FEMS Yeast Research, 15, 2015, fov050

doi: 10.1093/femsyr/fov050
Advance Access Publication Date: 1 July 2015
Minireview

MINIREVIEW

Advances in yeast systematics and phylogeny and their use as predictors of biotechnologically important metabolic pathways

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One sentence summary: Next generation sequencing enables improved phylogenetic analysis of yeasts and enables prediction of metabolic properties of different yeast species.
Editor: Jens Nielsen

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ABSTRACT

Detection, identification and classification of yeasts have undergone a major transformation in the last decade and a half following application of gene sequence analyses and genome comparisons. Development of a database (barcode) of easily determined DNA sequences from domains 1 and 2 (D1/D2) of the nuclear large subunit rRNA gene and from ITS now permits many laboratories to identify species quickly and accurately, thus replacing the laborious and often inaccurate phenotypic tests previously used. Phylogenetic analysis of gene sequences is leading to a major revision of yeast systematics that will result in redefinition of nearly all genera. This new understanding of species relationships has prompted a change of rules for naming and classifying yeasts and other fungi, and these new rules are presented in the recently implemented *International Code of Nomenclature for algae, fungi, and plants* (Melbourne Code). The use of molecular methods for species identification and the impact of Code changes on classification will be discussed, as will use of phylogeny for prediction of biotechnological applications.

Keywords: yeasts; taxonomy; systematics; biotechnology; ecology

INTRODUCTION

Prior to phylogenetic analyses of DNA sequences, placement of yeasts among the fungi was guided by interpretation of phenotype, which suggested that yeasts are primitive fungi (e.g. Guilliermond 1912) or, alternatively, that they may be reduced forms of more mycelial taxa (Cain 1972; von Arx and van der Walt 1987). Initially, it was thought that all yeasts are ascomycetes, but discovery that some yeasts are basidiomycetes that produce ballistoconidia (Kluyver and van Niel 1924, 1927; Nyland 1949) and basidia with basidiospores (Banno 1963, 1967) markedly changed

Received: 24 February 2015; **Accepted:** 29 May 2015
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our perspective of the yeasts. With the discovery of such phylogenetic diversity, the working definition of a yeast has become that of an ascomycete or basidiomycete fungus which divides by budding or fission and the sexual state, if known, is not enclosed in a fruiting body (Kurtzman, Fell and Boekhout 2011).

In this review, we will discuss the advances that have arisen from application of DNA sequence analysis for identification of yeast species and for determining phylogenetic relationships among species. These new approaches are showing that the number of currently known yeast species is likely to be a small fraction of the total to be found in nature and that determination of phylogenetic relationships will result in a major revision of yeast classification. Furthermore, correct identification of species will significantly revise our understanding of yeast ecology, clinical microbiology and other areas of science that require accurate species identification. We have noted that some species groups share unique physiological characters and from this observation, we will present the hypothesis that certain biotechnological properties can be predicted from knowledge of phylogenetic relationships.

Species recognition

Rapid identification of individual yeasts species is now routinely determined from nucleotide sequence divergence in domains 1 and 2 (D1/D2) of the large subunit (LSU) rRNA gene. In practice, this is done by conducting a Blast Search of the newly determined sequence with deposited sequences that are maintained by GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and associated databases. The ca. 450–600 nucleotide D1/D2 region is bounded by highly conserved flanking sequences and essentially all species can be PCR amplified by a single set of primers (Kurtzman and Robnett 1998; Fell *et al.* 2000). The importance of a single gene diagnostic system is that as new species are discovered, the continuously expanding database provides documentation of all described species as well as evidence of undescribed species through absence of their sequences. A second database that is widely used is the internal transcribed spacer (ITS), which is located between the SSU and LSU rRNA genes. The ITS sequence is divided into two sections (ITS1, ITS2) by the 5.8S gene, which is highly conserved and should not be included when comparing substitutions in ITS. The D1/D2 and ITS sequences are often similar in length, but notable length differences have been reported for some ascomycetes (Kurtzman and Robnett 1998, 2003) and some basidiomycetes (Fell *et al.* 2000; Scorzetti *et al.* 2002), resulting in a different nucleotide length for determining percent substitutions. With appropriate PCR primers, ITS and D1/D2 may be PCR replicated as a single amplicon. When conducting a Blast Search, the comparison should be made with the sequence of the type strain of the species that gives the nearest match because deposits in the database include many misnamed strains.

Species resolution for both ascomycetes and basidiomycetes is based on the prediction that strains of a species diverge in D1/D2 and ITS sequences by no more than 1% (Kurtzman and Robnett 1998; Sugita *et al.* 1999; Fell *et al.* 2000; Scorzetti *et al.* 2002). For ITS, sequence divergence between closely related species may be relatively small in clades of the Saccharomycetaceae (Kurtzman and Robnett 2003), or quite large as seen for *Citeromyces* (Kurtzman 2012). Not surprisingly, exceptions have been found to the prediction of 1% or greater nucleotide divergence between species for D1/D2 and ITS sequences and this may result from interspecific hybridization, different substitution rates or other genetic changes. Among these exceptions are *Saccharomyces bayanus* and *S. pastorianus*, which share the same rRNA repeat (Peterson and Kurtzman 1991; Groth, Hansen and Piskur 1999), and *Clavispora lusitaniae*, in which some strains show greater than 1% divergence in D1/D2 (Lachance *et al.* 2003). When taken in perspective, the D1/D2 and ITS databases present a powerful tool for rapid species identification and for presumptive detection of previously unknown lineages. Use of DNA sequences for identification has more than doubled the number of known yeast species during the past decade (Kurtzman, Fell and Boekhout 2011). It should be noted that ITS has been selected as the universal DNA sequence for identification of fungi because D1/D2 was less resolving of certain non-yeast fungal lineages (Schoch *et al.* 2012).

Non-sequencing applications for species identification

For some applications, DNA sequencing may not be required. Molecular methods based on sequences of known species are available from GenBank and other databases and can be used to develop species-specific primer pairs and probes. Other applications include randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP) and restriction fragment length polymorphisms (RFLP). The following is a brief description of these methods.

Species-specific primers

The use of species-specific primer pairs is effective when used for PCR-based identifications involving a small number of known species or when a particular species is the subject of the search (Fell 1993; Mannarelli and Kurtzman 1998; Chapman *et al.* 2003; Hulin and Wheals 2014). Following the PCR reaction, the mixture is separated by gel electrophoresis to visually detect the band that identifies the target species.

PNA

Peptide nucleic acid (PNA) probes offer a means for detection and quantification of species in clinical samples, food products and other substrates through fluorescence *in situ* hybridization. PNA probes have a peptide backbone to which is attached nucleotides complementary to a species-specific target sequence, and a fluorescent label is added for detection by fluorescence microscopy (Stender *et al.* 2001; Rigby *et al.* 2002). If probes are complementary to rRNA, the whole cell of the target species will 'glow' when visualized, which will also allow quantification by cell counts.

RAPD/AFLP

Microsatellite-primed RAPDs (Gadanho, Almeida and Sampaio 2003) and AFLP fingerprints (de Barrios Lopes *et al.* 1999; Illnait-Zaragozí *et al.* 2012) have been effectively used for rapid preliminary identification of large numbers of isolates, and the pattern-based identification is then often followed by gene sequencing of representative strains from each group that has a unique pattern. One concern in using pattern-based identification techniques is reproducibility between laboratories, because small differences in PCR conditions may impact the species-specific patterns that serve as reference.

Real-time PCR

The technique of real-time PCR has also been widely studied for applications in medical mycology, especially those aiming to detect and quantify loads of *Candida albicans*. In typical assays, 5 cfu ml^{-1} could be detected. Commonly used primers have been based on sequences of the rDNA repeat, such as ITS 1 and 2, or

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the small subunit (SSU) rRNA gene (Loeffler *et al.* 2000; Klingspor and Jalal 2006; Bergman *et al.* 2007; Khlif *et al.* 2009; Wellinghausen *et al.* 2009). This technique is also becoming widely employed in food and beverage analyses and has been used for detection and quantification of spoilage yeasts in orange juice (Casey and Dobson 2004) as well as in wine fermentations (Cocolin, Heisey and Mills 2001).

DGGE

Denaturing gradient gel electrophoresis (DGGE) is a method that has been used for species identification and quantification of yeast populations in foods and beverages. The technique is based on separation of DNA fragments that differ in nucleotide sequences (e.g. species specific) through decreased electrophoretic mobility of partially melted double-stranded DNA amplicons in a polyacrylamide gel containing a linear gradient of DNA denaturants (i.e. a mixture of urea and formamide). A related technique is temperature gradient gel electrophoresis, in which the gel gradient of DGGE is replaced by a temperature gradient (Muyzer and Smalla 1998). Applications of DGGE have included identification and population dynamics of yeasts, e.g. sourdough bread (Meroth, Hammes and Hertel 2003), in coffee fermentations (Masoud *et al.* 2004) and on wine grapes (Prakitchaiwattana, Fleet and Heard 2004). Levels of detection are often around 10^3 cfu ml^{-1}, but 10^2 cfu ml^{-1} have been reported, which compares favorably with standard plate count methods (Prakitchaiwattana, Fleet and Heard 2004).

Flow cytometry

High-throughput probe hybridization methods are available for detection of multiple species in multiple samples. One method that is effective for yeasts (Diaz and Fell 2004; Page and Kurtzman 2005) is an adaptation of the Luminex xMAP technology (Luminex Corp), which consists of a combination of 100 different sets of fluorescent beads covalently bound to species-specific DNA capture probes. Upon hybridization, the beads bearing the target amplicons are classified in a flow cytometer by their spectral addresses with a 635-nm laser. The hybridized biotinylated amplicon is quantified by fluorescent detection with a 532-nm laser. Strains that differ by one nucleotide often can be discriminated and the assay can be performed, after amplification, in less than 50 min in a 96-well format with as many as 100 different species-specific probes per well.

The molecular detection methods just described have provided some remarkable capabilities for yeast identification, but a number of factors affect detection and quantification. These include (1) cellular copy number of the gene to be used, (2) whether the gene is sufficiently conserved to be PCR amplified by 'universal' primers that will detect all species of interest, (3) efficiency of DNA extraction from cells in the sample, (4) efficiency of DNA recovery from the sample, (5) sample components that may interfere with DNA recovery or PCR amplification and (6) level of cell population that is detectable.

The impact on identification of yeasts through use of DNA sequences has been remarkable. Earlier, yeast identification was based on comparative physiology in which the capability to ferment certain mono-, di- and trisaccharides was determined, together with the utilization patterns of sugars, organic acids, alcohols, sugar alcohols, starch and some nitrogen compounds. Molecular identification methods began with determination of guanine + cytosine (G + C) ratios (e.g. Nakase and Komagata 1968) and was soon followed by nuclear DNA reassociation techniques (e.g. Price, Fuson and Phaff 1978). However, the introduction of rDNA sequencing technology has contributed to the recent steep increase in number of yeast species being described (Kurtzman and Robnett 1998; Fell *et al.* 2000). From an analysis of species described over the past two centuries, it can be seen that yeast species discovery rapidly increased at the beginning of the 20th century (Fig. 1) with the use of physiological growth profiles resulting in the description of 460 yeast species by the 1930s. This was followed by a decrease in the 1940–1950s due to the Second World War and it economic aftermath. A decline observed in the 1990s may be due to an awareness of the limitation of the use of physiological growth patterns in species distinctions, and hence reluctance emerged in using such data for the distinction of new species. However, with the introduction of D1/D2 and ITS databases for both ascomycete and basidiomycete yeasts (Kurtzman and Robnett 1998; Fell *et al.* 2000; Scorzetti *et al.* 2002; and the many publications that have followed), the description of new species rapidly increased. A major result from these barcoding studies was that more than 900 species were described in the first decade of the 21st century, and it is clear that this trend will continue in the future given that large parts of the earth's biomes have not yet been sampled for yeasts (Fig. 2). The fifth edition of *The Yeasts, a Taxonomic Study* (Kurtzman, Fell and Boekhout 2011) accepted >1400 yeast species belonging to 85 ascomycetous genera and 61 basidiomycetous genera, but since the publication of this treatment >230 yeast species have been registered in Mycobank (http://www.mycobank.org/).

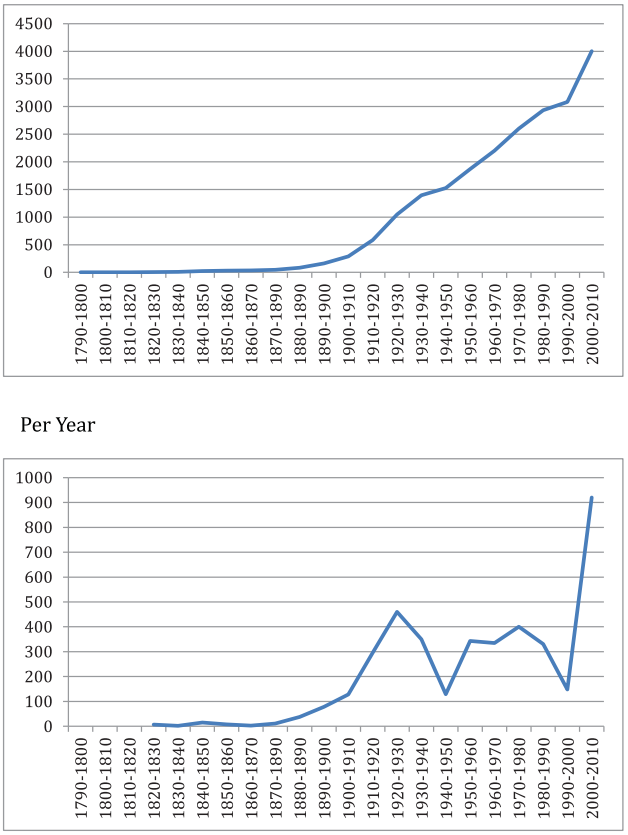

Figure 1. Number of yeast species being described from 1790–2000. Data are based on data given in Barnett, Payne and Yarrow (2000) and Kurtzman *et al.* (2011). Top: cumulative graph of species being described in time (decades); bottom: number of species described per decade.

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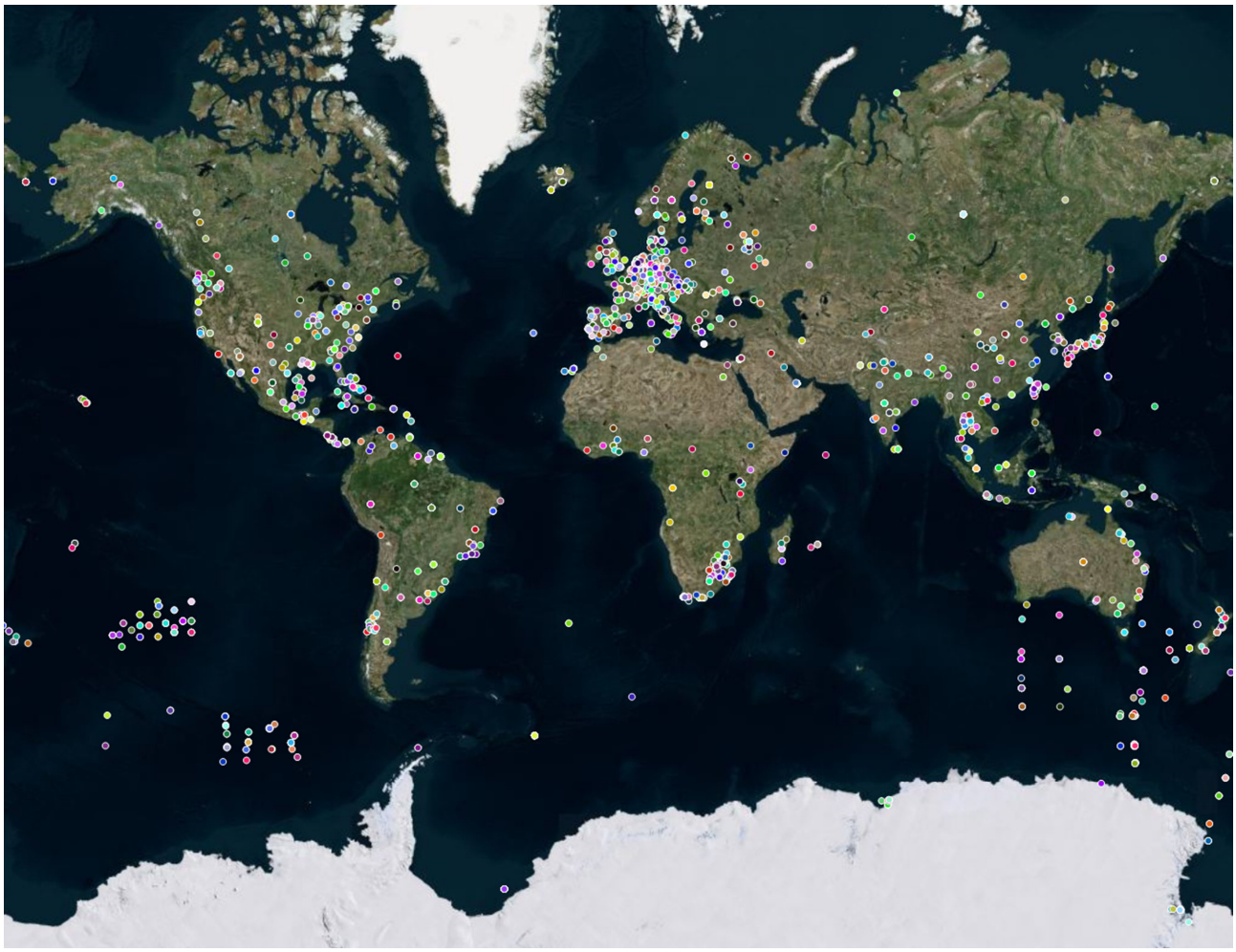

Figure 2. Geographical sources of approximately 7000 yeast strains present in the publicly available collection of CBS.

MALDI-TOF Mass Spectrometry, an alternative identification method for yeasts

MALDI-TOF MS-based identification has revolutionized microbial identification, including yeasts, in many laboratories worldwide. In comparison with DNA-based identification methods, such as sequence analysis of the D1/D2 domains of the LSU rDNA and the ITS 1 and 2 regions of the rDNA, MALDI-TOF MS gives identifications in short turnaround times (Tan *et al.* 2012; Cassagne *et al.* 2013). MALDI-TOF MS has been successfully applied to identify isolates of many clinically relevant yeasts, e.g. *Cryptococcus neoformans/C. gattii* species complex, *Ca. albicans* and non-*albicans Candida* species, arthroconidial yeasts of *Geotrichum* and *Trichosporon* spp. and *Malassezia* spp. (Marklein *et al.* 2009; McTaggart *et al.* 2011; Cendejas-Bueno *et al.* 2012; Firacative, Trilles and Meyer 2012; Kolecka *et al.* 2013, 2014; Hagen *et al.* 2015). With an increasing coverage of yeast species in the databases, the utility of the technique will further increase.

Phylogenetic placement of the ascomycete yeasts

Although it was seen from sexual states that yeasts may be ascomycetes or basidiomycetes, their placement within these two fungal phyla was largely unknown before molecular comparisons. An early study employed 5S rRNA sequences (Walker 1985) to examine relationships among the fungi, and this comparison divided the Ascomycota into three groups: (1) *Schizosaccharomyces* and *Protomyces* (Taphrinomycotina), (2) budding yeasts (Saccharomycotina) and (3) 'filamentous fungi' (Pezizomycotina). Berbee and Taylor (1993) analyzed representative members of the Mycota from SSU rRNA gene sequences and showed the same three major ascomycete lineages. This analysis also revealed that yeasts and 'filamentous fungi' are sister taxa and that *Schizosaccharomyces* and relatives represent an early diverging lineage. Kurtzman and Robnett (1994) showed from partial LSU and SSU rRNA sequences that all then accepted ascomycetous yeast genera were members of a single clade, which was separate from *Schizosaccharomyces* and members of the 'filamentous fungi'. The findings from single gene analyses have been supported by multigene sequence analyses (Fitzpatrick *et al.* 2006; James *et al.* 2006; Kuramae *et al.* 2006a,b; Sugiyama, Hosaka and Suh 2006; Schoch *et al.* 2009; Rosling *et al.* 2011; Kurtzman and Robnett 2013).

Sequence analyses are now providing an understanding of relatedness between both species and genera (Kurtzman 2011). Figure 3 shows phylogenetic relationships among genera of the Saccharomycotina as determined from a five-gene sequence analysis of type species from most presently accepted genera (Kurtzman and Robnett 2013). In this analysis, members of the Lipomycetaceae (Fig. 3, Clade 11) are the earliest diverging

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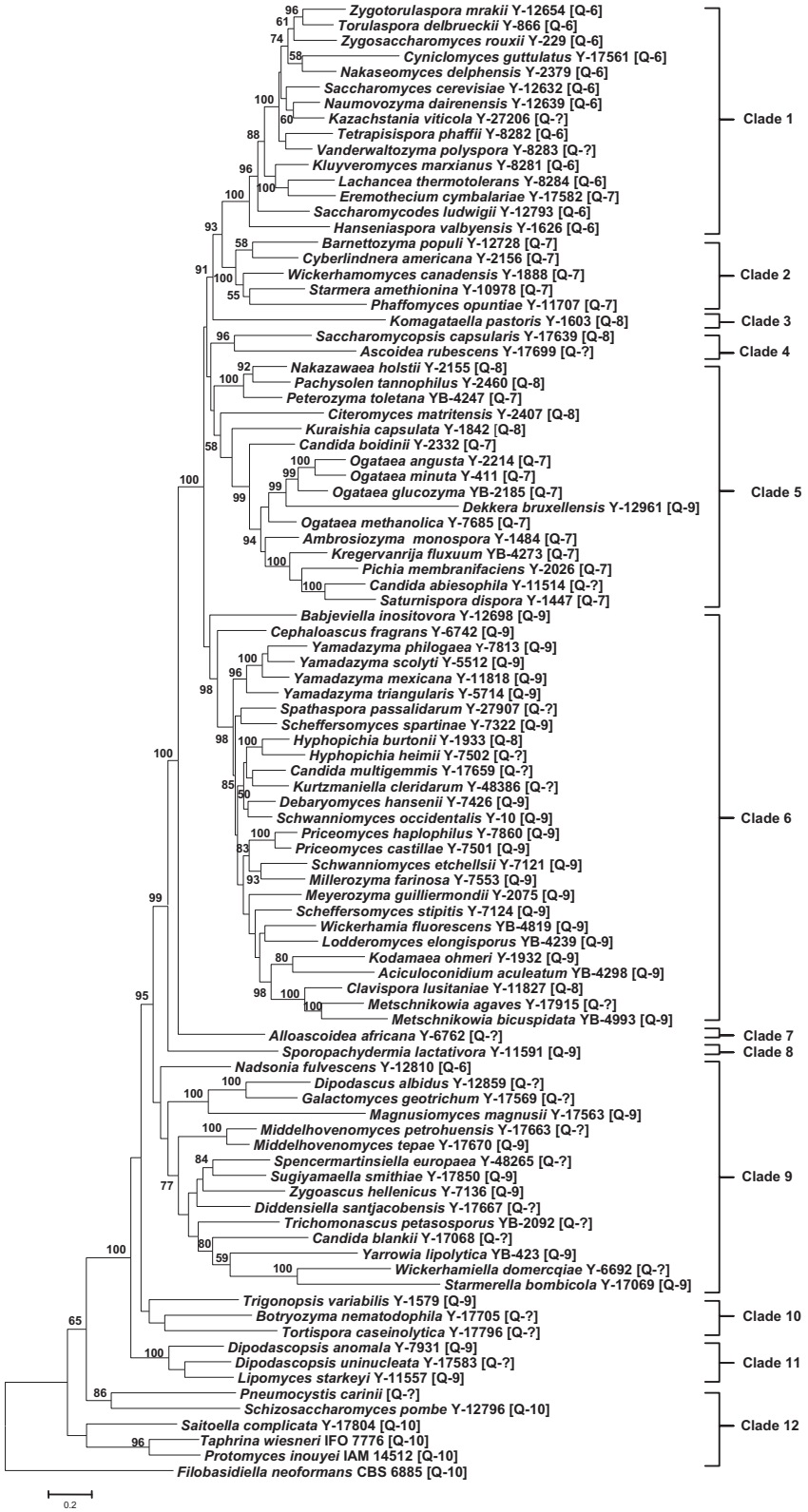

Figure 3. Phylogenetic relationships among type species of ascomycete yeast genera and reference taxa determined from maximum likelihood analysis of concatenated gene sequences for LSU rRNA, SSU rRNA, translation elongation factor-1α, and RNA polymerase II, subunits B1 and B2. The basidiomycete *Filobasidiella* (*Cryptococcus*) *neoformans* was the designated outgroup species in the analysis. Bootstrap values (1000 replicates) >50% are given at branch nodes and the final dataset included 11 773 positions. Strain accession numbers are NRRL unless otherwise indicated. Reproduced from Kurtzman and Robnett (2013).

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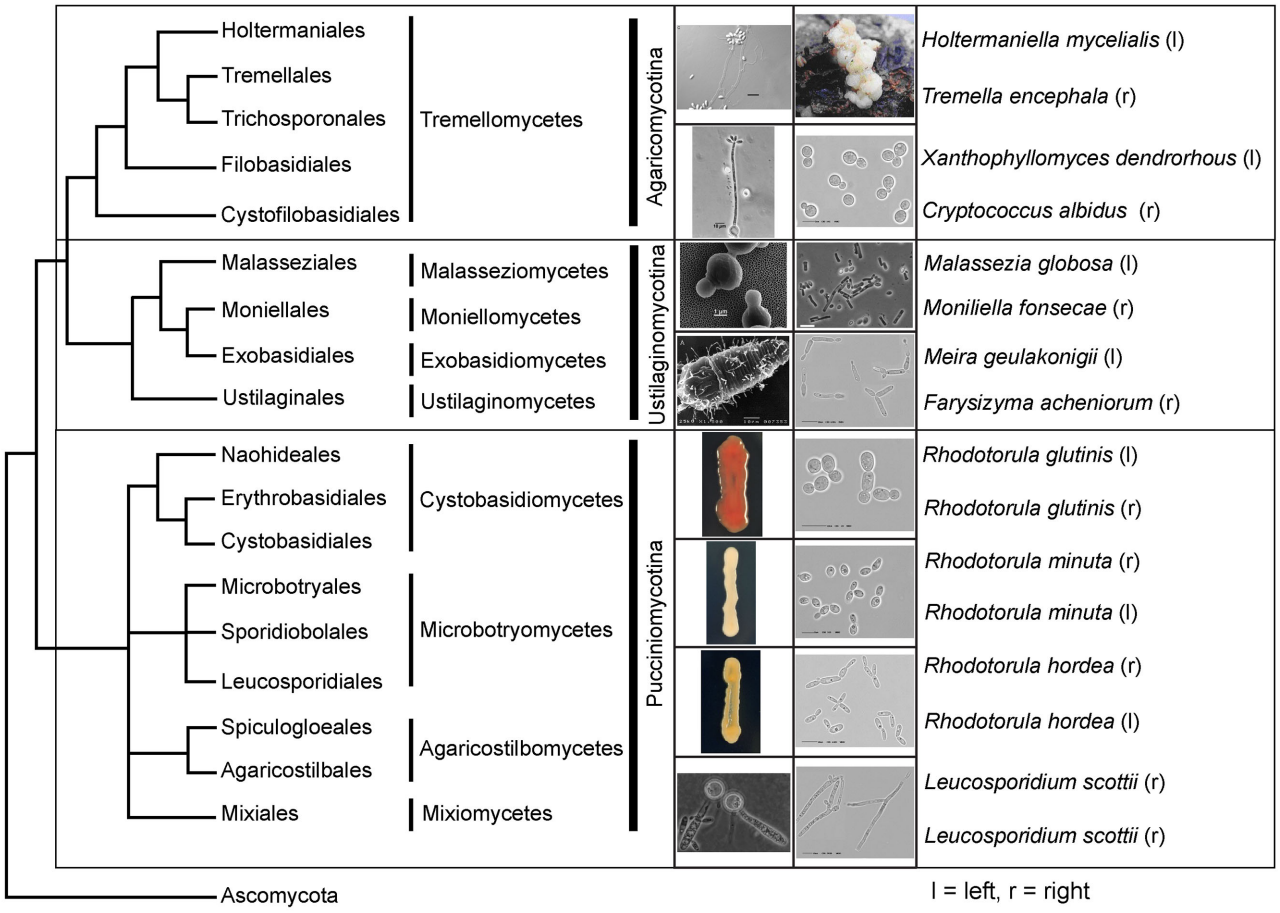

Figure 4. Schematic representation of the three subphyla, Agaricomycotina (top) Ustilaginomycotina (middle) and Pucciniomycotina (bottom) of Basidiomycota. Fungi with a yeast state, either as part of the life cycle or the entire known life cycle, occur in all three lineages. Courtesy: *Holtermannia mycelialis* (hyphae), *Tremella encephala* (basidiocarp), *Xanthophyllomyces dendrorhous* (=*Phaffia rhodozyma*, basidium), *Malassezia globosa* (budding yeast cell), *Moniliella fonsecae* (yeast cells and arthroconidia), *Meira geulakonigii* (pseudohyphae on mite) were taken from *The Yeasts, a Taxonomic Study*, 5th ed. (Kurtzman *et al.* 2011). *Cryptococcus albidus* (yeast cells), *Farysizyma acheniorum* (yeast cells), *Rh. glutinis*, *Rh. minuta* and *Rh. hordea* (both colonies and yeast cells) and *Leucosporidium* scottii (germinating teliospore with basidia, and pseudohyphae) were taken from the CBS website (http://www.cbs.knaw.nl/Collections/Biolomics.aspx?Table=CBS%20strain%20database).

members of the Saccharomycotina. Species of *Lipomyces* are commonly isolated from soil and the genus represents one of the few groups of yeasts for which soil is the primary habitat. *Saccharomyces* and related genera appear to be among the most diverged members of the Saccharomycotina (Fig. 3, Clade 1), and this clade includes the vigorous sugar fermenting yeasts often used for ethanol production. Phenotypic characters previously used to infer relatedness, such as ascospore morphology and nitrate assimilation, are found in many clades.

Support for many lineages in present phylogenetic trees is often weak and more robust analyses of relationships among the Saccharomycotina will require whole genome comparisons. At present, genome sequences are available for less than 100 yeasts and these are primarily from species of genetic, medical or biotechnological interest. Analyses of whole genomes, sometimes based on ca. 400 orthologous genes, have presented species relationships much like those determined from far fewer genes but, in contrast, phylogenetic trees derived from these analyses usually have much greater branch support (e.g. Fitzpatrick *et al.* 2006; Kuramae *et al.* 2006a,b). As demonstrated by Rokas *et al.* (2003) from analysis of genomes from *Saccharomyces* species, a minimum of 20 concatenated genes were needed to provide strong support of species placement in resulting phylogenetic trees. Because *Saccharomyces* is a small genus, strong resolution of species relationships in larger genera will require additional genes. Similarly, resolution of families from current datasets is often uncertain. Placement of *Cyniclomyces, Eremothecium, Kazachstania, Kluyveromyces, Lachancea, Nakaseomyces, Naumovozyma, Saccharomyces, Tetrapisispora, Torulaspora, Vanderwaltozyma, Zygosaccharomyces* and *Zygotorulaspora* in the family Saccharomycetaceae (Fig. 3, Clade 1) seems strongly supported as do some other clades that represent families, but other clades may represent several families, such as the large Clade 6 (Fig. 3), which is presently identified as the Debaryomycetaceae.

Phylogenetic placement of the basidiomycete yeasts

Basidiomycetous yeasts are polyphyletic and occur in all three subphyla of Basidiomycota, namely Pucciniomycotina, Agaricomycotina and Ustilaginomycotina (James *et al.* 2006; Hibbett *et al.* 2007; Boekhout *et al.* 2011) (Fig. 4). Within Pucciniomycotina, a biologically highly diverse group that is mainly unified by molecular phylogeny data, nine classes are recognized, but only four have species with a yeast state. These are Microbotryomycetes, Cystobasidiomycetes, Agaricostilbomycetes and Mixiomycetes. The latter contains only one species *Mixia osmundae*,

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which is a fern parasite that in culture grows with a yeast state (Nishida, Robert and Sugiyama 2011). Within Agaricostilbomycetes two major clades occur, Agaricostilbales with the yeast genera *Kondoa, Agaricostilbum*, some *Bensingtonia* species, *Kurtzmanomyces*, some *Sporobolomyces* species, *Sterigmatomyces* and Spiculogloeales with asexual yeasts presently classified in *Sporobolomyces*, namely *Sp. subbrunneus*, *Sp. coprosmicola* and *Sp. linderae* (Aime *et al.* 2006; Bauer *et al.* 2006). The Cystobasidiomycetes include mostly pink-colored asexual yeasts classified in the genera *Bannoa, Cyrenella,* part of *Rhodotorula* species, part of *Sporobolomyces* species and *Erythrobasidium,* and a diverse group of sexual and dimorphic species classified in the genera *Cystobasidium, Occultifur, Naohidea* and *Sakaguchia*. The Cystobasidiomycetes have three orders: (1) Cystobasidiales with *Cystobasidium* and *Occultifur,* and some *Rhodotorula* species, including *Rhodotorula minuta* and *Rh. slooffiae*; (2) Erythrobasidiales with *Erythrobasidium* and *Bannoa, Rh. lactosa* and some *Sporobolomyces* species such as *Sporobolomyces ogasawarensis* and (3) Naohideales with the genus *Naohidea* that forms cream-colored colonies in culture. The Microbotryomycetes include many species of so-called red yeasts belonging to *Rhodotorula* and *Rhodosporidium*. Yeast taxa mainly belong to two orders, Leucosporidiales and Sporidiobolales. The former maintains two sexual and teliospore-forming genera, i.e. most *Leucosporidium* species and *Mastigobasidium*, and their asexual counterparts. Sporidiobolales include the pink-colored species in the sexual and teliospore-forming genera *Rhodosporidium* and *Sporidiobolus* and their asexual equivalents, *Rhodotorula* and *Sporobolomyces*, respectively. The classification of these fungi, especially that of the anamorphic genus *Rhodotorula*, is in great need of revision and we expect that multigene-based and whole genome-based phylogenies in the near future will contribute to this.

Among subphylum Agaricomycotina, yeasts or dimorphic taxa with yeast states occur only in class Tremellomycetes that has four (or five, depending on the view of the taxonomist) orders, i.e. Cystofilobasidiales, Filobasidiales, Holtermanniales Tremellales and Trichosporonales that all contain yeast and yeast-like taxa (Fell *et al.* 2001; Sampaio 2004; Boekhout *et al.* 2011; Wuczkowski *et al.* 2011). The most basal lineage is Cystofilobasidiales (Fell, Roeijmans and Boekhout 1999; Boekhout *et al.* 2011) with seven well-circumscribed lineages and genera, such as *Phaffia/Xanthophyllomyces*, *Cystofilobasidium*, *Itersonilia, Udeniomyces, Guehomyces* and *Mrakia*. Taxonomic issues to be solved are the relationships between *Tausonia* and *Guehomyces, Udeniomyces pannonicus* and *Itersonilia* and the position of *Mrakia curviuscula*. Some species of Cystofilobasidiales, namely *Mrakia* spp. and *Phaffia* sp. are able to ferment sugars, which is a very rare trait among basidiomycetous yeasts and further known from *Filobasidium capsuligenum* belonging to Filobasidiales and some *Bandoniozyma* spp. belonging to Tremellales (Valente *et al.* 2012). Filobasidiales comprise all *Filobasidium* species as well as many species currently classified as *Cryptococcus*. Several clades seem well defined and may represent separate genera, such as the aerius clade, albidus clade, gastricus clade and cylindricus clade (Boekhout *et al.* 2011). The floriforme clade comprises *Filobasidium* species together with some Cryptococci, i.e. *C. magnus, C. oeirensis, C. chernovii, C. stepposus* and *C. wieringae*. The taxonomic relationship of *F. uniguttulatum* with the other species of *Filobasidium* requires further investigation.

The largest order in the Tremellomycetes is the Tremellales that contains many mushroom-forming taxa that are mycoparasitic and dimorphic. Molecular phylogenetic data strongly support the inclusion of anamorphic taxa (Fell *et al.* 2001; Scorzetti *et al.* 2002; Sampaio 2004; Millanes *et al.* 2011; Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm). The genus *Tremella* also turned out to be polyphyletic and needs revision (Millanes *et al.* 2011; Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm). Multigene-based phylogenies showed the presence of 25 well-supported clades within Tremellales (Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm). This and other studies (see above) showed that species of the anamorphic genera *Cryptococcus* and *Bullera* are intermingled with many teleomorph genera, such as *Tremella*, *Auriculibuller*, *Bulleromyces*, *Bulleribasidium*, *Filobasidiella* and *Kwoniella,* once again stressing the need for a major taxonomic revision. Some genera have been proposed to accommodate some species of *Bullera* and *Cryptococcus* that formed monophyletic lineages. *Dioszegia* has been reintroduced and *Derxomyces* and *Hannaella* were described (Takashima, Deak and Nakase 2001; Wang and Bai 2008). Family Cuniculitremaceae (Kirschner *et al.* 2001) accommodates species of the teleomorphic genus *Cuniculitrema* (with *Cun. polymorpha* = *Sterigmatosporidium polymorpha*) and the anamorphic genera *Fellomyces* and *Kockovaella,* which need to be taxonomically revised.

Order Trichosporonales (Fell *et al.* 2000) contains species of *Trichosporon*, except *Guehomyces pullulans* (formerly known as *Trichosporon pullulans*), that is classified in Cystofilobasidiales (Fell and Scorzetti 2004), but also species presently classified in genera such as *Cryptococcus* and *Bullera*, but also *Tetragoniomyces uliginosis* (Weiss *et al.* 2014) and the monotypic genus *Cryptotrichosporon* (Okoli *et al.* 2007). *Tetragoniomyces* represents the first teleomorphic genus in Trichosporonales and has a mycoparasitic life style and a close affinity to *Cryptotrichosporon* (Oberwinkler and Bandoni 1981; Weiss *et al.* 2014). Within this order, seven well-supported clades could be distinguished from a multigene-based phylogeny (Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm.). The question remains whether the order is a sister group to Tremelalles or whether it is part of Tremellales (Hibbett *et al.* 2007; Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm.), and what will be the taxonomic status of the clades within the group? One of the clades, namely the humicola clade was renamed as *Asterotremella* (Prillinger *et al.* 2007), but it seems that the name *Vanrija* is more appropriate as it has nomenclatural priority (Okoli *et al.* 2007; Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm.) and was used also in recent monographic treatments (Weiss *et al.* 2014). A small but well-supported distant clade comprising a mushroom-forming species, *Holtermannia corniformis*, and five anamorphic species, namely *C. festucosis, C. mycelialis, C. nyarrowii, C. takasimae* and *C. watticus* formed the basis to propose order Holtermanniales (Wuczkowski *et al.* 2011).

A further point of attention is how to use the name *Cryptococcus* when one applies the One-Fungus = One name principle. The major pathogen *C. neoformans* is proposed to contain at least seven species and a number of interspecies hybrids (Hagen *et al.* 2015). These authors proposed to limit the use of the name *Cryptococcus* to the *Filobasidiella* lineage, and to recombine the *Filobasidiella* species *Filobasidiella depauperata* and *Fi. lutea* in *Cryptococcus*. A major consequence is that all other cryptococcal species have to be renamed. Despite some initial inconvenience, we think that reclassification along phylogenetic lines will be beneficial to applied microbiology because potentially interesting species presently classified as *Cryptococcus* will no longer be linked to this pathogen species, which may ease registration for applied processes.

Until recently, subphylum Ustilaginomycotina comprised two classes, Ustilaginomycetes and Exobasidiomycetes (Begerow, Bauer and Boekhout 2000; Begerow, Stoll and Bauer 2006, 2014; Hibbett *et al.* 2007, Wang *et al.* 2014). However,

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multigene phylogenies identified both the *Moniliella* clade and the *Malassezia* clade to represent deep lineages that were recognized as classes, namely Moniliellomycetes and Malasseziomycetes, respectively (Wang *et al.* 2014). In all classes, yeasts and yeast-like taxa occur. Order Ustilaginales contains a number of anamorphic species presently classified in the polyphyletic genus *Pseudozyma*. Species of *Farysizyma* and *Pseudozyma* may represent anamorphs of some teleomorphic plant pathogens. Based on a multigene analysis, *Pseudozyma prolifica*, the type species of the genus, seems identical to *Ustilago maydis* (Wang *et al.* 2015) and *Farysizyma acheniorum* may represent *Farisia thuemenii* (Inácio *et al.* 2008; Wang *et al.* 2015). Among Exobasidiomycetes, species with a yeast or yeast-like state occur in Entylomatales, Microstromatales, Georgefischerilaes, Malasseziales and Exobasidiales (Wang *et al.* 2015). The genus *Tilletiopsis* is polyphyletic. *Tilletiopsis cremea, Ti. lilacina and Ti. washingtonensis* occur in the Entylomatales, *Ti. derxii, Ti. oryzicola, Ti. flava, Ti. fulvescens*, *Ti. minor* and *Ti. penniseti* belong to Georgefischerales with the teleomorph species *Tilletiaria anomala* forming a basal lineage, and *Ti. albescens* and *Ti. pallescens* represent two separate deep lineages (Wang *et al.* 2015). *Sympodiomycopsis paphiopedili,* together with the red yeasts *Rh. bacarum* and *Rh. hinnulea,* clusters in Microstromatales, and the former species seems to be identical to *Microstroma album* (de Beer *et al.* 2006; Wang *et al.* 2015). Some species with biocontrol properties against citrus mites were classified in *Meira* and *Acaromyces* (Boekhout *et al.* 2003).

From the above, it is clear that the classification of yeast taxa in the Basidiomycota urgently needs to be revised. Attempts to use multigene data for this purpose are underway and have been partially published (Wang *et al.* 2014; Xinzhan Liu, Feng-Yan Bai & Teun Boekhout, pers. comm.). For the other taxa, similar studies are underway. We expect that such data will be the basis for an improved taxonomy that will benefit taxonomists and the user communities.

Impact of molecular comparisons and the new Code of Nomenclature on species assignments

As discussed above, identification and classification of yeasts and other fungi is now almost exclusively done through use of DNA sequence analysis, which has resulted in discovery of many new species as well as changes in the genus assignment of species. These name changes affect our ability to communicate species identity and to conform to regulations governing the presence of pathogens and toxigenic species. One other event has had a major impact on naming of yeasts and all other fungi. Sequence comparisons demonstrated that sexual and asexual species are often members of the same genus, but previous editions of the *International Code of Botanical Nomenclature* required that sexual (teleomorphic) and asexual (anamorphic) species be assigned to different genera. This paradox was resolved with introduction of a new Code entitled the *International Code of Nomenclature for algae, fungi, and plants* (Melbourne Code) (McNeill *et al.* 2012), which requires that related species, anamorphic or teleomorphic, be assigned to the same genus. For some groups of yeasts, there will be little change in classification, but for others, major changes will result. The anamorphic genus *Candida* falls in the latter category. Species assigned to *Candida* divide by multilateral budding and may or may not form hyphae and pseudohyphae. This broad definition provided a convenient 'dumping ground' for placement of new asexual species and the result has been a large polyphyletic genus with 434 species (Daniel, Lachance and Kurtzman 2014).

One aspect of the new Code is that teleomorphic genera do not have priority over anamorphic genera. Priority will be established by the rules of nomenclature and also from other considerations, such as a history of common usage. *Candida* is a well-known genus based on the type species *Ca. vulgaris* (=*Ca. tropicalis*). Other members of the *Ca. tropicalis* clade include *Ca. albicans* and *Ca. dubliniensis*, as well as nearly 30 additional species. The little known *Lodderomyces elongisporus* is the sole ascosporic member of this clade, and it seems likely that the name *Candida* will be retained for the clade rather than reassigning species to the teleomorphic genus *Lodderomyces*, which would have had priority under the old Code. With recircumscription of *Candida*, over 400 species now classified in *Candida* must be reassigned to other genera (Daniel, Lachance and Kurtzman 2014). Some will be placed in extant genera, but other species will need to be assigned to new genera, as was done for those *Candida* species now assigned to the new genera *Deakozyma*, *Danielozyma* and *Middelhovenomyces* (Kurtzman and Robnett 2014). Consequently, the *Candida* species shown in Fig. 8 will need to be transferred to *Yarrowia* because they are not members of the *Ca. tropicalis* clade. This change in the Code will undoubtedly cause some confusion, but the long-term goal is to classify phylogenetically related species in the same genus, and the impact will be that genus names will then convey the genetic and thus the physiological properties of assigned species.

Geographical distribution of the yeasts

The increased accuracy of species identification determined from DNA sequences allows a fresh look at biodiversity and ecology. Figure 2 displays the origin of approximately 7000 publicly available yeast isolates present in the CBS collection and this proxy of yeast diversity clearly shows that some regions of the world, such as Europe, Japan, South Africa and parts of North America are better sampled than, for example, India, China, Brazil and Australia. Russia, large parts of Africa, the Middle East and the northern part of North America, Greenland, Antarctica and the Amazon region of South America, one of the main biodiversity hotspots on earth, are poorly sampled. When comparing the description of new yeast species during three decades in the period 1980–2010 with the economic status of the countries represented by gross domestic product per capita as listed by IMF (http://www.imf.org/external/pubs/ft/weo/2013/01/weodata/weoselgr.aspxpresenbias), it is clear that most activity in yeast taxonomy is located in the wealthy, more technologically advanced countries (Fig. S1, Supporting Information). However, the so-called emerging countries, such as Brazil, Thailand and China, have shown much increased taxonomic activity in the years 2000–10.

Next to geographical limitations in data collection, ecological limitations also occur. For instance, large biomes, such as the tropical lowland rainforests in the Amazon, Indonesia and Central Africa, the Saharan, Kalahari, Arabian, Chinese and Australian deserts, the arctic forests and tundras in Eurasia, Northern America and Patagonia, montane and submontane ecosystems, such as those present in the Himalaya, the Rockies and the Andes, the Arctic, Antarctic and montane glaciers, and most marine ecosystems, including all oceans, are poorly studied with respect to yeast diversity. The substrates where yeasts have been isolated also show a bias, especially towards human-derived products and production processes. Taking the origin of the approximately 7000 publicly available yeast strains in the CBS collection as a proxy for 'diversity of yeast habitats', more than half originated from plant-related, soil-related, insect-related or

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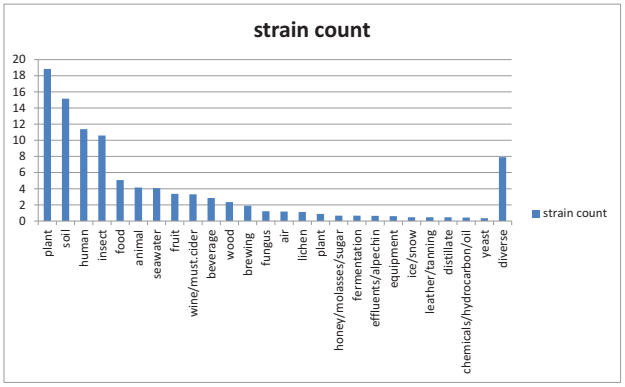

Figure 5. Origin of approximately 7000 publicly available yeast strains form CBS.

human-related (i.e. clinical) sources (Fig. 5). Further important origins were (fermented) foods, (diseased) animals, seawater, fruits, wine and must fermentations, beverages, wood and beer and brewing-related habitats (together approximately 27%).

Phylogeny as a predictor of biotechnologically important metabolic pathways

Since the first observation of yeasts by Antonie van Leeuwenhoek, it took until the 19th century before yeasts were demonstrated to occur in beer and wine by researchers such as Desmazières, Schwann and Kützing, but it was Pasteur who demonstrated that yeasts were responsible for fermentation (from Guilliermond 1912), thus paving the way for further advances in biotechnology. Other major steps forward were the isolation of yeasts in pure culture, a technique developed by Chr. Hansen and the isolation of an enzyme involved in fermentation, 'zymase alcoolique', by Buchner (from Guilliermond 1912). The use of pure cultures allowed researchers to differentiate between different yeast isolates and to distinguish species, genera and higher taxa. The introduction of comparative physiology to zymology, in which the capability of strains to ferment and to assimilate various compounds was determined, provided some discrimination of species, and the data also gave an overview of the metabolic capabilities of species and their potential for biotechnological applications.

Some recent reviews (Johnson and Echavarri-Erasun 2011; Johnson 2013a,b) presented the yeast diversity involved in both traditional biotechnology (i.e. fermented drinks, foods and feeds), and in enzyme and metabolite production, development of pharmaceuticals and in such processes as pro- and prebiotics and bioremediation. It is estimated that the global annual production of *S. cerevisiae* is >1 million tons (Hansen 2004; Verstrepen, Chambers and Pretorius 2006). Although *S. cerevisiae* remains the work horse for many biotechnological processes, there is increased interest in the application of other, so-called non-conventional yeasts. In part, this is because yeast biodiversity represents huge evolutionary distances, and one can expect significant differences in proteins and metabolic pathways among divergent species. In the Saccharomycotina, the evolutionary distance between *S. cerevisiae* and *Ca. glabrata,* which are considered to be 'closely' related species (Kurtzman and Robnett 2003), mirrors the evolutionary distance between humans and fish (Dujon 2006).

About 200 species are known to be involved in either agriculture or traditional food/feed/drinks fermentations and an additional 200 species have applications in biotechnological processes or have potential for such applications (data from Kurtzman, Fell and Boekhout 2011). The arsenal of enzymes produced by yeasts are highly diverse and range from alpha-amylases, D-aminoxidases, N-benzyl-3-pyrrolidinol dehydrogenases, cellulases, chitinases, dextranases, epoxyhydrolases, glucanases, glutaminases, beta-glucosidases, inulinases, laccases, lipases, pectinases, phytases, proteases, xylanases to xylosidases (data from Kurtzman, Fell and Boekhout 2011). Also of special interest is production of enzymes at lower temperatures by psychrophilic yeasts (Buzzini and Margesin 2014).

The diversity of compounds being produced by yeasts is extensive and includes pigments, such as asthaxanthin and carotenoids, polyunsaturated fatty acids, cellobiose, lipids that seem to play a role in biocontrol against powdery mildews, hydroxy fatty acids, sophorolipids, triacylglycerols (up to 40–60% dry weigth), extracellular polysaccharides, flavors and volatiles, oxylipins, pyridines, polyphosphate, polyols, vitamins (e.g. riboflavin) and pharmaceuticals such as (R)-phenylacetylcarninol, 5-bromouridines and arabinofuranosylcytosine (data from Kurtzman, Fell and Boekhout 2011).

Among the species that have potential application in bioremediation are *Blastobotrys adeninivorans, C. humicola, C. musci, C. phenolicus, C. terreus, Mastigobasidium intermedium* and *Rh. mucilaginosa* (including waste water). Other interesting species are *Nakaseomyces delphensis* (waste water), *Lipomyces kononenkoae, L. starkeyi, G. pullulans* (the previous three degrade herbicides), *Saccharomycopsis fibuligera and T. cutaneum* (agricultural waste), *Ca. parapsilosis, Ca. maltosa, Ca. tropicalis* and *C. marinus* (the previous four utilize hydrocarbons). *Geotrichum klebahnii, G. pullulans and Sporidiobolus metaroseus* are of interest for lignin degradation (data from Kurtzman, Fell and Boekhout 2011).

Only a small part of the yeast domain has been screened for applications, but the biotechnological importance of yeasts is increasing. Ongoing sequencing projects, such as the 1002 yeast genomes project (mainly *S. cerevisiae*) by J. Schacherer (Strasbourg, France) and G. Liti (Nice, France), the 'The Making of Biodiversity Across the Yeast Subphylum' genome project that aims to sequence the genomes of 1000 Saccharomycotina species (http://www.nsf.gov/awardsearch/showAward?AWD_ID=1442148&HistoricalAwards=false), and the dikaryome and iGenolevures projects (http://gryc.inra.fr) will further boost such applications as *in silico* screens will allow searching genomes for interesting metabolic pathways and the enzymes involved.

When the foregoing applications are placed in the context of phylogenetically defined taxa, it is clear that many metabolic pathways are widely shared among species, such as glucose fermentation, which occurs among species of the Saccharomycotina, the Taphrinomycotina and the Basidiomycota. However, certain other pathways, such as methanol utilization (*Komagataella*, *Kuraishia*, *Ogataea*) or D-xylose fermentation (*Pachysolen*, *Scheffersomyces*, *Spathaspora*) appear restricted to specific phylogenetic lineages. The following are examples of apparent lineage-specific metabolic pathways.

Biosynthesis of sophorolipids has been reported for several yeast species (Gorin, Spencer and Tulloch 1961; Tulloch, Spencer and Deinema 1968; Chen *et al.* 2006), but the greatest number of high-producing species occurs in the *Starmerella* clade (Fig. 6), and many of the species tolerate high sugar concentrations (Table 1). Sophorolipids are composed of the sugar sophorose and a fatty acid such as oleic acid, and this combination imparts strong detergent properties to the molecule (Van Bogaert *et al.* 2007). *Candida kuoi*, *Ca. batistae*, *Ca. riodocensis* and *Ca. stellata* produce sophorolipids that have an open chain structure in contrast to the closed chain sophorolipids produced by *Starmerella*

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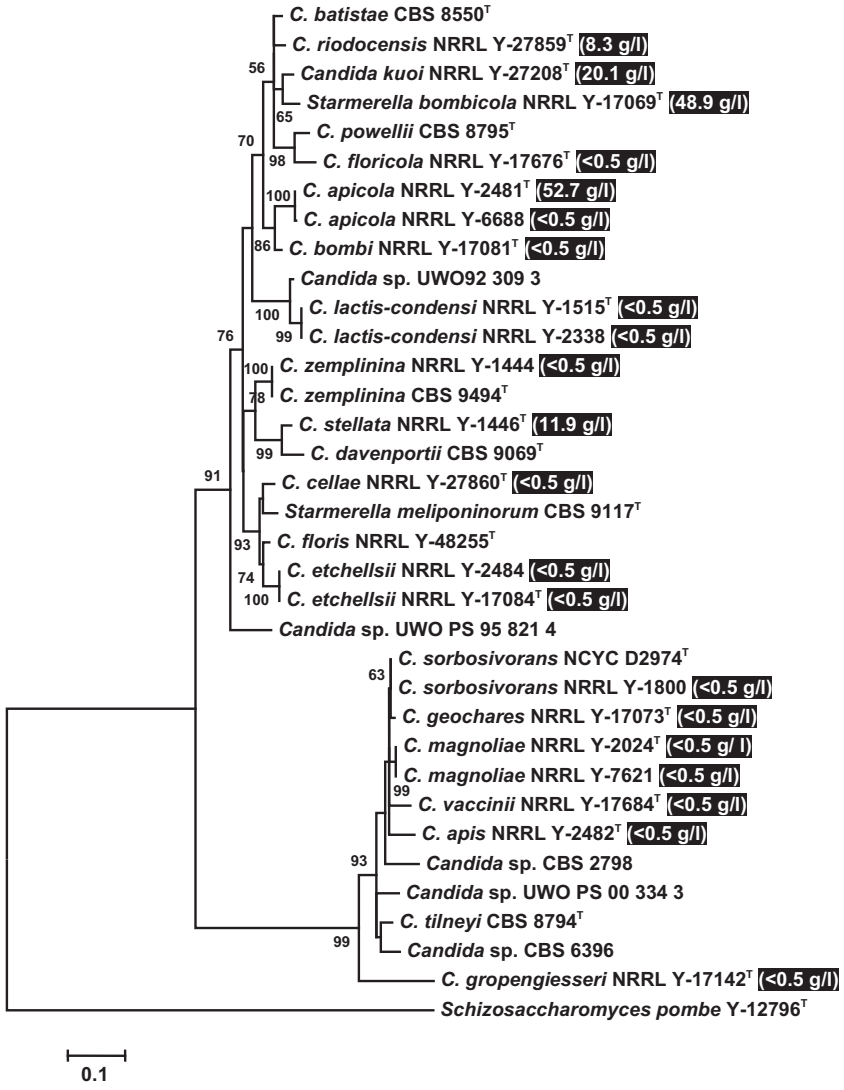

Figure 6. Production of sophorolipids by species of the *Starmerella* clade (Kurtzman *et al.* 2010). Phylogenetic relationships were determined from maximum likelihood analysis of D1/D2 LSU rRNA gene sequences (Kurtzman *et al.* 2010) with bootstrap values from 1000 replicates. Species of the *Starmerella* clade separate into two subclades, which suggests the possibility of two genera. Prefixes for strain numbers: CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; NRRL, ARS Culture Collection, Peoria, IL, USA; UWO, University of Western Ontario, London, Ontario, Canada.

bombicola and *Ca. apicola* (Kurtzman *et al.* 2010). It appears that the open chain form has greater detergent properties (Price *et al.* 2012), but both forms offer a 'green' alternative to other types of detergents that are often petroleum based.

Species of *Trichomonascus* and its anamorph *Blastobotrys* metabolize uric acid, isoleucine, adenine and certain other compounds that are not utilized for growth by most other yeasts (Middelhoven and Kurtzman 2003). In view of the unique properties of this clade, assigned species would seem to have biotechnological potential. In one example, three species of the clade were found to glucosylate T-2 toxin (McCormick, Price and Kurtzman 2012), a metabolite produced by species of *Fusarium* that parasitize maize, oats and wheat (Fig. 7). T-2 toxin is hazardous to plants, humans and livestock. Plants have developed a defense mechanism against toxicity by glucosylating the toxin. The glucosylated T-2 toxin is known as a 'masked' mycotoxin because it is undetected by analytical methods developed for unmodified toxin, thus making its impact on food safety uncertain. The finding that T-2 toxin can be glucosylated by species of *Blastobotrys* has resulted in a large supply of T-2 glucoside for development of analytical methods for detection of T-2 glucoside and the demonstration that under conditions of a simulated mammalian gut, the glucoside is metabolized, thus freeing T-2 toxin (McCormick *et al.* 2015).

Yarrowia lipolytica is well known for production of citric acid, lipases, proteases and cells with high oil content (Groenewald *et al.* 2014, and references therein). The widespread use of molecular identification methods has resulted in the discovery of 11 new species in the *Yarrowia* clade (Fig. 8). Although some of the species are of clinical origin, others are not, raising the possibility that other members of the *Yarrowia* clade have yet undiscovered biotechnological potential.

The biotechnological applications of basidiomycetous yeasts lag behind those of the ascomycetous yeasts. Presently, several interesting biotechnological applications of these microbes are being explored. Mycosporines (MYCs) are water-soluble UV-absorbing compounds consisting of an aminocyclohexenone unit bound to an amino acid or an amino alcohol group that may

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Table 1. Yeasts known to grow at high glucose concentrations[1].

Species name	Number of strains	Concentration of glucose		
		50%	60%	70%
Blastobotrys adeninivorans	6	6	6	2
Blastobotrys raffinosifermentans	1	1	1	1
Blastobotrys terrestris	2	1	1	1
Candida allociferrii	2	2	2	1
Candida apicola	12	12	11	8
Candida apis var. *apis*	1	1	1	1
Candida bombi	14	11	9	4
Candida bombiphila	2	1	1	1
Candida etchellsii	11	11	10	8
Candida floricola	2	2	2	2
Candida gropengiesseri	3	3	2	2
Candida kungkrabaensis	1	1	1	1
Candida magnoliae	11	11	11	5
Candida sorbosivorans	3	3	3	3
Candida tolerans	1	1	1	1
Candida versatilis	16	16	16	9
Cryptococcus amylolentus	2	1	1	2
Debaryomyces hansenii var. *hansenii*	11	9	6	1
Debaryomyces marama	14	12	10	2
Debaryomyces melissophilus	4	4	4	1
Debaryomyces robertsiae	4	3	3	1
Debaryomyces subglobosus	5	5	4	1
Filobasidium capsuligenum	10	2	1	1
Hyphopichia burtonii	14	14	14	14
Millerozyma farinosa	25	23	22	1
Moniliella acetoabutens	5	5	3	2
Moniliella pollinis	1	1	1	1
Moniliella suaveolens var. *nigra*	32	1	1	1
Rhodotorula glutinis	14	4	2	1
Schizosaccharomyces octosporus	10	8	8	8
Schizosaccharomyces pombe	60	23	20	6
Schwanniomyces etchellsii	5	4	3	1
Schwanniomyces polymorphus var. *africanus*	3	3	2	1
Starmerella bombicola	5	3	2	1
Starmerella meliponinorum	1	1	1	1
Sterigmatomyces elviae	6	5	5	2
Sugiyamaella smithiae	6	1	1	1
Trichosporonoides megachiliensis	3	3	3	3
Trichosporonoides nigrescens	2	1	1	1
Wickerhamiella australiensis	2	2	1	1
Wickerhamomyces ciferrii	7	5	5	1
Zygosaccharomyces mellis	12	12	12	6

[1]Strains compared are from the CBS Yeast Collection.

have a potential use as a sunscreen (Libkind *et al.* 2005a). Basidiomycetous yeasts are a major source for these compounds and their production correlates with phylogeny. Among pucciniomycetous yeasts, MYCs are not known to occur in class Microbotryomycetes (orders Sporidiobolales, Heterogastridiales, Microbotryales and Leucosporidiales), Classiculomycetes, Atractiellomycetes and Pucciniomycetes, but they do occur in Cystobasidiomycetes (orders Cystobasidiales and Erythrobasidiales, but not in order Nahoideales), Agaricostilbomycetes (orders Spiculogloeales and Agaricostilbales) and Mixiomycetes (Mixiales) (Libkind *et al.* 2005b; Libkind, Moline and van Broock 2011). Among Agaricomycotina, they were found in species of Tremellales, Filobasidiales (namely *F. floriforme*) but not in most species of Cystofilobasidiales (Libkind *et al.* 2005b, 2011), except *Phaffia rhodozyma* (Libkind, Moline and van Broock 2011) that represents a deep lineage in this group of fungi. Another high-value pigment that shows a narrow phylogenetic distribution is astaxanthin, an important carotenoid pigment used in aquaculture (salmon feed), animal feed and with possible beneficial health aspects related to the prevention of cancer and cardiovascular diseases (Johnson and Echavarri-Erasun 2011; Johnson 2013a,b). Among the yeasts, the pigment is known to be produced only by isolates of the *Ph. rhodozyma* complex (teleomorph *Xanthophyllomyces dendrorhous*) (Fell, Johnson and Scorzettii 2011; Johnson and Echvarri-Erasun 2011; Johnson 2013b) that belong to Cystofilobasidiales (Agaricomycetes). Strains are usually found in association with tree sap from birch trees in temperate regions of the northern Hemisphere and in stromata of *Cyttaria* spp., which are parasites of *Nothofagus* trees on the southern hemisphere (Fell *et al.* 2007; Libkind *et al.* 2007).

Carotenoids, such as β-carotene, torulene, torularhodin and γ-carotene that may be of importance for the pharmaceutical,

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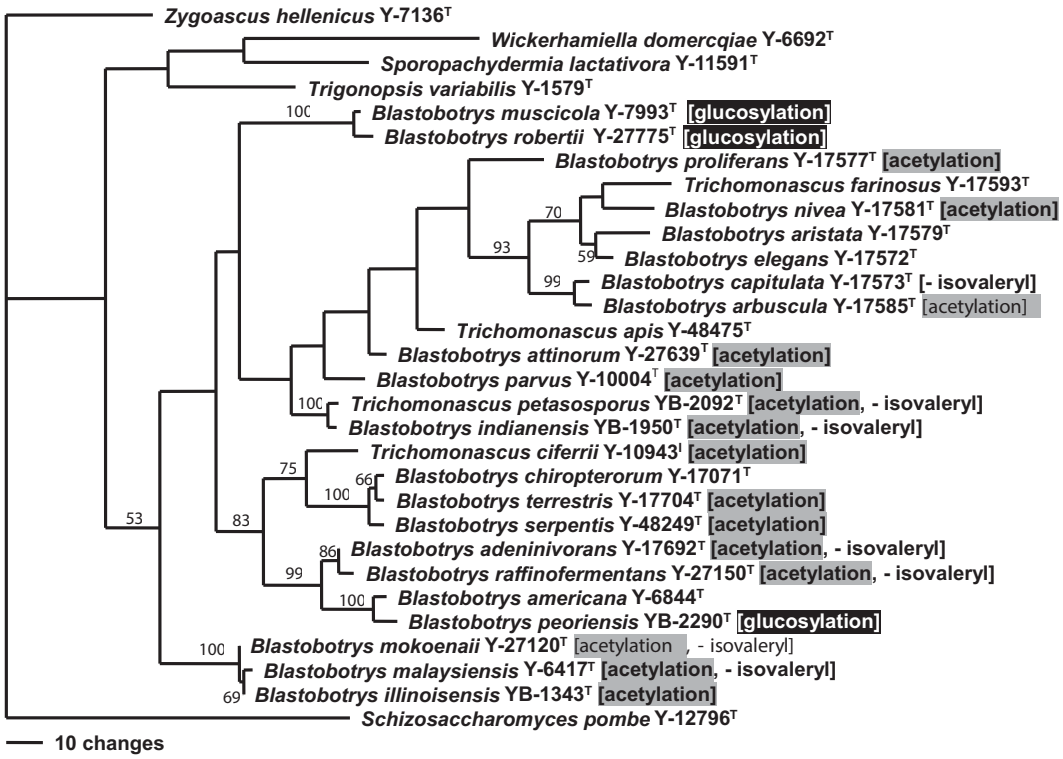

Figure 7. Modification of the *Fusarium*-produced T-2 mycotoxin by species of *Trichomonascus* and its anamorph *Blastobotrys*. Phylogenetic relationships were determined from maximum likelihood analysis of D1/D2 LSU rRNA gene sequences (Kurtzman and Robnett 2007) with bootstrap values from 1000 replicates. Glucosylation, black highlight; acetylation, gray highlight; removal of isovaleryl sidechain, absence of highlighting.

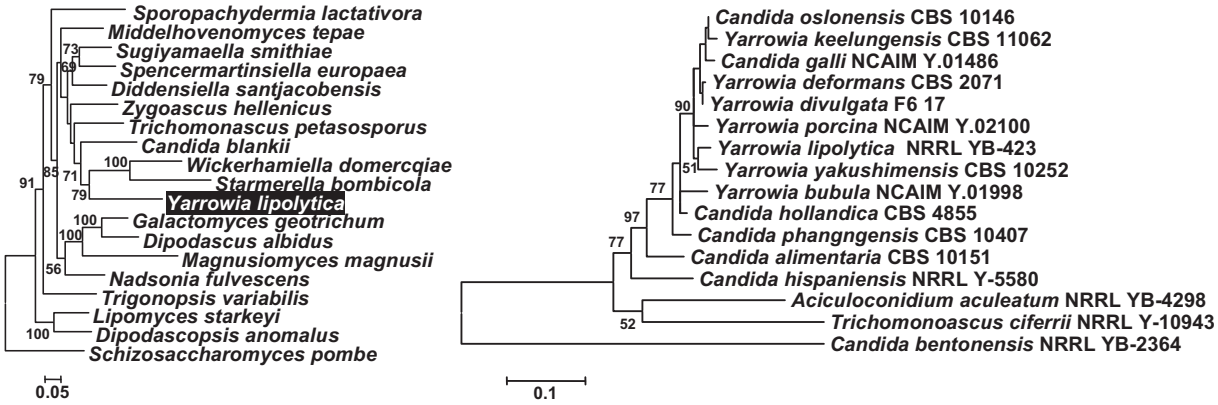

Figure 8. Phylogenetic relationships among species of *Yarrowia*. Left panel shows placement of *Yarrowia* among neighboring genera (from Kurtzman and Robnett 2013) and the right panel shows relationships of species assigned to *Yarrowia* as determined from maximum likelihood analysis (1000 bootstrap replicates) of D1/D2 LSU rRNA gene sequences (C. P. Kurtzman, unpublished). In species order in the tree, D1/D2 sequences have the following accession numbers: AM268477, EF621561, AY346454, EF405984, EU194451, KF649289, U40080, AM268472, JN256212, AM268482, AB304772, AM268481, AY789654, U40087, U40138, AY789653. Species presently placed in *Candida* will need to be transferred to *Yarrowia* to conform to the new Code. The divergent *Ca. hispaniensis* may not be a member of the *Yarrowia* clade.

food and feed, and chemical industries, are mainly formed by so-called red yeasts belonging to Pucciniomycotina, order Sporidiobolales and Erythrobasidiales (Frengova and Beshkova 2009; Thakur and Azmi 2013).

Itaconic acid is a compound of interest for the plastic/polymer industry with a global market estimate of 10 000–15 000 metric tons per year (Willke and Vorlop 2001) and representatives of Ustilaginales, such as *U. maydis* and *P. antarctica*, have been reported to produce this compound (Haskins, Thorn and Boothroyd 1955; Willke and Vorlop 2001; Levinson, Kurtzman and Kuo 2006; Klement *et al.* 2012). Other *Pseudozyma* spp. tested were found not to produce the compound (Levinson, Kurtzman and Kuo 2006). *Pseudozyma* is a polyphyletic genus so it may be possible that this inability to produce itaconic acid in part reflects the phylogeny, but likely also strain differences may occur as a second isolate of *P. antarctica* was unable to produce the compound (Levinson, Kurtzman and Kuo 2006). Finally, various members of Moniellomycetes, a black osmophilic yeast in the Ustilaginomycotina (Wang *et al.* 2014), are known as producers of erythritol that find application as a low-calorie sweetener (Cho, Yamagish and Mikawa 1998).

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Table 2. Species that can grow on 60% glucose and 16% sodium chloride [1].

Species	CBS number
Candida andamanensis	10 859
Candida boleticola	7847
Candida etchellsii	8147
Candida kungkrabaensis	10 927
Candida saraburiensis	11 696
Candida suratensis	10 928
Debaryomyces subglobosus	792

[1]The CBS database lists 942 species with salt tolerance data and 123 species have strains that are able to grow at 10% NaCl and 50 species have strains that grow at 16% NaCl. There are only seven strains belonging to seven different species that are able to grow at 60% glucose and 16% sodium chloride.

The examples above do not provide an exhaustive overview of the biotechnological potential of basidiomycetous yeasts, but these examples do demonstrate that an improved and reliable phylogeny may have predictive value for some properties of biotechnological interest.

Applications of oleaginous yeasts, especially some Basidiomycota, such as *C. curvatus, T. cacaoliposimilis, T. oleaginosis* and some red yeasts, especially *Rhodosporidium toruloides*, for the production of biofuels, such as biodiesel, as well as food and feed additives, such as cocoa butter equivalents or single cell oils, are highly promising (Holdsworth, Veenhuis and Ratledge 1988; Hassan *et al.* 1995; Gujjari *et al.* 2011; Thiru, Sankh and Rangaswamy 2011; Zhu *et al.* 2012; Lin *et al.* 2014; Tchakouteu *et al.* 2015). Of note, oleaginous ascomycetous yeasts, e.g. *Yarrowia* and *Lipomyces* (Groenewald *et al.* 2014; Tchakouteu *et al.* 2015) are also explored for the same purpose.

High temperature and high osmotic tolerance

Tolerance of yeasts to high concentrations of sugars, salt and acetic acid and growth at high temperatures are often desirable traits in biotechnology and some of these traits are more common in certain phylogenetic lineages than in other lineages. The use of yeasts tolerant to these stresses that are present in bioreactors increases the efficiency of the industrial process and reduces the chances of contamination by other microorganisms. However, resistance of some yeasts to these extreme conditions may promote spoilage in the food industry since the species can grow in the presence of common preservatives and high osmotic conditions (Tables 1 and 2), thus leading to large economic loss in this industrial sector (Boekhout and Robert 2003; Blackburn 2006).

Osmotolerant yeasts are able to grow in environments with high osmotic pressures, such as media with high concentrations of salt or sugar. Since the molecular mechanisms involved in the tolerance to sugar and salt are different and the tolerance to one stress does not require the tolerance to the other, very often the terms sugar and salt tolerance are preferred against osmotolerance (Tokuoka 1993).

High osmotic pressure is one of the common stresses in industrial fermentations. This stress is caused by the sugars used as a substrate and it is even more relevant in very high gravity fermentations where high concentrations of sugars (up to 30% w/v) are used and therefore, sugar-tolerant yeasts are needed (Puligundla *et al.* 2011). Most yeast species grow well in glucose concentrations up to 40% (w/v) whereas the number of yeast growing at 50–70% is much lower. From data taken from the CBS yeast database (http://www.cbs.knaw.nl/Collections/Biolomics.aspx) (Table 1), all isolates of 324 species are able to grow at 50% glucose, but only 140 species have strains able to grow at 60% glucose. Within a species, variation occurs in this trait and for only a few species are all isolates able to grow at 50%. Forty-two species had strains that are able to grow at 70% sugar with *Zygosaccharomyces rouxii* being the most 'extreme' species as strains may tolerate growth at 90% glucose (Martorell *et al.* 2007).

High concentrations of salt are also a limiting factor in industrial fermentations. High concentrations of Na+ in a bioreactor can be caused by high salt substrates such as molasses-enriched sugar cane stalks (Maharjan *et al.* 2012), some food wastes (Moukamnerd, Kawahara and Katakura 2013) and marine biomass (Khambhaty *et al.* 2012), or due to water recycling (Roush, Elias and Mormile 2014). The use of sea water for bioethanol production has also been suggested and that would require the use of salt-tolerant microorganisms (Zaky *et al.* 2014). The CBS database lists 942 species with salt tolerance data and 123 species have strains that are able to grow at 10% NaCl and 50 species have strains that grow at 16% NaCl. There are only seven strains belonging to seven different species that are able to grow at 60% glucose and 16% sodium chloride (Table 2). Because not all strains were tested for growth at these high levels of glucose and sodium chloride, it is anticipated that other species having these characteristics will be found. For example, *Debaryomyces hansenii* is not listed in Table 2 because CBS has no data for 16% NaCl, but Norkrans (1968) reported that some strains are able to grow in concentrations of NaCl up to 24% (w/v).

Industrial processes done at higher temperatures have the advantage of reducing cooling costs, especially in tropical countries. During ethanol fermentation, heat is generated and a cooling system is needed to maintain the optimal temperature if a mesophilic microorganism is used (Kumar *et al.* 2013). Additionally, high temperatures reduce risk of contamination and allow continuous ethanol harvest and better mixing of the fermentation compounds (Lee 1997; Banat *et al.* 1998). The CBS database reports growth at 45°C for 1260 species but only 22 species have strains that can grow at 45°C, and for growth at 50°C, this number is reduced to two species, *Kluyveromyces marxianus* and *Ogataea polymorpha*.

Tolerance of acetic acid is currently a limiting factor in bioethanol production since weak acid is cytotoxic for yeast cells. Acetic acid is produced during the hydrolysis of the raw material and concentrations range between 5 and 10 g l^{-1} (Lindberg *et al.* 2013). The CBS database contains information about growth at 1% (v/v) acetic acid for 1040 strains and 31 species have strains that grow at this concentration. Further, tests performed at CBS showed that at 2% acetic acid only four species have strains that grow well, namely *Z. bailii* var. *bailii*, *Kregervanrija fluxuum*, *Pichia membranifaciens* and *Schizosaccharomyces pombe*. When the concentration is increased to 2.5% acetic acid, only *Z. bailii* grows.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSYR online.

ACKNOWLEDGEMENTS

The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by

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FUNDING

This publication was made possible by NPRP grant 5-298-3-086 from the Qatar National Research Fund (a member of the Qatar Foundation) to Teun Boekhout. The authors thank Marie Curie Initial Training Network CORNUCOPIA (FP7-PEOPLE- 2010-ITN, grant agreement nr. 264717) for the funding.

Conflict of interest. None declared.

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