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Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses

X.-Z. Liu[1,4], Q.-M. Wang[1,4], B. Theelen[2], M. Groenewald[2], F.-Y. Bai[1,2*], and T. Boekhout[1,2,3*]

[1]*State Key Laboratory for Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China;* [2]*CBS Fungal Biodiversity Centre (CBS-KNAW), Uppsalalaan 8, Utrecht, The Netherlands;* [3]*Shanghai Key Laboratory of Molecular Medical Mycology, Changzheng Hospital, Second Military Medical University, Shanghai, PR China*

**Correspondence*: F.-Y. Bai, baify@im.ac.cn; T. Boekhout, t.boekhout@cbs.knaw.nl

[4]These authors contributed equally to this work.

Abstract: The Tremellomycetes (Basidiomycota) contains a large number of unicellular and dimorphic fungi with stable free-living unicellular states in their life cycles. These fungi have been conventionally classified as basidiomycetous yeasts based on physiological and biochemical characteristics. Many currently recognised genera of these yeasts are mainly defined based on phenotypical characters and are highly polyphyletic. Here we reconstructed the phylogeny of the majority of described anamorphic and teleomorphic tremellomycetous yeasts using Bayesian inference, maximum likelihood, and neighbour-joining analyses based on the sequences of seven genes, including three rRNA genes, namely the small subunit of the ribosomal DNA (rDNA), D1/D2 domains of the large subunit rDNA, and the internal transcribed spacer regions (ITS 1 and 2) of rDNA including 5.8S rDNA; and four protein-coding genes, namely the two subunits of the RNA polymerase II (*RPB1* and *RPB2*), the translation elongation factor 1-α (*TEF1*) and the mitochondrial gene cytochrome *b* (*CYTB*). With the consideration of morphological, physiological and chemotaxonomic characters and the congruence of phylogenies inferred from analyses using different algorithms based on different data sets consisting of the combined seven genes, the three rRNA genes, and the individual protein-coding genes, five major lineages corresponding to the orders Cystofilobasidiales, Filobasidiales, Holtermanniales, Tremellales, and Trichosporonales were resolved. A total of 45 strongly supported monophyletic clades with multiple species and 23 single species clades were recognised. This phylogenetic framework will be the basis for the proposal of an updated taxonomic system of tremellomycetous yeasts that will be compatible with the current taxonomic system of filamentous basidiomycetes accommodating the ‘one fungus, one name’ principle.

Key words: Fungi, Basidiomycota, Tremellomycetes, Yeasts, Multigene phylogeny.

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INTRODUCTION

Unicellular basidiomycetes and dimorphic basidiomycetes with a stable free-living unicellular state during their life cycles are recognised as basidiomycetous yeasts (Boekhout *et al.* 2011). They occur in all three subphyla of the Basidiomycota, namely Agaricomycotina, Pucciniomycotina and Ustilaginomycotina, which are presently recognised on the basis of molecular phylogenetic analyses (Fell *et al.* 2000, Scorzetti *et al.* 2002, James *et al.* 2006, Hibbett *et al.* 2007, Wuczkowski *et al.* 2011). Yeast taxa in the Agaricomycotina occur only in the basal Tremellomycetes lineage (Hibbett 2006, Boekhout *et al.* 2011, Weiß *et al.* 2014). Phenotypic and molecular analyses revealed a close affiliation of basidiomycetous yeasts with various groups of filamentous basidiomycetes (Millanes *et al.* 2011). However, yeasts and filamentous fungi have conventionally been studied by different scientific communities and classified using different criteria, resulting in the developments of hitherto independent taxonomic systems of the two groups of fungi. Recent molecular phylogenetic studies have shown the incompatibility between the taxonomic system of basidiomycetous yeasts and that of filamentous basidiomycetes. Furthermore many currently recognised genera of tremellomycetous yeasts, which are mainly defined based on phenotypic characters, are strikingly polyphyletic. For example, species of the genus *Cryptococcus* are located in all currently recognised orders of Tremellomycetes and occur intermingled with species of other genera, such as *Bullera* (Fell *et al.* 2000, Boekhout *et al.* 2011, Fonseca *et al.* 2011, Millanes *et al.* 2011, Weiß *et al.* 2014). The polyphyletic nature of the traditionally defined teleomorphic genus *Tremella*, which is usually dimorphic, is also remarkable. Several monophyletic clades have been recognised among *Tremella* species which occur interspersed with other teleomorphic and anamorphic genera (Boekhout *et al.* 2011, Millanes *et al.* 2011, Weiß *et al.* 2014). These problems existing in the current taxonomic systems of both yeasts and filamentous fungi in the Tremellomycetes remain to be resolved.

The high-level classification of Basidiomycota has been updated with results from the Assembling the Fungal Tree of Life (AFTOL) project that used a multigene sequence analysis approach (Lutzoni *et al.* 2004, James *et al.* 2006, Hibbett *et al.* 2007). However, only a limited number of basidiomycetous yeast taxa were included in that project, making it impossible to propose a corresponding revision of the taxonomic system of basidiomycetous yeasts. Consequently, the artificial classification system of these organisms largely remained in the latest edition of ‘The Yeasts, a Taxonomic Study’ [hereafter referred to as ‘The Yeasts’] (Kurtzman *et al.*

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2011), due to the lack of reliable multigene phylogenetic studies of these yeasts.

The requirement for revising the taxonomic system of tremellomycetous yeasts has been emphasised by recent progress in biodiversity studies of yeasts and by the change of fungal nomenclature adopting the 'one fungus = one name' concept (Hawksworth 2011). Molecular taxonomic studies have resulted in the availability of a comprehensive sequence database of the large subunit of the ribosomal RNA gene (LSU rDNA) D1/D2 domains and the ITS (including 5.8S) regions of rDNA for almost all known basidiomycetous yeast species (Fell *et al.* 2000, Scorzetti *et al.* 2002, Wang & Bai 2008, Schoch *et al.* 2012). The rDNA sequence databases have become a molecular platform for rapid identification of yeasts, resulting in continued discovery of new taxa in recent years (Boekhout 2005, Wang & Bai 2008, Wuczkowski *et al.* 2011). The addition of these new species has contributed to the increase of the polyphyletic nature of many basidiomycetous yeast genera. For example, the distribution of *Bullera* species has expanded from Tremellales and Filobasidiales (Boekhout & Nakase 1998) to Trichosporonales (Nakase *et al.* 2002, Fungsin *et al.* 2006). With each new species being taxonomically misplaced, the chaos of the taxonomic system increases. Therefore, an updated taxonomic system is imperative for the correct placement of the vast amount of hidden yeast diversity.

Similar to filamentous fungi, many yeast species have separate teleomorphic and anamorphic names. As regulated by the new International Code of Nomenclature for algae, fungi and plants (Melbourne Code) (McNeill *et al.* 2012), after January 1 2013, only one name is legitimate regardless of whether or not a sexual state exists. An updated taxonomic system, especially the redefinition of genera based on a robust multigene phylogeny, will be required for the name choices and to minimise the possibility of name changes in the future. The purpose of this study is to confidently resolve the phylogenetic relationships among tremellomycetous yeasts and dimorphic fungi based on multiple gene sequence analyses, resulting in a framework that allows us to update the taxonomic system of yeasts and related taxa in the Tremellomycetes.

MATERIALS AND METHODS

Taxon sampling

A total of 294 tremellomycetous yeast strains were included in this study, which covered the type strains of 286 currently recognised species and varieties, the type strains of six synonyms, and two additional strains with mating types opposite to those of the type strains (Table 1). From the 240 tremellomycetous yeast species and varieties included in the latest edition of 'The Yeasts' (Kurtzman *et al.* 2011), 234 were included in this study. In addition, 52 tremellomycetous yeast species which were published too late for inclusion in the book were also employed in this study. The taxa sampled covered 16 teleomorphic and 19 anamorphic genera. The type strains of two pucciniomycetous and one ustilaginomycetous yeast species were employed as outgroup (Table 1).

DNA extraction, PCR, and sequencing

Genomic DNA was extracted from yeast cells actively growing on YPD medium using the method described in Bolano *et al.* (2001) with minor modifications. The UltraClean® Microbial DNA Isolation Kit (MO BIO, CA) was used when high quality DNA templates were required for PCR amplification of some protein genes. A set of six genes was selected and sequenced based on previous studies of the Assembling the Fungal Tree of Life (AFTOL-1) project (James *et al.* 2006, Hibbett *et al.* 2007). These genes included three rRNA genes, namely the small subunit (SSU or 18S) of the ribosomal DNA (rDNA), D1/D2 domains of the large subunit (LSU or 26S) rDNA, and the internal transcribed spacer regions (ITS 1 and 2) of the rDNA, including the 5.8S

Table 1. List of tremellomycetous yeasts and dimorphic taxa employed. The sequences with GenBank numbers in bold are determined in this study.

Lineage/clade	Species	Strain	ITS (5.8S)	D1D2	SSU	*RPB1*	*RPB2*	*TEF1*	*CYTB*
Cystofilobasidiales									
Cystofilobasidium	*Cystofilobasidium bisporidii**	CBS 6346^{T}	**KF036597**	EU085532	AB072225	**KF036419**	**KF036832**	**KF037103**	**KF423238**
	*C. capitatum**	CBS 6358^{T}	AF139627	AF075465	D12801	**KF036420**	**KF036833**	**KF037104**	/
	*C. ferigula**	CBS 7202^{T}	**KF036598**	CBS database	AB032628	/	/	**KF037105**	**KF423239**
	*C. infirmominiatum**	CBS 323^{T}	AF444400	AF075505	AB072226	/	**KF036834**	**KF037106**	**KF423240**
	*C. lacus-mascardii**	CBS 10642^{T}	EF613495	AY158642	**KF036665**	**KF036421**	**KF036835**	**KF037107**	**KF423241**
	*C. macerans**	CBS 10757^{T}	EU082231	EU082225	**KF036666**	**KF036423**	**KF036837**	**KF037109**	/
	*C. macerans**	CBS 2206	AF444329	AF189848	AB032642	**KF036422**	**KF036836**	**KF037108**	**KF423242**
Guehomyces	*Guehomyces pullulans*	CBS 2532^{T}	AF444417	EF551318	AB001766	**KF036478**	**KF036892**	**KF037155**	AF175778
	Tausonia pamirica	CBS 8428^{T}	**KF036600**	EF118825	**KF036692**	/	/	/	/
huempii	*Cryptococcus huempii*	CBS 8186^{T}	AF444322	AF189844	AB032636	**KF036377**	**KF036790**	**KF037062**	**KF423200**
	*Mrakia curviuscula**	CBS 9136^{T}	**KF036599**	EF118826	**KF036684**	**KF036510**	**KF036924**	**KF037185**	**KF423313**
Itersonilia	*Itersonilia perplexans*	CBS 363.85^{T}	AB072233	AJ235274	AB072228	/	**KF036900**	/	**KF423296**
	Udeniomyces pannonicus	CBS 9123^{T}	AB072229	AB077382	AB072227	**KF036579**	**KF036996**	**KF037251**	/
Mrakia	*Mrakia blollopis**	CBS 8921^{T}	AY038826	AY038814	**KF036683**	**KF036509**	**KF036923**	**KF037184**	/
	*M. frigida**	CBS 5270^{T}	AF144483	AF075463	D12802	**KF036511**	**KF036925**	**KF037186**	/