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Molecular Taxonomy of the Yeasts

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The term 'yeast' is often taken as a synonym for *Saccharomyces cerevisiae*, but the phylogenetic diversity of yeasts is illustrated by their assignment to two taxonomic classes of fungi, the ascomycetes and the basidiomycetes. Subdivision of taxa within their respective classes is usually made from comparisons of morphological and physiological features whose genetic basis is often unknown. Application of molecular comparisons to questions in yeast classification offers an unprecedented opportunity to re-evaluate current taxonomic schemes from the perspective of quantitative genetic differences. This review examines the impact of molecular comparisons, notably rRNA/rDNA sequence divergence, on the current phenotypically defined classification of yeasts. Principal findings include: 1) budding ascomycetous yeasts are monophyletic and represent a sister group to the filamentous ascomycetes, 2) fission yeasts are ancestral to budding and filamentous ascomycetes, 3) the molecular phylogeny of basidiomycetous yeasts is generally congruent with type of hyphal septum, presence or absence of teliospores in the sexual state, and occurrence of cellular xylose.

KEY WORDS — Yeasts; systematics; ribosomal RNA/DNA; molecular evolution; phylogeny.

INTRODUCTION

The yeasts represent a unique group of fungi characterized by vegetative growth that is predominantly unicellular, and by the formation of sexual states which are not enclosed in fruiting bodies. *Saccharomyces cerevisiae* has been recognized as an ascomycete for well over a century, but it was not until much later that some yeasts were thought to be basidiomycetes (Kluyver and van Niel, 1927). This supposition was confirmed by Banno (1967) with the description of the heterobasidiomycetous species *Rhodosporidium toruloides*. With this finding came the realization that the yeasts are phylogenetically quite divergent. The classification system presently used for the yeasts is based predominantly on phenotypic characters such as physiological reactions and the morphology of vegetative and sexual states. Because little is known of the genetic basis for many of these characters, current taxonomy is unlikely to accurately predict evolutionary relationships.

The use of molecular methods to estimate genetic relatedness among the yeasts had its beginnings in comparisons of nuclear DNA complementarity. Strains defined as conspecific from measurements of DNA relatedness were shown to differ in glucose fermentation, nitrate assimilation, and formation of pseudohyphae and true hyphae (Kurtzman and Phaff, 1987). These characters had been considered phylogenetically important and were used to define species and genera. Because of these findings, the genus *Torulopsis* (absence of pseudohyphae) became a synonym of *Candida* (presence of pseudohyphae) and *Hansenula* (assimilation of nitrate) became a synonym of *Pichia* (inability to assimilate nitrate). Comparisons of nuclear DNA relatedness resolve only the genetic distance of sibling species and thus provide no information about broader relationships (Kurtzman, 1987).

Comparisons of ribosomal RNA (rRNA) and its template ribosomal DNA (rDNA) have been used extensively in recent years to assess both close and distant relationships among many kinds of organisms. The interest in rRNA/rDNA comes from two important properties: 1) ribosomes are present in all cellular organisms and appear to share a common evolutionary origin, thus providing a molecular history shared by all organisms, 2) some rRNA/rDNA sequences are sufficiently conserved that they are homologous for all organisms and serve as reference points that enable alignment of the less conserved areas used to measure evolutionary relationships.

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In this review, I will describe the impact that rRNA/rDNA comparisons are beginning to have on our understanding of evolutionary relationships among the yeasts. Additionally, I will discuss application of this new information to the practical goal of rapid yeast identification.

METHODS FOR ISOLATION AND CHARACTERIZATION OF rRNAs AND rDNAs

rRNAs occur in several size classes in eukaryotes; the genes coding for large (25S to 28S), small (18S), and 5·8S rRNAs occur as tandem repeats with as many as 100 to 200 copies. The separately transcribed 5S rRNA gene may also be included in the repeats (Garber *et al.*, 1988). Each of the rRNA size classes has been examined for extent of phylogenetic information present.

The large quantities of rRNAs expressed by cells make isolation and purification of these molecules relatively easy despite the nearly ubiquitous occurrence of stable RNases. Numerous methods for isolation and purification of RNAs have been described. The procedures of Chirgwin *et al.* (1979) and their modification by Kurtzman and Liu (1990) are generally satisfactory. Techniques for the isolation and characterization of rDNA have been described by White *et al.* (1990) and Vilgalys and Hester (1990).

Methods for sequencing nucleic acids are now commonplace. Techniques for 5S rRNA sequencing have been summarized and discussed by Walker (1984, 1985). The procedures used for sequencing large and small subunit RNAs and DNAs are based on the dideoxy method of Sanger *et al.* (1977). Lane *et al.* (1985) have described the application of this method to rRNA sequencing through use of oligonucleotide primers and reverse transcriptase. Most initial comparisons of yeasts and other microorganisms were based on reverse transcriptase mediated sequencing of rRNAs because of the relative simplicity of this method over earlier rDNA sequencing techniques. Complete sequences were not often determined because McCarroll *et al.* (1983) and Lane *et al.* (1985) demonstrated that partial sequences of small subunit rRNAs provided essentially the same phylogenies as complete sequences.

White *et al.* (1990) and Kaltenboeck *et al.* (1992) provided protocols for sequencing rDNA using specific oligonucleotide primers and the polymerase chain reaction (PCR). rDNA sequencing results in fewer artifacts than rRNA sequencing, and the method offers the opportunity to sequence both strands of the rDNA genes, thus further reducing errors. Recently, methodologies again evolved with the introduction of automated sequencing.

ESTIMATES OF RELATEDNESS FROM rRNA - rDNA COMPARISONS

rRNA - rDNA reassociation

The first extensive use of rRNA comparisons for yeast systematics was described by Bicknell and Douglas (1970), who measured species divergence from the extent of reassociation between tritium-labeled 25S rRNA and complementary sites of filter-bound nuclear DNA. This and similar methods have been used by other workers but, because all species pairs must be tested, the comparison of large numbers of taxa is quite laborious. Another aspect of this procedure is that as evolutionary distances increase, a point is reached at which there is insufficient base sequence similarity to allow duplexing of paired molecules. It has been suggested that sequences must exhibit 75 to 80% or greater similarity before reassociation can occur (Bonner *et al.*, 1973).

Restriction fragment length polymorphisms of rDNA

rDNAs occur in multiple copies and lend themselves to analysis based on restriction fragment length polymorphisms (RFLP). Magee *et al.* (1987) treated rDNAs from several medically important *Candida* species with a variety of restriction endonucleases and concluded that *Candida guilliermondii*, *C. tropicalis*, and *C. albicans* produced sufficiently different digestion patterns to allow recognition of each species. Similar results were obtained by Vilgalys and Hester (1990) for several species of the genus *Cryptococcus*. Lachance (1990) used RFLP patterns to map the genetic profiles of 125 isolates of the cactus yeast *Clavispora opuntiae* that had been collected worldwide. Nearly all of the restriction sites that allowed discrimination of individual strains were located in the hypervariable intergenic spacer region.

Data from the preceding studies show that RFLP patterns allow recognition of individual species, as well as individual strains of a species. Consequently, the method has considerable diag-

nostic value. Estimates of evolutionary relationships from RFLP patterns have been reported for species assigned to *Candida* (Magee *et al.*, 1987) and *Cryptococcus* (Vilgalys and Hester, 1990). Such estimates would be expected to be less accurate than estimates derived from sequence comparisons because as evolutionary distances increase, the extent of pattern similarities becomes less certain.

5S rRNA

Because of the conserved nature and small size (ca. 120 nucleotides) of 5S rRNAs, their sequences are easily determined and have been widely used for estimating broad phylogenetic relationships (Hori and Osawa, 1979). Walker and Doolittle (1982) compared 5S rRNAs from eight basidiomycetes, including four yeasts, and concluded that sequence similarity correlated with the structure of hyphal septa (i.e., simple pores versus dolipores). The report of Gottschalk and Blanz (1984) that rust fungi, which have simple septal pores, cluster with the group defined by Walker and Doolittle as having dolipore septa proved incorrect because contaminating yeasts had been sequenced instead of the yeast stages of the rusts (P.A. Blanz, personal communication).

The studies of 5S rRNAs from ascomycetous yeasts have been less extensive than the studies of 5S rRNAs from basidiomycetous species. Mao *et al.* (1982) reported that the 5S sequence of *Schizosaccharomyces pombe* differed sufficiently from that of *Saccharomyces cerevisiae* to suggest that these two organisms are phylogenetically quite divergent. Similar results were obtained by Walker (1985), who further showed the ascomycetes to be divided among three groups: 1) *Schizosaccharomyces* and *Protomyces,* 2) budding yeasts, and 3) filamentous fungi.

Small subunit and large subunit rRNA - rDNA sequences

Close relationships The compilation of large subunit RNA sequences by Gutell and Fox (1988) demonstrated the 5' end of this molecule to be quite variable and of potential use for detection of closely related species. Peterson and Kurtzman (1991) examined sequence divergence in this region for sibling species pairs from several yeast genera. These data showed that nucleotide differences in region 25S-635 of Peterson and Kurtzman (domain D2, Guadet *et al.*, 1989) are sufficient to separate nearly all sibling species (Table 1). One exception is the pair *Saccharomyces bayanus/S. pastorianus*. It is believed that the latter species arose as a partial amphidiploid following chance hybridization between *S. cerevisiae* and *S. bayanus*, and that it retains the rDNA of *S. bayanus* (Kurtzman and Robnett, 1991; Peterson and Kurtzman, 1991; Vaughan Martini and Kurtzman, 1985). *Williopsis saturnus* and its variety *sargentensis*, which show no nucleotide differences in the region sequenced, are insufficiently studied to comment on their apparent lack of divergence. Some sibling species pairs show a five-fold difference in substitutions over that of other pairs, but this may not be definitive evidence for proposing unequal rates of nucleotide substitutions among species until the genetic processes that initiate species formation are better understood. With few exceptions, the D2 region is sufficiently variable to recognize ascomycetous and basidiomycetous yeast species, including most sibling pairs. Conspecific strains ordinarily show 0–1% divergence; the distantly related species *Pichia bimundalis* and *Schizosaccharomyces japonicus var. versatilis* exhibit ca. 47% substitutions (Peterson and Kurtzman, 1991).

Distant relationships

— Ascomycetous yeasts

The phylogeny of the ascosporogenous yeasts has been vigorously debated since the time of Guilliermond (1912) and before. Some have viewed the yeasts as primitive fungi while others perceived them to be reduced forms of more evolved taxa. Cain (1972) has been a proponent of this latter idea, arguing that hat (galeate)-spored genera such as *Pichia* and *Cephaloascus* are likely to be reduced forms of the perithecial euascomycete genus *Ceratocystis*. Redhead and Malloch (1977) and von Arx and van der Walt (1987) accepted this argument and commingled yeasts and mycelial taxa in their treatments of the Endomycetales and Ophiostomatales.

Examination of rRNA/rDNA sequence divergence from a limited number of taxa indicated that the ascosporogenous yeasts, with the exception of *Schizosaccharomyces*, form a monophyletic group (clade) distinct from the filamentous species (Barns *et al.*, 1991; Bruns *et al.*, 1991; Hausner *et al.*, 1992; Hendriks *et al.*, 1992; Kurtzman, 1993; Nishida and Sugiyama, 1993; Walker, 1985; Wilmotte *et al.*, 1993). Kurtzman and Robnett

Table 1. Extent of ribosomal RNA sequence divergence among sibling yeast species*

Species Pair	Percent DNA Relatedness†	Percent Nucleotide Differences in Region 25S-635‡
Heterothallic Species		
Saccharomyces cerevisiae X *S. pastorianus*	58	5·4
Saccharomyces cerevisiae X *S. bayanus*	10	5·4
Saccharomyces pastorianus X *S. bayanus*	70	0·0
Pichia mississippiensis X *P. amylophila*	25	2·0
Pichia bimundalis X *P. americana*	21	1·0
Issatchenkia scutulata X var. *exigua*	25	5·1
Homothallic Species		
Debaryomyces melissophilus X *D.* sp. n.	30	0·7
Saturnospora saitoi X *S. ahearnii*	30	2·0
Williopsis saturnus X var. *sargentensis*	43	0·0

* Data from Kurtzman, 1984; Kurtzman and Robnett, 1991; Kurtzman *et al.*, 1980a, b; Liu and Kurtzman, 1991; Peterson and Kurtzman, 1990, 1991; Vaughan Martini and Kurtzman, 1985.

† Conspecific strains generally exhibit in excess of 70% nuclear DNA relatedness. Varietal designations have been accorded those strains showing 40-70% DNA relatedness unless genetic comparisons demonstrate the absence of interfertility. *I. scutulata* and its variety *exigua* show some intervarietal fertility and, at 25% DNA relatedness, appear to be exceptional because limited fertility would not be expected.

‡ Ca. 300 nucleotides in region 25S-635.

(1994a) analyzed rRNA sequence divergence from type species of all cultivatable ascomycetous yeasts and yeast-like taxa. This work demonstrated the yeasts, as well as yeast-like genera such as *Ascoidea* and *Cephaloascus,* to comprise a clade sister to the 'filamentous' ascomycetes (euascomycetes). *Eremascus*, which forms asci unenclosed in a fruiting body, aligned with the euascomycete clade and may represent a genus close to the phylogenetic demarcation of the hemiascomycetes (excluding *Schizosaccharomyces*) and the euascomycetes. These results substantiate the long-held observation that yeasts cannot be defined solely on the basis of presence or absence of budding. Such members of the yeast clade as *Ascoidea*, *Ashbya* and *Eremothecium* show no typical budding, whereas *Aureobasidium*, *Phialophora* and certain other genera of euascomycetes are usually dimorphic. Budding is also a common mode of vegetative reproduction among many basidiomycetous genera. Similarly, vegetative reproduction by fission is shared by *Dipodascus* and *Galactomyces,* members of the yeast clade, as well as by the distantly related genus *Schizosaccharomyces*. Sexual states of all members of the yeast clade are characterized by asci unenclosed in a fruiting body. This feature is shared by only a few taxa outside the yeast clade such as *Eremascus* and *Schizosaccharomyces*. *Myriogonium* and *Trichomonascus* form unenclosed asci but may be euascomycetes as well.

Phylogenetic relationships among the ascomycetous yeasts, calculated from partial sequences of small and large subunit rRNAs, are depicted in Fig. 1. Although there are insufficient phylogenetically informative sites to resolve many of the genera, the comparison gives an overview of relationships. Tree topology is similar to that presented by Wilmotte *et al.* (1993), who examined fewer species, but used nearly complete 18S sequences. In the comparison of partial sequences, most taxa having coenzyme Q with the same number of isoprene units tend to group, but this is not true of the closely related genera *Ashbya*, *Eremothecium, Holleya* and *Nematospora* which show a variation in coenzyme Q ranging from 5-9 isoprene units. Some congruence is found between location of taxa on the rRNA gene tree and the type of hyphal septal pore produced. The two known genera (*Ambrosiozyma, Hormoascus*) that

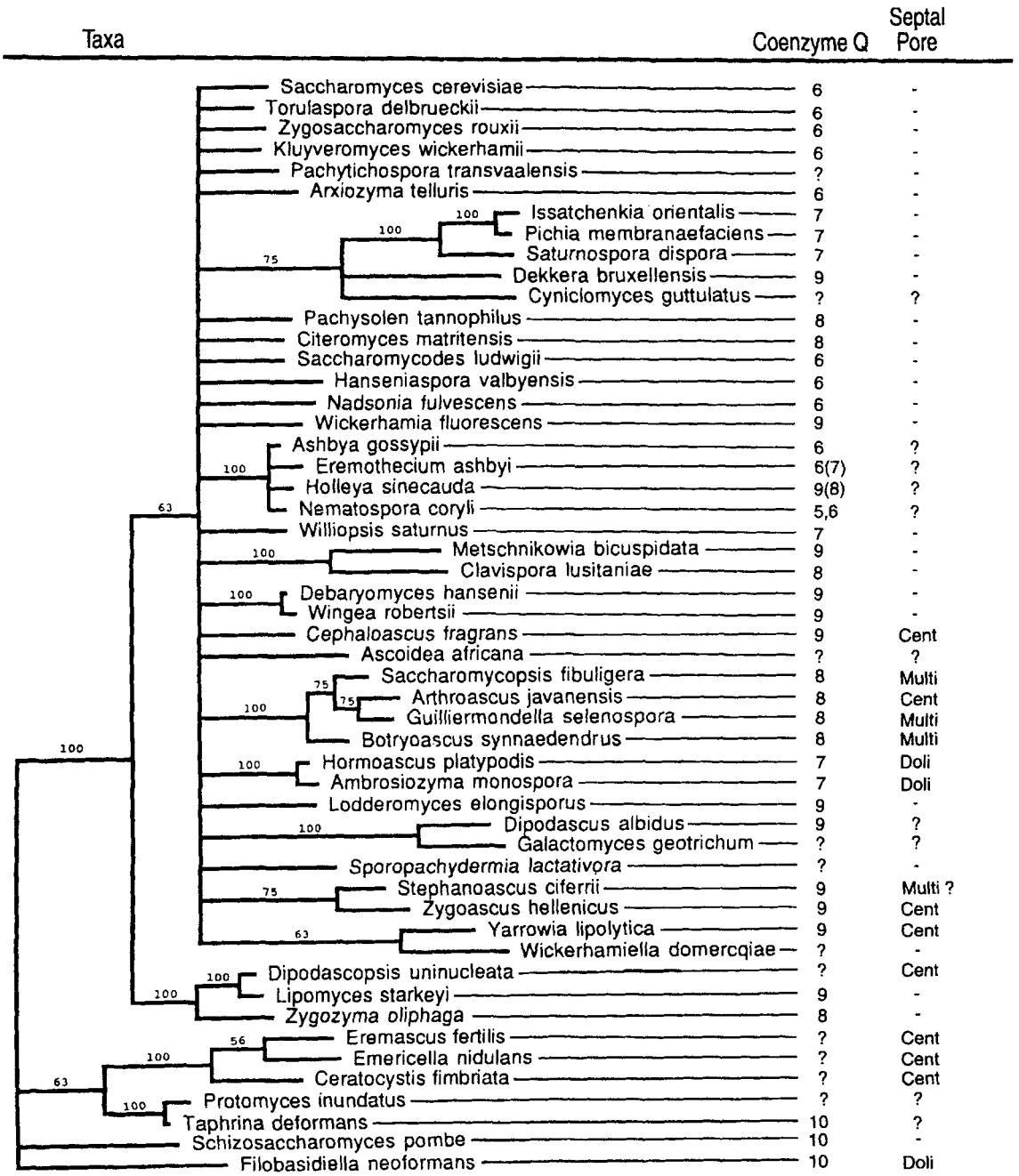


Figure 1. A phylogenetic tree derived from maximum parsimony analysis depicting the ascomycetous yeasts, yeast-like fungi, and various reference species. The phylogram was calculated from combined small and large subunit rRNA partial sequences as described by Kurtzman and Robnett (1994a). Branch lengths are proportional to nucleotide differences, and the numbers given on branches are the percentage of frequencies with which a given branch appeared in 100 bootstrap replications. Branches without numbers had frequencies of less than 50%. Coenzyme Q data are from the compilation of Barnett *et al.* (1990) and refer to the number of isoprene units in the sidechain on the parent molecule. Information on septal pores is from Kreger-van Rij and Kurtzman (1984) and Barnett *et al.* (1990).
Cent = central pore, Multi = multiperforate septum, Doli = dolipore-like.

form dolipore-like septa are closely associated. However, *Arthroascus* produces septa with a simple central pore whereas *Guilliermondella* has multiperforate septa, and yet the two genera closely cluster. These incongruities may be resolved by sequencing the complete 18S molecule and by including all known species for each of the genera under study.

rRNA/rDNA sequence comparisons have been quite helpful for understanding species relationships within genera. For example, *Schwanniomyces occidentalis* (Kurtzman and Robnett, 1991), *Wingea robertsii* (Kurtzman and Robnett, 1994b), and the *Pichia* species *P. carsonii* and *P. etchellsii* (Yamada *et al.*, 1992), were found to be members of the genus *Debaryomyces* on the basis of rRNA relatedness (Fig. 2). The initial assignment of these species to other genera resulted from misinterpretation of the phylogenetic significance of ascospore morphology. Species originally placed in *Debaryomyces* are characterized by spheroidal ascospores that are roughened by wartlike or ridgelike outgrowths of wall material. An exception is *D. marama* which has ellipsoidal ascospores with wartlike outgrowths and spiral ridges. In contrast, *Schwanniomyces* forms spheroidal ascospores with surface projections and a prominent equatorial ring, *Wingea* has smooth, lenticular ascospores, and the two former *Pichia* species produce smooth, spheroidal ascospores. In contrast, *Saturnospora* and *Williopsis* both form saturnoid ascospores, yet the two genera are only distantly related (Liu and Kurtzman, 1991).

The impact of rRNA/rDNA comparisons on the taxonomy of ascomycetous yeasts is just being felt and will require additional work to fully realize its potential. Major findings to date include: 1) yeasts and yeastlike species are phylogenetically separate from the euascomycetes, 2) the fission yeast genus *Schizosaccharomyces* is phylogenetically distant from the 'budding' yeast clade and from the euascomycetes, resulting in the reassignment of the fission yeasts to a separate order, the Schizosaccharomycetales (Eriksson *et al.*, 1993; Kurtzman, 1993) and, 3) the demonstration that many phenotypic characters such as ascospore morphology are poor indicators of phylogeny.

— Basidiomycetous yeasts

Anamorphic (asexual) basidiomycetous yeasts may be morphologically indistinguishable from anamorphic ascomycetous yeasts. The discovery that the inner cell walls of basidiomycetous yeasts are lamellar when viewed in thin section under the transmission electron microscope, in contrast to the uniform inner layer of ascomycetes, has provided a reliable means for separation of the two taxonomic classes when sexual states are not found (Kreger-van Rij and Veenhuis, 1971). A second method of separation, more easily applied, is the Diazonium Blue B (DBB) staining technique (van der Walt and Hopsu-Havu, 1976). Colonies of basidiomycetes stain a magenta color in the presence of DBB whereas colonies of ascomycetes remain unstained. From these findings, it has become apparent that basidiomycetes make up a large part of the yeast domain.

There are two general types of teleomorphic (sexual) states found among the basidiomycetous yeasts (Fell and Kreger-Van Rij, 1984; Boekhout *et al.*, 1993). In the first, teliospores are formed and germinate to produce a basidium that bears basidiospores. This type of sexual cycle shows considerable similarity to the rust and smut fungi. The second type of sexual state lacks teliospores. Basidia develop on hyphae or yeast cells and give rise to basidiospores in a manner similar to the Tremellales (jelly fungi).

Several other characteristics are added to the dichotomy of sexual states. Some taxa produce carotenoids, and the presence of the pigments has been used as a criterion for genus assignment. Ballistoconidia, forcibly ejected vegetative cells, are common to some taxa and their presence is a defining character of genera. Additionally, the hyphal septal pore of basidiomycetous yeasts may be either simple or the ultrastructurally more complex dolipore. Finally, some taxa exhibit the presence of cellular xylose, evidently arising from extracellular polysaccharides (Golubev, 1991), whereas other species do not.

Guého *et al.* (1989) presented an overview of the phylogeny of basidiomycetous yeasts from measurements of divergence among partial sequences of large and small subunit rRNAs. Three major groups were resolved: 1) teliospore formers with hyphae having simple septal pores, 2) teliospore formers with hyphae having dolipore septa and, 3) non-teliospore formers with hyphae having dolipore septa. On the basis of 18S sequence comparisons, Suh and Sugiyama (1993) placed the smut fungus *Ustilago maydis*, a teliospore former, near the clade comprising representative genera of all basidiomycetous yeasts. In turn, the basidiomycetous yeasts appear to be a sister group to the Agaricales (Berbee and Taylor, 1993).

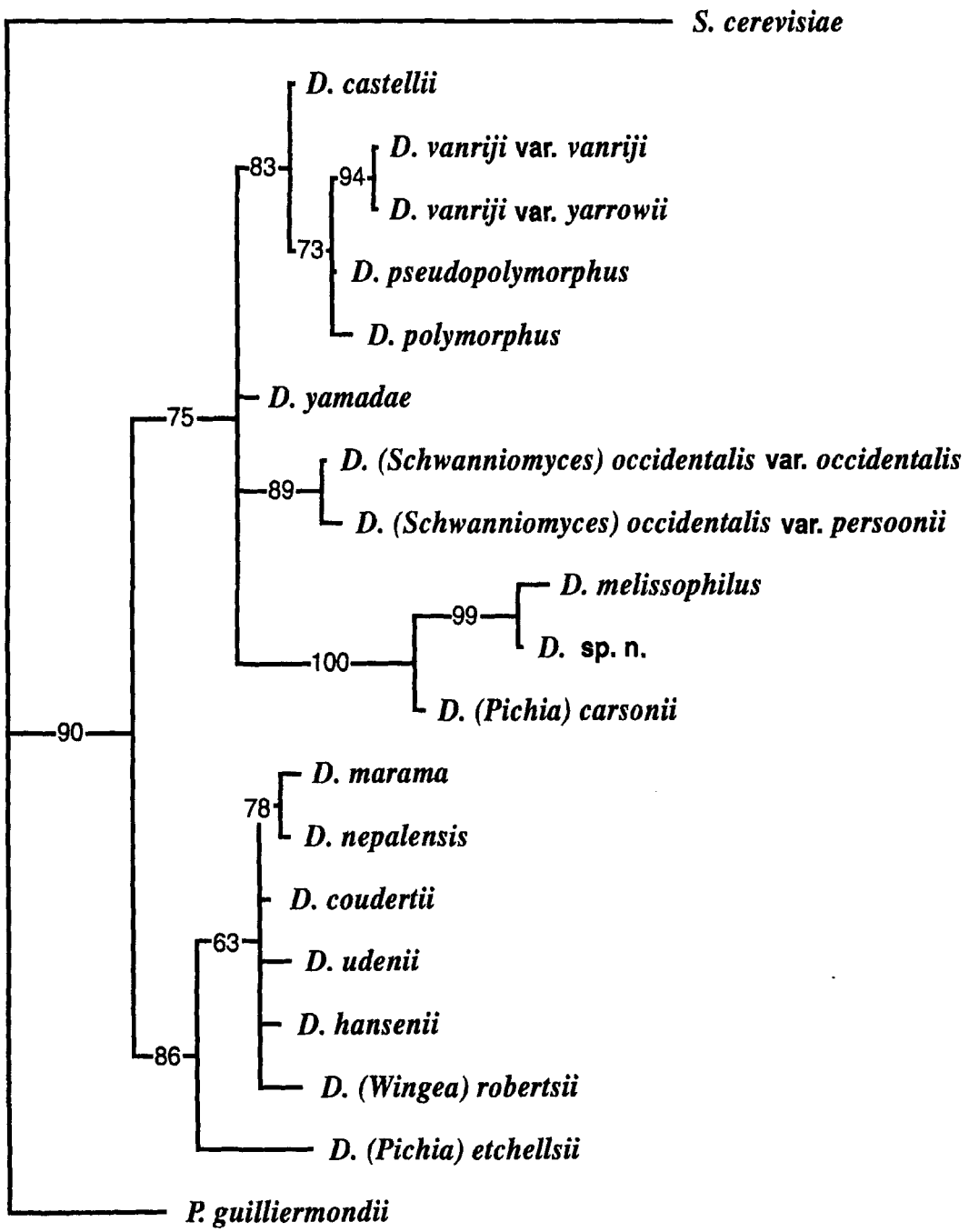
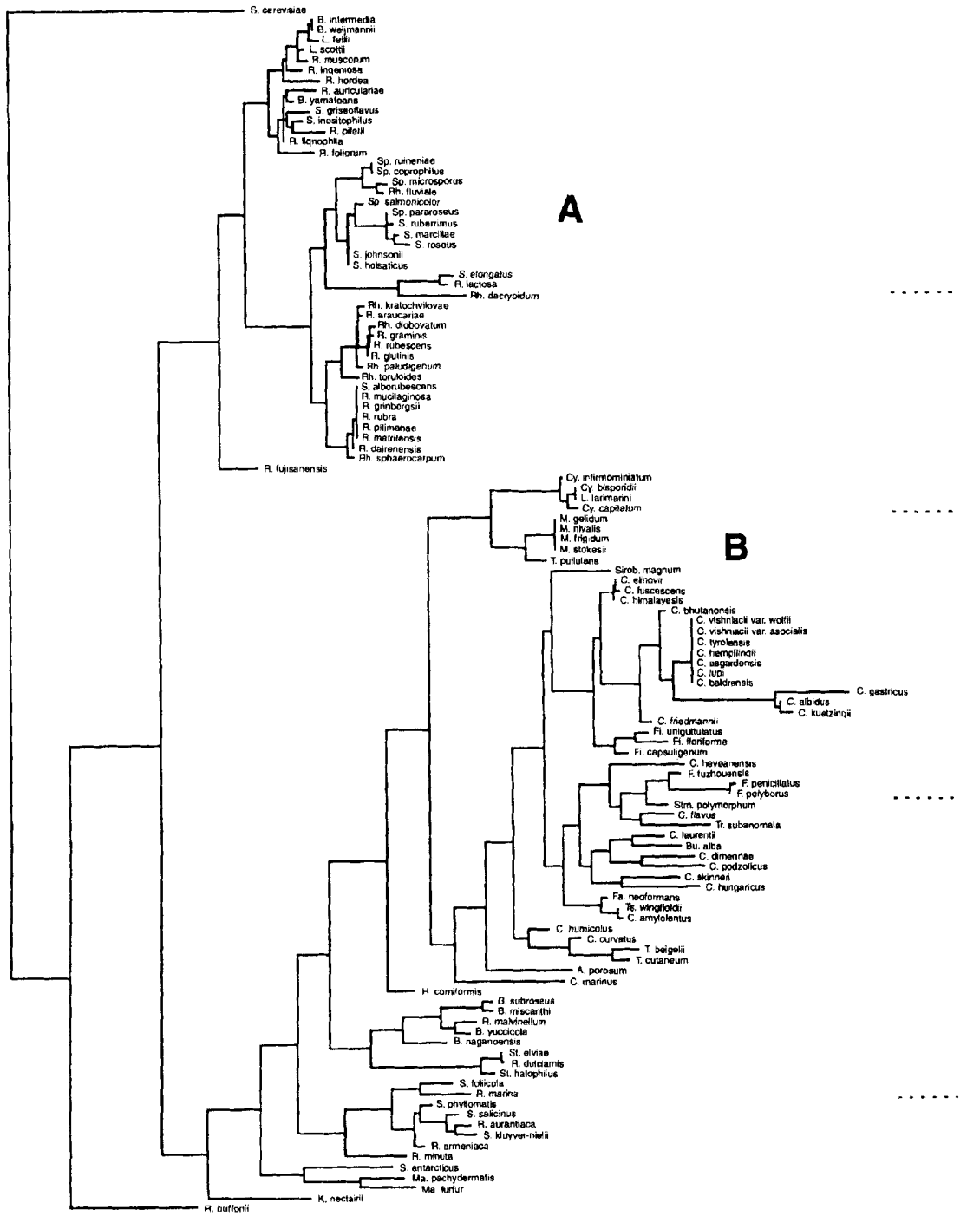


Figure 2. A phylogenetic tree of the genus *Debaryomyces* derived from maximum parsimony analysis of combined small and large subunit rRNA partial sequences as described by Kurtzman and Robnett (1991, 1994b). Branch lengths are proportional to nucleotide differences, and the numbers given on branches are the percentage of frequencies with which a given branch appeared in 1000 bootstrap replications. Sequence analysis showed *Pichia carsonii*, *P. etchellsii* and species of the monotypic genera *Schwanniomyces* and *Wingea* to be members of the genus *Debaryomyces* despite differences in their ascospore morphology. *Debaryomyces* is comprised of two subclades.

Phylogenetic relationships among the species and genera



Key morphological and biochemical characters of the genera

Genus	Telio-spores	Ballisto-conidia	Septal pore	Co-Q	Cellular xylose
Leucosporidium (L)[T]	+	-	Simple	9, 10	-
Sporidiobolus (Sp)[T]	+	+	Simple	10	-
Sporobolomyces (S)[A]	?	+	Simple	10	-
Erythrobasidium[T]	-	-	Simple	10 (H_2)	-
Rhodotorula (R)[A]	?	-	Simple	9, 10	-
Rhodosporidium (Rh)[T]	+	-	Simple	9, 10	-
Cystofilobasidium (Cy)[T]	+	-	Doli	8	+
Phaffia[A]	?	-	?	10	+
Mrakia (M)[T]	+	-	Doli	8	+
Sirobasidium (Sirob)[T]	-	+?	Doli	?	?
Cryptococcus (C)[A]	?	-	?	9, 10	+
Filobasidium (Fi)[T]	-	-	Doli	9, 10	+
Fellomyces (F)[A]	?	-	?	10	+
Kockovaella[A]	?	+	?	10	+
Sterigmatosporidium (Stm)[T]	+?	-	?	10	+
Tremella (Tr)[T]	-	-	Doli	10	+
Bulleromyces (Bu)[T] (Bullera)[A]	-	+	Doli	10	+
Filobasidiella (Fa)[T]	-	-	Doli	10	+
Tseuchiyaea (Ts)[A]	?	-	?	10	+
Trichosporon (T)[A]	?	-	Simple/Doli	9, 10	+
Apiotrichum (A)[A]	?	?	?	?	?
Holtermannia (H)[T]	-	?	Doli	?	?
Kondoa (=Rh. malvinellum)[T]	+	-	Simple	9	-
Bensingtonia (B)[A]	?	+	Simple	9	-
Sterigmatomyces (St)[A]	?	-	?	9	-
Ballistosporomyces[A]	?	+	?	10	-
Malassezia (Ma)[A]	?	-	?	?	-
Kurtzmanomyces (K)[A]	?	-	?	10	-

At present, the most extensive phylogenetic comparison of basidiomycetous yeasts is that of Fell *et al.* (1992) who examined 117 species assigned to 23 genera. A 247-nucleotide segment in the D2 region was sequenced; this region resolves closely related species, but may have too few phylogenetically informative sites to accurately assess more distant relationships. The phylogram from that work (Fig. 3) shows the species to be divided between two major clades. The analysis generally supports the concept that taxa assigned to the Tremellales are characterized by dolipore septa and cellular xylose, whereas taxa placed in the Ustilaginales form teliospores, have simple septal spores, and lack cellular xylose. Some exceptions are apparent. The teleomorphic genus *Erythrobasidium* does not form teliospores as do other members of the clade. *Cystofilobasidium* and *Mrakia*, both members of the Tremellales, form teliospores. Another inconsistency concerns the genera located in the lower portion of the Tremellales clade (Fig. 3, B). From what is known of their septal pore structure and lack of cellular xylose, the genera would be expected to group with the Ustilaginales. If their placement is correct, this would suggest that the Tremellales arose from within an already highly diversified group that now represents the Ustilaginales.

Ballistoconidia and carotenoids are found among many genera of the basidiomycetous yeasts suggesting these traits to be ancestral, but not always expressed, thus rendering them of little value for defining taxa. These conclusions were also drawn by Nakase *et al.* (1993).

Heterogeneity of coenzyme Q composition occurs in many currently defined genera and will require additional study before its taxonomic significance is fully understood. rRNA sequence analysis demonstrates *Rhodotorula*, *Sporobolomyces*, *Cryptococcus*, and *Bensingtonia* to be polyphyletic, further confirming that commonly used phenotypic characters are insufficient for defining anamorphic genera.

A strong start has been made to understand the phylogeny of basidiomycetous yeasts from rRNA/rDNA sequence comparisons, but additional sequencing must be done to better resolve taxa. This will include sequences of greater length as well as inclusion of all known species of a group. For example, the anamorphic genera *Tseuchiyaea* and *Ballistosporomyces*, which were recently defined from differences in partial sequences, may overlap with some earlier described genera (Fig. 3).

RELIABILITY OF rRNA/rDNA GENE TREES TO INFER PHYLOGENY

With such great emphasis being placed on rRNA/rDNA gene trees to reconstruct phylogenies, it must be asked if there is other evidence to corroborate the conclusions drawn. There are presently only a few comparisons of other molecular sequences that allow this question to be addressed. Sequence analysis of orotidine 5'-monophosphate decarboxylase by Radford (1993) demonstrated the budding yeasts and the euascomycetes to be sister groups as shown from rRNA/rDNA sequences. One unusual aspect of this work was placement of the Mucorales as a sister group to the basidiomycetes. Tsai *et al.* (1994) showed that phylogenetic relationships among species of *Epichloë* (Clavicipitaceae) were the same when analyzed from either rDNA sequences or those from the β-tubulin gene. Relationships among species of *Dekkera* and its anamorph *Brettanomyces* were essentially identical when analyzed from either nuclear rDNA sequences or from sequences of the mitochondrially encoded cytochrome oxidase subunit II gene (Boekhout *et al.*, 1994).

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Figure 3. A phylogenetic tree derived from maximum parsimony analysis depicting the basidiomycetous yeasts. The phylogram, which represents the most parsimonious tree, was calculated from rRNA sequences of the D2 region of the large subunit as described by Fell et al. (1992). Branch lengths are proportional to the number of nucleotide differences. Bootstrap values were not determined. Clade A is comprised of taxa placed in the Ustilaginales. Taxa in Clade B are predominantly assigned to the Tremellales, but see text for discussion. The strain of the outlying species *Rhodotorula buffonii* that was examined was shown to be a misidentified ascomycete. Genera preceded by a broken line were not in the original analysis and have been placed in the present phylogram on the basis of other studies (Guého *et al.*, 1989; Nakase *et al.*, 1993; and Sugiyama and Suh, 1993). Teleomorphic genus names are followed by the letter T and anamorphic genera are designated with the letter A. Morphological and biochemical characters are from Barnett *et al.* (1990), Boekhout *et al.* (1993) and Fell and Kreger-van Rij (1984).

Simple = simple septal pore, Doli = dolipore septum.

Another aspect of gene tree reliability is the method used for its construction. Most investigators now analyze data using phylogeny inference programs based on cladistic principles. These programs often include a statistics package to test the robustness of competing phylogenetic trees. Several recent reviews address these important issues (Avise, 1989; Felsenstein, 1988; Hillis *et al.*, 1994; Saitou and Imanishi, 1989).

RAPID MOLECULAR METHODS FOR YEAST IDENTIFICATION

The specificity of nucleic acid sequences has prompted development of several methods for rapid species identification. Because these techniques can detect single nucleotide changes, they should first be tested on a large variety of genetically defined strains to understand species variation. Aberrant strains may represent different species.

The RFLP technique was discussed earlier in the review and has been used extensively in some laboratories. Bruns *et al.* (1991) listed some of the factors that require attention when using RFLPs. Random amplified polymorphic DNA (RAPD) is another methodology that promises widespread application. The technique is based on amplification of genomic DNA in the presence of one or more short (ca. 10-15-mers) oligonucleotide primers of random sequence. The amplified products are visualized on an agarose gel and strains identified from matching band patterns. Hadrys *et al.* (1992) discussed details of this procedure noting points of technical difficulty. Fell (1993) applied a three-primer, PCR-based technique to yeast identification that appears species-specific. The reaction mix includes genomic DNA, two external primers for the D1/D2 region of large subunit rDNA, and a species-specific internal primer. The external primers allow amplification of the ca. 600-nucleotide D1/D2 region, but in the presence of a species-specific primer (third primer), the amplification product is shorter and easily detected on an agarose gel. There is currently considerable activity in the field of molecular probe development and rapid methods appear nearly ready for widespread use.

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