvinella* appears close to *Bensingtonia yuccicola* and *Rhodotorula dacryoides* is in a large cluster of species that includes *Rhodotorula lactosa* and *Erythrobasidium hasegawianum* (Fell *et al.*, 1992, 1995). Emendation of *Kondoa* was done by Fonseca *et al.* (2000) and from the analysis of the D1/D2 region of the 26S rRNA gene, it was placed in the 'Agaricostilbum clade' of the class Urediniomycetes (Fonseca *et al.*, 2000; Fell *et al.*, 2000). In cytochrome *b* phylogenetics, *Kondoa malvinella* was closely related to *Bensingtonia yuccicola* and *Rhodotorula dacryoides* to *Erythrobasidium hasegawianum*.

In conclusion, the cytochrome *b* phylogeny of the basidiomycetous yeasts correlates with cell wall biochemistry and septal ultrastructure findings. The results show the effectiveness of the use of cytochrome *b* sequences for both species identification and study of phylogenetic relationships among basidiomycetous yeasts.

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