Chapter 2
Basidiomycetous Yeasts: Current Status

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Contents

Abstract The approach to yeast identification has significantly changed in just a few decades due to rapid increase in basic biological knowledge, increased interest in the practical applications and biodiversity of this important microbial group, and enormous technological advances especially in the sphere of molecular tools. While some conventional methods are still tenable, many molecular techniques have been developed that allow for strain classification at all taxonomic levels. However, the oldest tool of microbiology, the microscope, is still a fundamental accessory for studies involving yeast biology, biodiversity and taxonomy.

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The basidiomycetous yeasts, are currently recognized, in three classes of the Basidiomycota: Ustilaginomycetes, Urediniomycetes and Hymenomycetes. These yeasts have considerable economic, agricultural and medical importance and estimates suggest that the number of known yeasts represents only about 1 to 5% of the species that exist in nature. There is an increased interest in exploration of these species for economic exploitation and there is a need to understand their biodiversity and ecological roles.

Identification and phylogenetic placement of the basidiomycetous yeasts is not always easy, partly because of their polyphyletic nature. The unifying characteristic of these fungi is a predominant unicellular growth phase. Separation of yeasts into the three classes of fungi is based on septal morphology, cell wall composition and rDNA analysis. Generic diagnosis is based on sexual and vegetative biology, in addition to physiological tests such as growth on inositol or D-glucuronic acid and formation of extracellular starch-like compounds. Species are usually differentiated by physiological attributes, particularly the utilization of carbon and nitrogen sources, and by measurement of DNA reassociations between closely related species. Currently approximately 50 genera and 250 species of basidiomycetous yeasts are known. Molecular methods used in their identification include, species-specific PCR primers, analysis of RFLPs PFGE, randomly amplified polymorphic DNA (RAPD) and single-stranded conformational polymorphisms (SSCP). Significant advances in basidiomycete systematics have resulted from sequence analysis of the large and small subunits of rDNA.

Basidiomycetous yeast species are associated with living plants, *viz*., *Sporobolomyces* and *Phaffia.* Several species have been found to play a prominent role in biocontrol of plant disease whereas others have application in agro based industry. For example, *Phaffia rhodozyma* produces a pigment astaxanthin that has considerable market in aquaculture industry. On the other hand several species produce polysaccharases and can store lipids in amounts reaching upto 65% of their biomass. Some species of *Cryptococcus, Rhodotorula* and *Trichosporon* can degrade varied aromatic compounds and thus are a candidate in bioremediation. On the negative side is the pathogenic *Filobasidiella neoformans* that poses medical problem since both the varieties of this basidiomycetous yeast infect the lungs which can result in pneumococcal-type pneumonia. Lipophilic *Malassezia* spp. are associated with skin surfaces but can cause serious pulmonary and other infections.

Diversity searches in the natural environment have resulted in description of new species within the basidiomycetous yeasts at a rapid pace and the field is wide open to global exploration.

Keywords Basidiomycetous yeasts, systematics, molecular methods, biocontrol, Astaxanthin, polysaccharases

2.1 Introduction and Definition

Yeasts are described as unicellular fungi and are generally characterized by the absence of coenocytic hyphae. They are represented usually by small cells which reproduce by budding or by the formation of a cross wall, followed by fission. During budding, the cell wall of the mother cell inflates and blows out to form a 'bud', which is subsequently released as a daughter cell. However, several yeast exhibit formation of hyphae or pseudohyphae, made up of chains of elongated bud.

The term 'yeast' has no taxonomic standing of its own *per se* and represents a growth form in several groups of unrelated fungi. Some fungi are dimorphic (two life stages) and exhibit a 'yeast' stage that shifts to mycelial growth under certain cultural conditions. Yeasts include fungi with sexual forms (basidiomycetous yeast and ascomycetous yeasts) and asexual forms. Basidiomycetous yeasts can be differentiated from the ascomycetous yeasts based on urease test which is positive in the former group.

The basidiomycetes are divided into subclasses: Hymenomycetes (mushroom-forming basidiomycetes), Urediniomycetes (rusts), and the Ustilaginomycetes (smuts). Some basidiomycetous members grow in culture with budding cells.

In this chapter, the diversity, taxonomy and systematics, and importance of basidiomycetous yeast are stressed. Beginning as a loose group of asporogenous forms, basidiomycetous yeasts now stand on firm footing by way of diversity, ecology and phylogenetic consideration.

2.2 Diversity and Ecology

The unicellular nature of yeasts makes them better suited for deep liquid substrates or moist and uneven surfaces. Therefore, yeasts grow typically in moist environments where there is an abundant supply of simple, soluble nutrients such as sugars and amino acids. This explains why they are common on leaf and fruit surfaces, on roots and in various types of food. Exceptions are those that degrade polymers, such as starch and cellulose. Basidiomycetous yeasts are widely distributed on a variety of substrates such as angiosperm wood and bark, weathered and dead inflorescence and mushrooms (McLaughlin et al., 2004).

Yeasts are found in widely different aquatic and terrestrial sources, as also in certain restricted habitats (Table 2.1). They are also found associated with the body of certain animals where they act as intestinal commensals (Lachance and Starmer, 1998). The type of nutrients reaching the soil determines the yeast microflora although some forms are permanent residents in soil, *viz., Cryptococcus, Rhodotorula* species, and *Sporobolomyces* species (Spencer and Spencer, 1997; Lachance and Starmer, 1998).

In a survey of basidiomycetous yeast, *Fellomyces fuzhouensis* was considered as potentially pathogenic; this species exclusively reproduces by conidiogenesis (Gabriel

Table 2.1 Distribution of major genera of basidiomycetous yeasts in various habitats

Habitat	Genera
Plants	*Sporobolomyces, Rodotorula, Cryptococcus* sp.
Tree exudates	*Phaffia rhodozyma*
Insect	*Cryptococcus* sp.
Soil	*Cryptococcus neoformans, Rhodotorula, Sporobolomyces* sp.
Water body	*Cryptococcus, Rhodotorula, Trichosporon* sp., *Rhodosporodium*
Animal	*Cryptococcus, Rhodotorula, Trichosporon* sp.
Atmosphere	*Cryptococcus neoformans, Rhodotorula, Sporobolomyces*

et al., 2000). *Sporobolomyces roseus* Kluyver & van Neil is a common ballistoconidium-forming yeast species which occurs in several different habitats, but most frequently in the phyllosphere (Nakase, 2000).

The distribution of yeast species in various water sources varies quite widely from a few cells ml^{-1} in unpolluted water to more than a million ml^{-1} in effluents. In polluted water the number of yeast increases proportionally with the degree of pollution (Lachance and Starmer, 1998). Some, such as red yeasts have been used as indices of pollution. Fresh water yeasts as well as those found in sea include those from other ecosystems, which have been washed into these water sources (Vincent, 1988). Aquatic species of basidiomycetous forms generally include species of *Cryptococcus, Rhodotorula and Sporobolomyces* (Hagler and Ahearn, 1987).

The diversity of ballistoconidium – forming yeasts has been studied extensively in phyllosphere in north-east China. As many as 250 yeast strains were isolated from 39 leaf samples of various plants collected from Changbai Mountain, Jilin Province, north-east China; of these six strains were classified into one group by conventional and chemotaxonomic characterization. Two separate groups, representing two novel *Bensingtonia* species *viz., Bensingtonia changbaiensis* sp. nov. (type strain CB 346^T = AS 2.2310^T = CBS 9497) and *Bensingtonia sorbi* sp. nov. (type strain, CB 288^T = AS 2.2303^T = CBS = 9498^T), were recognized based on 26S rDNA D1/D2 domain, ITS region and 18S rDNA sequence analyses (Wang et al., 2003).

The *cactus*-yeast-*Drosophila* system has been used as an example (Starmer et al., 1991) to show the role of dispersal as a factor that is highly specific since it may involve insects that feed only on certain Cactus species (Lachance, 2003). The yeasts present in certain habitats provide sufficient benefits such as food enrichment and detoxification to the vector. This ensures further dispersal of the yeast community to a new environment. Furthermore, the compatibility of yeast species within a niche influences the species found in that habitat (Lachance and Starmer, 1998).

Yeast habitats are often rich in simple organic carbon, sometimes very high in moisture, acidic or occasionally alkaline. This diversity in habitat-types confirms that yeasts are able to grow over a broad range of growth conditions. These features enable one to predict their distribution; however, new yeast species isolated from

the varying habitats are formed due to selection pressures exerted by the environment. The observed similarities and differences in yeasts found in a particular environment play a vital role in observing the evolution as it progresses. Thus, ecology of yeasts is believed to involve the effects of the physical environment on the yeast cells and the interaction of the yeast species with other microorganisms (Spencer and Spencer, 1997).

2.3 Isolation and Maintenance

2.3.1 Isolation of Basidiomycetous Yeasts

Yeasts, like other heterotrophic living organisms require carbon, nitrogen, phosphorus, trace elements and growth factors as sources of nutrition. Yeasts rarely occur in the absence of either molds or bacteria and since they do not occur naturally as pure cultures it is important to analyse the components that are crucial. Hence, selective techniques are often used for the recovery of yeasts, using media that permit the yeast to grow while suppressing molds and bacteria (Yarrow, 1998).

For isolation purposes, the direct streak plating technique is recommended. The preferred medium is yeast-malt (YM) agar which is acidified to pH 3.7 using either hydrochloric acid or phosphoric acid. Acidification is preferred over the incorporation of antibiotics and fungistatic agents (Lachance and Starmer, 1998). Fungistatic agents are used with caution as some of these compounds may also inhibit certain yeasts (Yarrow, 1998). Cultures are usually incubated between 20°C and 25°C since most basidiomycetous yeasts are mesophilic. Optimum temperatures for growth are higher for some yeasts and lower for others. The psychrophilic taxa require temperatures of between 4°C and 15°C as their optimum temperatures. Higher temperatures, in the range of 30–37°C, are often required for yeasts that are strictly associated with warm-blooded animals (Yarrow, 1998).

Recovery of yeasts present in low numbers requires use of enrichment media and conditions that favour their growth over other microorganisms. Usually a sample is inoculated into a liquid medium with a pH of 3.7 to 3.8. Air can be excluded from the culture to discourage the development of moulds although this method leads to the appearance of fermentative strains excluding the aerobes. Sterile pharmaceutical paraffin can be poured on the surface of the media to a depth of 1 cm to exclude air (Yarrow, 1998).

The use and ability of some yeasts to either transform toxic compounds to valuable compounds or to detoxify these compounds has been well documented (Copley, 1998; Fetzner, 1998).

The carbon sources usually utilized by microorganisms contain glucose or other carbohydrates and are used as carbon and energy source. Microbes with the ability

to utilize non-carbohydrate carbon sources such as alkanes, branched alkanes (Demain et al., 1998), low molecular weight aromatics and cyclic alkanes are important because these compounds are environmental pollutants (Van Beilen et al., 1998, 2003). In addition, the degradation of cyclic alkanes by microbes is important in nature and in technological applications such as wastewater, waste gas treatment, bioremediation and biocatalysis (Sikkema et al., 1995). In particular, cyclohexane is becoming increasingly important as an industrial solvent because it is relatively nontoxic compared to benzene, a known carcinogen, used previously as an industrial solvent (Uribe et al., 1990; Sikkema et al., 1995).

Monoterpenes are branched chain C_{10} hydrocarbons widely distributed in nature. The most widespread terpene in the world is limonene, which is formed by over 300 plants (Colocousi et al., 1996; van der Werf and de Bont, 1998; King and Dickinson, 2000, 2003). The biotransformation of limonene by microorganisms with the potential production of more valuable natural flavour compounds, has been reported in bacteria and basidiomycetous yeasts (van der Werf et al., 1999). This degradation pathway was determined by biochemical studies (van der Werf et al., 1999). Some yeast strains, all belonging to the alkane-utilizing yeasts, can hydroxylate monoterpenes (Van Rensburg et al., 1997), but ascomycetous yeast strains in general do not utilize monoterpenes as sole carbon source. The only report on the isolation of yeasts able to grow on monoterpenes has been on basidiomycetous yeasts (Thanh et al., 2004).

2.3.2 Maintenance of Cultures

The best medium for maintaining yeast cultures requires the addition of glucose to the media as sole source of carbon. This is preferred since the risk of changes in growth and fermentative pattern, due to the selection of mutants is minimized (Scheda and Yarrow, 1966). However, an unstable strain can change its properties within a few days due to the selection pressure when grown on media containing malt extract. Consequently, YM agar slopes as well as yeast-glucose-peptone or malt agar are used to maintain yeast flora (Yarrow, 1998).

Numerous yeast strains are stored at temperatures between 4°C and 12°C by sub-culturing at intervals of six to eight months. The frequency of subculturing differs among yeasts, with some such as *Arxiozyma* and *Malassezia* requiring subculturing every month because they are more sensitive to prolonged storage. The teleomorphic members of the ascomycetous and basidiomycetous yeasts are known to lose the ability to sporulate on successive cultivation on laboratory media. The extent to which yeasts lose their ability to sporulate differs among yeasts and may range from a few weeks to several years (Yarrow, 1998). As a result, it is best to preserve important strains using techniques such as lyophilization (Kirsop and Kurtzman, 1988), L-drying (Mikata and Banno, 1989) and freezing in either liquid nitrogen or a mechanical freezer at temperatures between −60°C and −135°C (Yarrow, 1998). The method currently being mostly used is freezing in liquid

nitrogen (cryopreservation), which uses a cryoprotectant such as glycerol to ensure high rates of survival as well as genetic stability.

2.4 Cytological Characteristics

Basidiomycetous yeasts are characterized by electron dense, layered cell walls (Simmons and Ahearn, 1987) and septal morphology. Septal morphology has been used as a primary phylogenetic character for discrimination within the basidiomycetous yeasts (Moore, 1998). For example, species belonging to urediniomycetous forms have septa with simple pores (Fig. 2.1) in which the cell wall is attenuated towards the central pore during the hyphal state. Multiple pores have however been reported in *Kriegeria eriophori* instead of single pore (McLaughlin et al., 1995). Mannose is excessively present in the cell wall, glucose to some extent; fucose and rhamnose are rarely present and xylose is entirely absent (Prillinger et al., 1991). Starch-like compounds and inositol are absent in urediniomycetous yeasts.

Ustilaginomycetous yeasts are characterized by 'micropore-like' septa (Fig. 2.2) which possesses an inflated margin. The occurrence of inflated margin can vary in species. The micropore septa do not have tapering cell walls and lacks a true pore (Bauer et al., 1997). The level of glucose is high; galactose and mannose are present to a certain extent whereas xylose is absent (Prillinger et al., 1990).

Hymenomycetous yeasts possess dolipore septum (Fig. 2.3) wherein cell walls contain glucose, mannose and xylose (Roeijmans et al., 1998). Inositol is usually assimilated and starch-like compounds are produced by a majority of them.

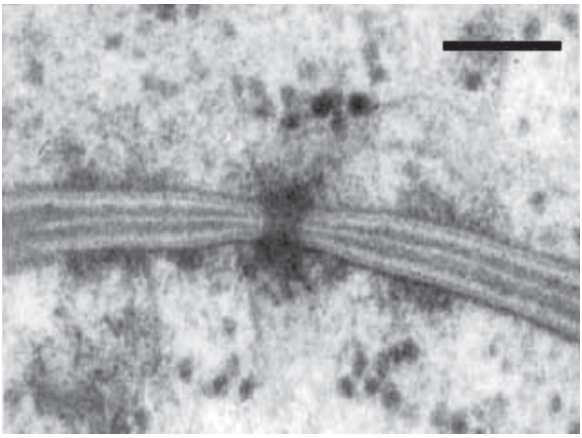

Fig. 2.1 Septal pore type in Urediniomycetes. From Bauer, R. et al. 1997. *Can. J. Bot.* **75**: 1273–1314. Copyright NRC Research Press. Reproduced with permission

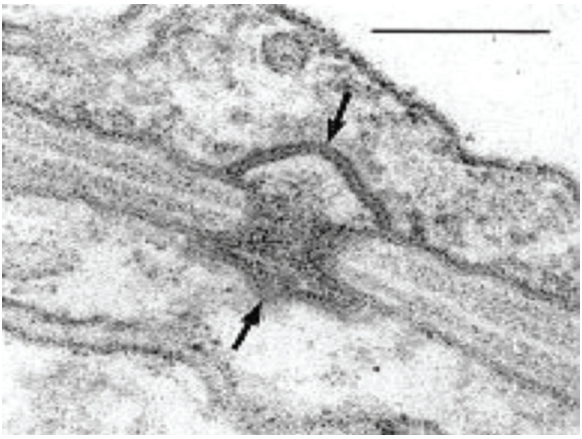

Fig. 2.2 Transmission electron micrograph showing a typical septal pore apparatus of the Ustilaginomycetes (*Entyloma callitrichis*) with two membrane caps (arrows). Scale bar = 0.1 μm. From Bauer, R. et al. 1997. *Can. J. Bot.* **75**: 1273–1314. Copyright NRC Research Press. Reproduced with permission

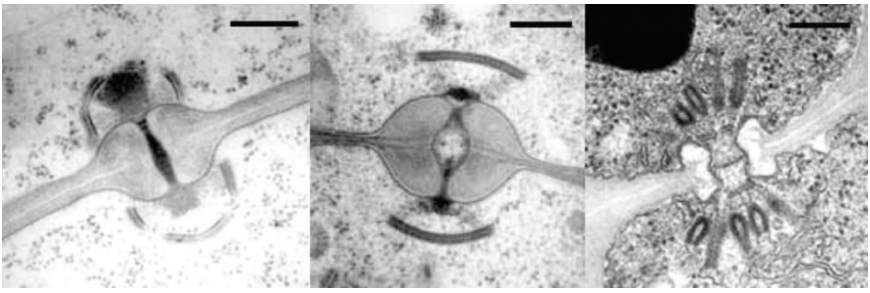

Fig. 2.3 Septal pore types in the Hymenomycetes. Left: Dolipore with perforated parenthesomes of *Schizophyllum commune* (Bar = 0.25 μm) Center: Dolipore with continuous parenthesomes of *Tulasnella* sp. (Bar = 0.25 μm). Right: Dolipore with cup-shaped parenthesomes of *Tremella* sp. (Bar = 0.2 μm) From Bauer, R. et al. 1997. *Can. J. Bot.* **75**: 1273–1314. Copyright NRC Research Press.Reproduced with permission

Sampaio et al. (2002) described two new genera *Bullerobasidium* and *Papiliotrema* with three new species, *B. oberjochense, P. bandonii* and *B. murrhardtense* by integrated analysis of morphological, ultrastructural, physiological and molecular data. They placed these new genera and species into the order Tremellales since they compared with those of closely related taxa.

Two general types of teleomorphs are found among the basidiomycetous yeast (Boekhout et al., 1993). In the first, teliospores are formed and germinate to produce a basidium that bears basidiospores. The second type of sexual cycle has no

teliospores. Basidia develop on hyphae or yeast cells and give rise to basidiospores in a manner similar to jelly fungi (Kurtzman and Fell, 1998). When these sexual structures are not present, basidiomycetous yeasts are morphologically indistinguishable from ascomycetous yeasts, except that morphology of the bud scars can sometimes be different.

More useful diagnostic characteristics of the basidiomycetous yeasts are the presence of clamp connections, the red stained colonies with diazonium blue B (colonies of ascomycetes remain unstained) (van der Walt and Hopsu-Hava, 1976), + ve urease reaction and a high G + C content. TEM studies have demonstrated that the inner walls of the basidiomycetous yeasts are typically lamellar, in contrast to the uniform inner layer of ascomycetes (Kreger-van Rij and Veenhuis, 1971). TEM is also useful to demonstrate dolipore (complex barrel-shaped structures formed in the septa, which are covered on both sides by a membrane called parenthosome) in basidiomycetous yeast. The presences of carotenoids and ballistoconidia (forcibly ejected vegetative cells) in some taxa have been used as a criterion for assignment to a genus.

Ultrastructural features correlate well with chemosynthetic characteristics and are regarded as reliable systematic criteria in higher taxonomic rank. However, at lower level, rRNA sequence analysis shows that *Rhodotorula, Sporobolomyces*, and *Cryptococcus* are polyphyletic, confirming that commonly used phenotypic characteristics are insufficient for defining anamorphic genera (Nakase et al., 1991).

Prillinger and coworkers (1990, 1991) and Dorfler (1990) investigated carbohydrate composition of the purified cell wall in yeast states of Basidiomycetes. They found that xylose, rhamnose, fucose and galactose as well as the ratio of glucose to mannose were important in differentiation of higher taxa. Employing GLC-analysis of purified cell wall hydrolysates in basidiomycetous yeast, Prillinger et al. (1991) identified three different carbohydrate profiles, Glucose-Mannose-Galactose (*Ustilago*-type that exhibited high amount of glucose and balanced amount of mannose and galactose), Mannose-Galactose-Fucose (*Microbotryum*-type, that showed greater amount of mannose compared to that of glucose and balanced amount of galactose and fucose) and Glucose-Mannose-Xylose (*Tremella*-type wherein higher amount of glucose and balanced amount of mannose and xylose was observed); a fourth type in the systematics of basidiomycetous yeast, was the so-called *Dacrymyces*-type that exhibited presence of xylose and balanced amounts of glucose and mannose in the cell walls, e.g. *Dacrymyces* and *Calocera.*

2.5 Taxonomy of the Basidiomycetous Yeasts

The principles of yeast taxonomy encompass identification, naming and placing organisms in their proper evolutionary framework. Historically, basidiomycetous yeasts have been placed into three taxonomic classes namely the Hymenomycetes, Urediniomycetes and Ustilaginomycetes.

2.5.1 Natural Classification System

Natural classification systems are based on evolution, and have been the preferred method for systematics. This system addresses the species concept, and also shows the phylogeny or sequence of events that are involved in evolution. The species concept recognizes that different species have different phenotypic characters by which they can be recognized (van der Walt, 2000).

2.5.2 Biological Species Concept

Modern biologists have arrived at the biological species concept that is characterized by four distinct criteria. These are, a reproductive unit, an ecological unit, a genetic unit and, an evolutionary entity. This was further enhanced by another dimension of the species through the introduction of the so-called phylogenetic species concept that focused on the interpretation of the ribosomal nucleotide sequence analysis thereby excluding the phenotypic, genetic or ecological criteria. The two concepts namely the biological species concept and phylogenetic species concept have little in common. The biological species concept is not easily applied in practical systematics resulting in having to adhere to the type-based species and the extensive use of phenotypic differentiation (Kurtzman, 1987; Boekhout and Kurtzman, 1996). By definition, the biological species concept excludes the asexual (anamorphic) yeast species. Barnett and co-authors (2000) have listed 93 characters for identification purposes concentrating on just phenotypic characteristics.

To overcome the noted drawbacks from the two preceding concepts, yeast taxonomists had to consider analysis of the nuclear genome that had already been extensively used in prokaryotic systems. The base composition of the nuclear genome was believed to reflect the ancestral descent at molecular level reducing reliance on phenotypic characterization (van der Walt, 2000).

2.5.3 Conventional Identification

The criteria and tests for identification of basidiomycetous yeasts involve firstly, observation of culture characteristics, which include colour, shape and texture of the colonies. The production of extra-cellular polysaccharides is also observed by the resulting mucoid growth. There are distinctive colours such as yellow, orange and red those are peculiar to certain genera of basidiomycetous yeasts, viz., *Phaffia*, *Rhodosporidium* and *Sporidiobolus*. However, the colour produced by a majority of yeasts ranges from whitish through cream to buff (Yarrow, 1998). This is followed by observation of asexual structures, which include shape and size of the vegetative cells. The mode of conidia formation is important and provides information, which aids in the identification of a strain. Budding starts by forming a small outgrowth

at some point on the surface of the cell without the cell changing in size. The increase in size is seen in a newly formed bud, which eventually separates from the parent cell (mother). Holoblastic budding results from outgrowth of the entire cell wall of the parent cell, the bud separates from the narrow base leaving a scar through which no further budding occurs. This type of budding is characteristic of the Saccharomycetales and their anamorphic states while enteroblastic is characteristic of basidiomycetous yeasts and results in formation of a collaret due to recurrent formation and abscission of a succession of buds (Yarrow, 1998).

The vegetative cells have different shapes that include globose, subglobose, ellipsoidal, ovoidal, cylindrical, botuliform, elongate, apiculate, lunate and triangular. The shape may reflect the type of reproduction and in some cases it is peculiar to particular genera or species viz., the bottle-shaped cells of *Malassezia* (Yarrow, 1998).

Sexual structures are investigated with respect to arrangement, cell wall ornamentations, number, shape and size of basidiospores (Fig. 2.4). Other types of spores formed that aid identification include endospores which are vegetative cells, formed within discrete cells and hyphae. They cannot be stained selectively. However, they can also be observed in old cultures on YM agar. Asexual endospores are observed in strains of the genera *Cryptococcus, Cystofilobasidium,* and *Trichosporon* whereas they are uncommon in other genera.

2.5.4 Species Differentiation Based on ITS and D1/D2 Regions

van der Aa Kühle and Jespersen (2003) investigated phylogenetic analysis of closely related species by amplifying the region spanning two intergenic transcribed spacers (ITS1 & ITS2) and the 5.8S ribosomal subunit. This region is located between the 18S and the 28S rRNA genes in yeasts. The ITS region is subdivided into ITS 1 region which separates the conserved 18S and the 5.8S rRNA genes (Frutos et al., 2004). The ITS 2 region is found between 5.8S and 28S rRNA genes. The ITS 1 and 2 have been shown to play a role in primary rRNA processing (Musters et al., 1990).

D1/D2 domain: This region refers to the variable domain of the large subunit (26S) ribosomal DNA or the complete small subunit and is approximately 600 bases in size. Conspecific strains are separated by less than 1% nucleotide substitution whereas biological species are separated by greater than 1% nucleotide substitution. Kurtzman and Robnett (1998) have shown that most yeast species can be identified from sequence divergence in this region, which represents a partial sequence of the 26S rDNA (Kurtzman and Robnett, 1998; Fell et al., 2000).

The sequencing of the D1/D2 domain has been extensively used to identify yeasts (Phaff et al., 1999; Hong et al., 2001; Scorzetti et al., 2002). According to Frutos et al. (2004) it is accepted universally as the main tool for yeast taxonomy. Databases of the D1/D2 sequences are now available for all currently recognized ascomycetous and basidiomycetous yeasts (Kurtzman and Robnett, 1995, 1997, 1998; Guffogg et al., 2004). This extensive available database makes the task of species identification much easier (Kurtzman, 2001; Starmer et al., 2001; Wesselink

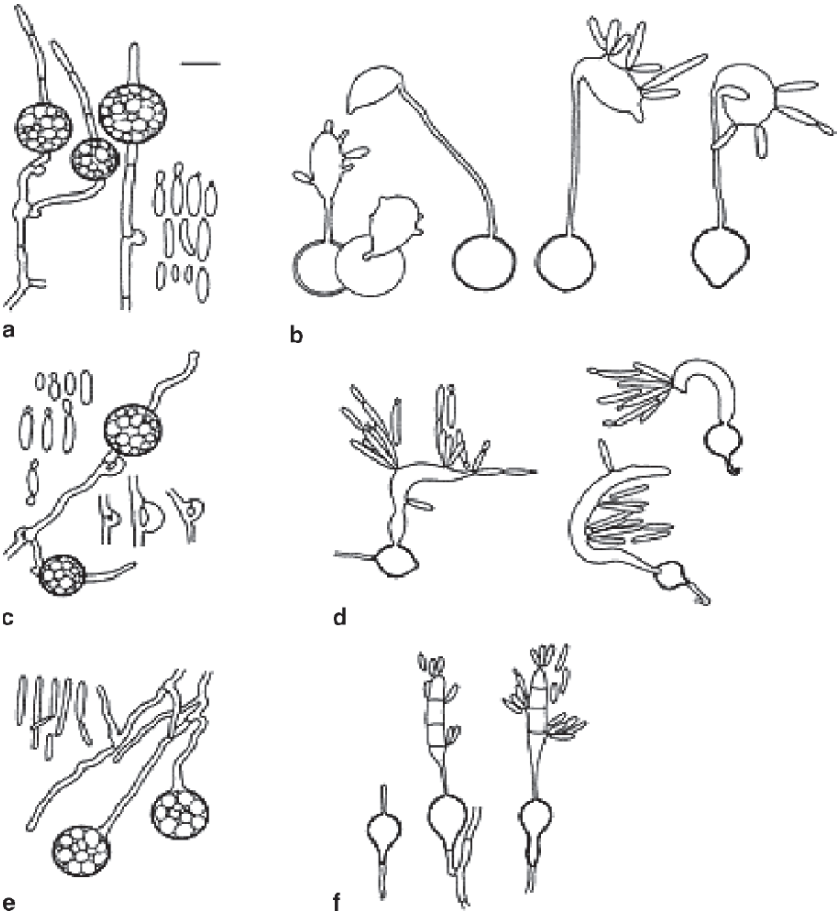

Fig. 2.4 Line drawings of different developmental stages of basidiomycetous yeast cells (after 4–6 days on MYP agar), mycelium and teliospores (after 1–2 weeks on PDA) (**a**, **c**, **e**) are shown; germinated teliospores, basidia and sessile basidiospores (**b**, **d**, **f**) are shown. From Sampaio, J.P. et al. 2004. *Int. J. Syst. Evol. Microbiol.* **54**: 1401–1407. Copyright IUMS. Reproduced with permission

et al., 2002) and serve as reliable and practical criteria for identification of most known yeasts (Abliz et al., 2004). It has been extensively used to characterize most of the basidiomycetous species at strain level.

2.6 Systematics of Basidiomycetous Yeasts

Basidiomycetous yeasts have been divided into different lineages based on the analysis of different region of rDNA viz., four main clusters according to 26S rDNA (Sporidiales, Tremellales, Filobasidiales and related taxa and, the

Ustilaginales; Fell et al., 1995); and three main lineages according to 18S rDNA or the D1/D2 region of the large subunit rDNA (Ustilaginomycetes, Urediniomycetes and Hymenomycetes; Fell et al., 2000).

Not only nuclear DNA (n DNA) approach but also another approach wherein characterization of genes present in extrachromosomal organelles provides a pivotal way to infer phylogeny of closely related species, viz., the rapid evolution of the mitochondrial genome, its lack of recombination and its maternal inheritance, makes it an attractive marker for inferring the phylogeny of closely related species (Manceau et al., 1999). Phylogenetic relationships of *Rhodotorula* spp. were studied by employing partial sequences of mitochondrial cytochrome b gene (Biswas et al., 2001). Based on *mt cyt* b gene, the basidiomycetous yeasts are distributed into two main clusters: one containing Tremellales, Filobasidiales and their anamorphs, and the other, Ustilaginales, Sporidiales and their anamorphs.

Fell et al. (2000) examined the phylogenetic diversity of yeasts among the Ustilaginomycetes, Urediniomycetes, and Hymenomycetes. The results obtained confirmed some accepted concepts, *viz.,* that yeasts are a heterogeneous group of organisms and that many genera are representative of artificial assemblages, e.g. the genus *Cryptococcus* occurs in the following Hymenomycetes clade: Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales. Similarly species of *Rhodotorula* occur in *Microbotryum, Sporidiobolus* and *Erythrobasidium* clades of the Urediniomycetes and Microstromatales clade of the Ustilaginomycetes.

Middelhoven et al. (2004) isolated three novel species, *Trichosporon vadense* sp. nov. (type strain CBS 8901^T), *Trihcosporon smithiae* sp. nov. (type strain CBS 8370^T) and *Trichosporon gamsii* sp. nov. (type strain CBS 8245^T), from soil and novel species *T. scarabaeorum* sp. nov. (type strain CBS 5601^T) and *T. dehoogi* sp. nov. (sp. nov. CBS 8686^T) from insect of unknown origin, respectively. These new species were quite different from other related species based on phylogenetic position and physiological characteristics.

A novel species of *Cryptococcus* was recovered from salt farm on the Taean Peninsula in Korea, i.e. *C. taeanensis*. The isolate exhibited typical physiology of the genus *C. vuillemin*, but was quite distinct from previously described species in the genus based on its large subunit rRNA D1/D2 domain sequence (Shin et al., 2005).

2.6.1 Yeast Species of the Hymenomycetes

Swann and Taylor (1995a, b, c) recommended two subclasses among the Hymenomycetes based on sequence analysis of small subunit rDNA, (a) the Hymenomycetidae, containing the non-yeast-like macrofungi, mushrooms and puffballs; and (b) the Tremellomycetidae. These authors analyzed D1/D2 region and kept them in four major clades (Fig. 2.5) of the Tremellomycetidae: the Tremellales, the Trichosporonales, the Filobasidiales and the Cystofilobasidiales. The hymenomycetous yeast genus, *Cryptococcus*, is polyphyletic and occurs in all four clades whereas remaining genera occur in single clades: a. the Tremellales – *Bullera,*

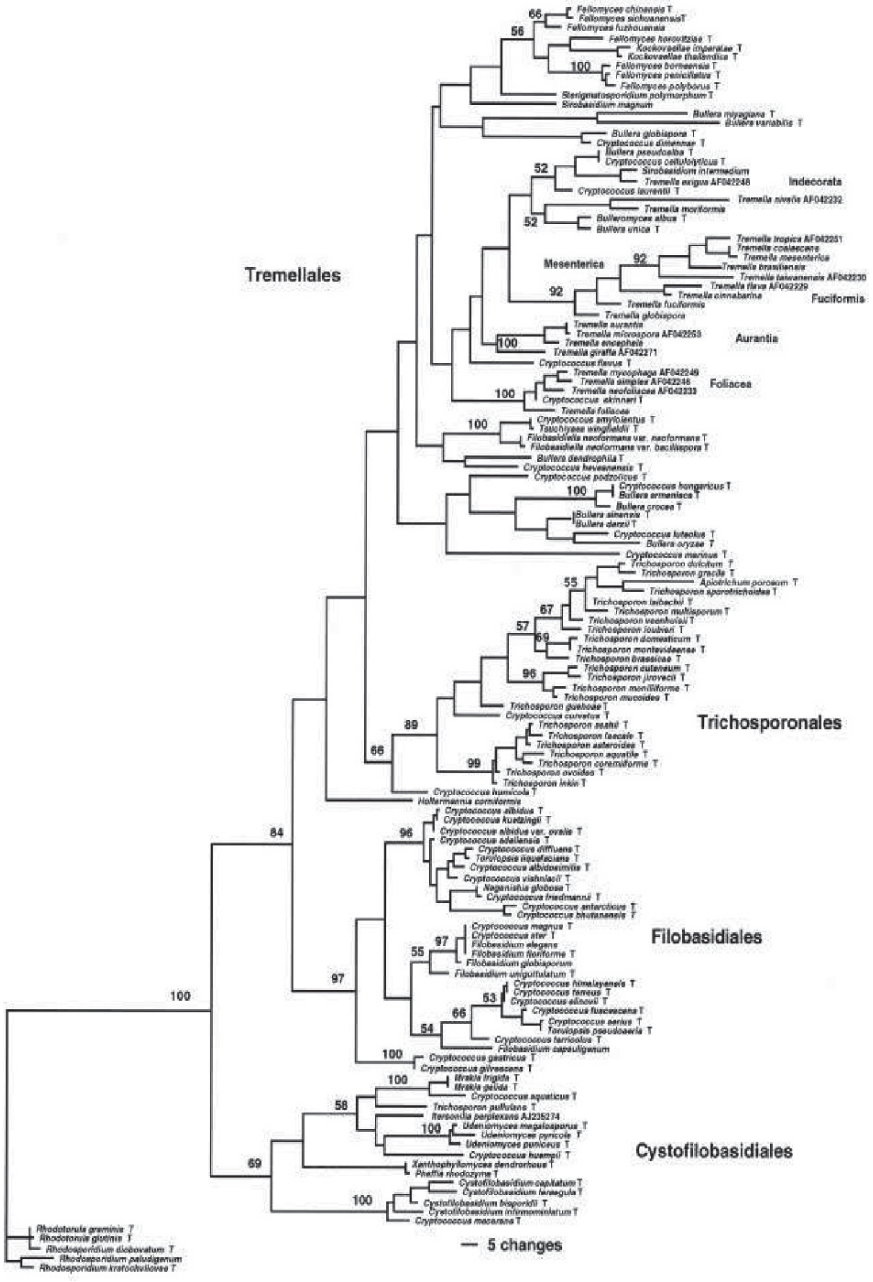

Fig. 2.5 Hymenomycetous yeasts: phylogenetic analysis of the D1/D2 region of the large-subunit rDNA (one of 100 equally parsimonious trees). From Fell et al. 2000. *IJSEM* **50**: 1351–1371. Copyright IUMS. Reproduced with permission

Bulleromyces, Fellomyces, Filobasidiella, Kockovaella and *Tsuchiyaea*; b. the Trichosporonales – all species of *Trichosporon* with the exception of *Trichosporon pullulans* which occurs in the cystofilobasidiales; c. the Filobasidiales – *Filobasidium*; and, d. Cystofilobasidiales – *Cystofilobasidium, Mrakia, Phaffia, Urediniomyces* and *Xanthophyllomyces.*

2.6.2 Yeast Species of the Urediniomycetes

The group urediniomycetes constitutes four major clades (Fig. 2.6) with the genera *Microbotryum, Sporidiobolus, Agaricostilbum* and *Erythrobasidium*. Three other genera once occupied two or more clades. *Bensingtonia* occurs in *Microbotryum* and *Agaricostilbum* clades; *Rhodotorula* in *Microbotryum, Sporidiobolus,* and *Erythrobasidium* and *Sporobolomyces* occur in all four clades. Genera found in single clade were:

a) Microbotryum clade – *Leucosporidium*
b) Sporidiobolus clade – *Rhodosporidium* and *Sporidiobolus*
c) Agaricostilbum clade – *Kondoa, Kurtzmanomyces* and *Sterigmatomyces*
d) Erythrobasidium clade – *Erythrobasidium, Sakaguchia* and *Occultifur*

2.6.3 Distribution of Yeasts among the Ustilaginomycetes

A majority of yeast species examined within the Ustilaginomycete consist of the D1/D2 sequences; originally generally isolated from plants, they were distributed into several clades (Fig. 2.7): *Pseudozyme* spp. and *Rhodotorula acheniorum* are in the subclass Ustilaginomycetidae, whereas *Rhodotorula phylloplana* and *Sympodiomycopsis paphiopedili* are in the subclass Exobasidiomycetidae. *Tilletia* is phylogenetically associated with the Tilletiales.

2.7 Basidiomycetous Yeast – Life Cycle

The life cycle of basidiomycetous yeast normally alternates between diplophase and haplophase. Both ploidies can exist as stable cultures. In heterothallic strains, haploid cells are of two mating types, a and α. Mating of a and α cells results in a/α diploids that are unable to mate but can undergo meiosis. The eight haploid (basidiospores) products resulting from meiosis of a diploid cell are contained within the wall of the mother cell (the basidium). Digestion of the basidium and separation of the basidiospores by micromanipulation yield the eight haploid meiotic products.

Fig. 2.6 Urediniomycetous yeasts, representing four clades (*Microbotryum*, *Sporidiobolus*, *Erythrobasidium* and *Agaricostilbum*): phylogenetic analysis of the D1/D2 region of the large-subunit rDNA (one of 100 equally parsimonious trees). From Fell et al. 2000. *IJSEM* **50**: 1351–1371. Copyright IUMS. Reproduced with permission

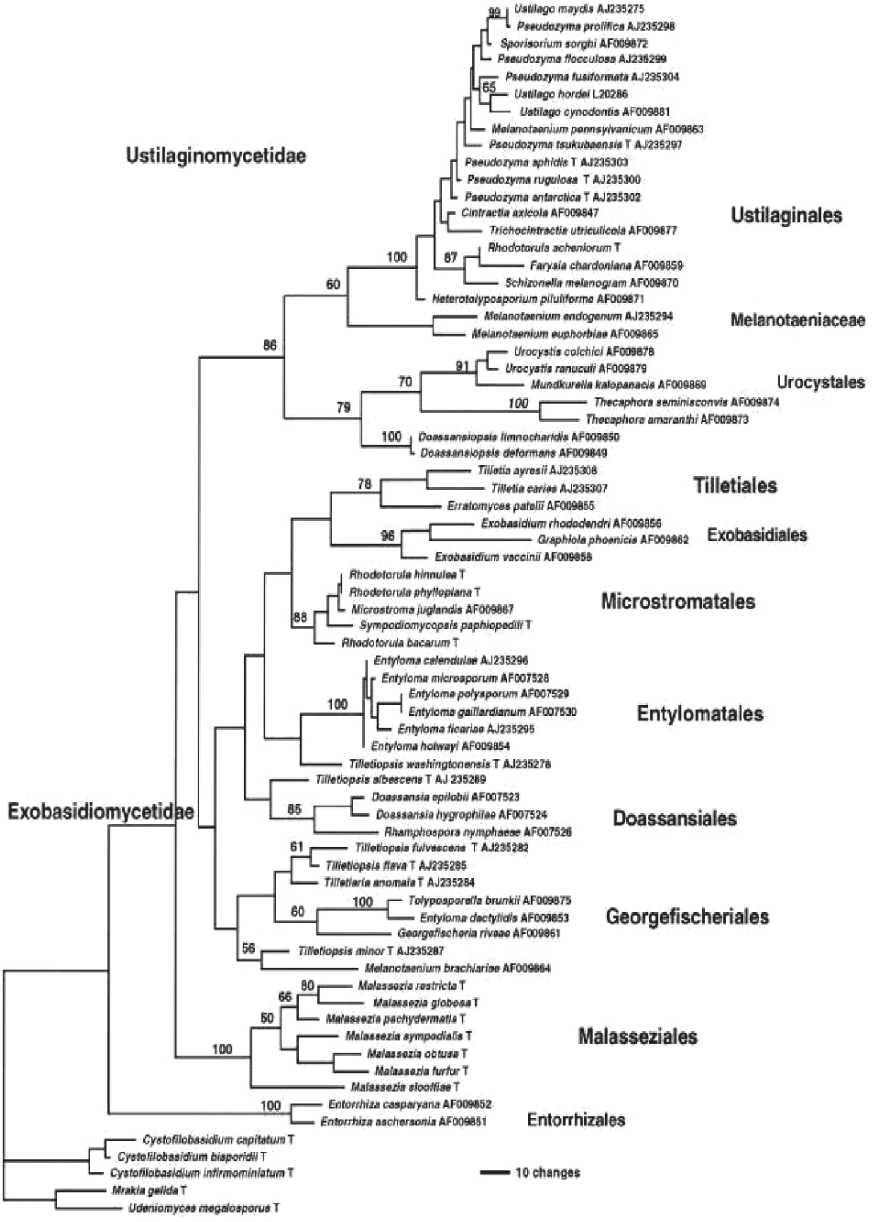

Fig. 2.7 Ustilaginomycetous fungi and associated yeasts: phylogenetic analysis of the D1/D2 region of the large-subunit rDNA (one of 100 equally parsimonious trees. From Fell et al. 2000. *IJSEM* **50**: 1351–1371. Copyright IUMS. Reproduced with permission

Analysis of the segregation patterns of different heterozygous markers among the four spores constitutes tetrad analysis and reveals the linkage between two genes (or between a gene and its centromere) (Mortimer and Schild, 1981).

On the whole, genetic distance in yeast appears to be remarkably proportional to physical distance, with a global average of 3 kb/cM. A large variety of protocols for genetic manipulation in yeast are available (Guthrie and Fink, 1991; Johnston, 1994). Yeast has a generation time of ca 80 min and mass production of cells is easy. Simple procedures for the isolation of high molecular weight DNA, rDNA, mRNA, and tRNA are at hand. It is possible to isolate intact nuclei or cell organelles such as intact mitochondria (maintaining respiratory competence). High efficiency transformation of yeast cells is achieved, for example, by the lithium acetate procedure (Ito et al., 1983) or by electroporation. A large variety of vectors have been designed to introduce and to maintain or express recombinant DNA in yeast cells (e.g. Guthrie and Fink, 1991; Johnston, 1994). Furthermore, a large number of yeast strains carry auxotrophic markers, drug resistance markers or defined mutations. Culture collections are maintained, for example, at the Yeast Genetic Stock Center (YGSC) and the American Type Culture Collection (ATCC). Mutant strains with defined gene deletions together with clones carrying the corresponding gene cassettes have emerged from the EUROFAN and TRANSATLANTIC projects. Ordered cosmid libraries using different vectors were constructed during the yeast-sequencing project (e.g. Thierry et al., 1995; Riles et al., 1993; Stucka and Feldmann, 1994).

2.8 Basidiomycetous Yeasts as Model System

2.8.1 Cryptococcus neoformans

Studies of non-vertebrate hosts during the recent past decade have yielded deeper insights into the molecular mechanisms of microbial pathogenesis and host defense. Apparently similar bacterial virulence factors are involved in pathogenesis in hosts that are evolutionarily far apart, *viz*., plants, nematodes, and mammals (Aballay and Ausubel, 2002). *Caenorhabditis elegans,* a nematode has proved particularly important host for study of bacterial pathogenesis.

Steenbergen et al. (2001) reported the use of the free-living amoeba *Acanthamoeba castellanii* as a model for study of survival strategies used by the human opportunistic fungal pathogen *Cryptococcus neoformans* after ingestion by macrophages. These workers observed that *C. neoformans* was phagocytosed by *A. castellanii*, and that once inside, the yeast replicated, eventually killing the amoeba. The process appeared remarkably similar to that in mammalian macrophages (Levitz, 2001); an acapsular strain of *C. neoformans* did not survive when incubated with *A. castellanii* and a phospholipase mutant exhibited decreased replication rate in amoeba, processes similar to those in macrophages.

These observations suggest that cryptococcal characteristics that contribute to mammalian virulence also promote fungal survival in free-living amoebae. In view of the successful use of *C. elegans* and *A. castellanii* to study bacterial and fungal pathogenesis, especially among immunocompromised patients (Marty and Mylonakis, 2002), such observations are of special relevance.

Study of the pathogenic mechanisms of *C. neoformans* has been enhanced substantially by the development of transformation protocols, homologous recombination for genetic manipulations, and reproducible animal models (Hua et al., 2000). The most important *C. neoformans* virulence factors identified so far include the polysaccharide capsule (Perfect et al., 1998), laccase (an enzyme essential for melanin production) (Jacobson, 2000), and at least two signal transduction cascades (Waugh et al., 2002). These observations suggest that the developmental aspects of *C. neoformans* pathogenesis in humans can be modeled using simple invertebrate nematode *C. elegans* as an experimental host.

2.8.2 Fellomyces fuzhouensis

A new, potentially pathogenic yeast which reproduces by conidiogenesis, can also act as model system

In conformity with other 'long neck yeasts', *Fellomyces* is characterized by formation of conidia, a unique vegetative structure in so far as yeasts are concerned. While, majority of yeast species reproduce vegetatively by budding, fission or by 'bud-fission', conidiogenesis, was discovered in the genus *Sterigmatomyces;* this involves the development of a sterigma, a tube-like, slender projection, that bears a single, spherical or ovoidal conidium (Fell, 1966). In the genus *Sterigmatomyces*, conidia separate from sterigmata by cleavage at the midpoint, whereas in the genus *Fellomyces* they separate at the distal ends of sterigmata (Hamamoto et al., 1998).

Compared to the budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*, *Fellomyces fuzhouensis* is rich in membranous organelles, especially long cisternae of the endoplasmic reticulum and large mitochondria. The density of cristae of mitochondria is related to the physiological activity (growth) of each cellular compartment, i.e. conidiogenous cell, sterigma and conidium. The multilamellar cell wall in conidiogenous, ageing cells is reminiscent of the cell wall of the Basidiomycetes (Kreger-van Rij and Veenhuis, 1971) and shows that this organism was correctly classified as a basidiomycetous yeast (Fell et al., 2000).

Based on the use of ultrastructural methodologies, the genus *Fellomyces* has been separated from *Sterigmatomyces* on the basis of breaking-off of the empty sterigma from the conidiogenous cell; this is in addition to differences in biochemical characterization and chemical composition of the wall. The long, empty sterigma visualised in *F. fuzhouensis* is due to the breakage that occurs in the narrowest part of the sterigma at the conidium base; this area also shows presence of an actin ring.

Conidia could easily be detached when cultured in liquid media on a shaker but, on solid media, subsequent cell generations remain connected in a network.

2.8.3 Isolation and Characterization of Carotenoid Hyperproducing Mutants of Yeast

The carotenoid pigment astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is an important component in feeds of aquacultural animals. It is produced as a secondary metabolite by the yeast *Phaffia rhodozyma*, and the isolation of rare mutants that produce increased quantities is limited by the lack of genetic selections. As a model system for enriching mutants increased in production of secondary metabolites, quantitative flow cytometry/cell sorting (FCCS) have been used to isolate astaxanthin hyperproducing mutants of this yeast (An et al., 1991).

2.9 Importance of Basidiomycetous Yeasts

Besides being a diverse group of fungi, basidiomycetous yeasts have considerable industrial and medical importance. Among these, *Cryptococcus* (Tremellales) is medically the most important genus. *Cryptococcus neoformans* is ubiquitous in the environment and serves as a model organism for fungal pathogenesis, and an opportunistic human pathogen of global importance.

This yeast encodes unique genes that contribute towards its unusual virulence properties; comparison of two phenotypically distinct strains reveals variation in gene content, in addition to sequence polymorphism between the genome. The genus *Cryptococcus* has 34 species of diverse relationships.

Virulence in *C. neoformans* is mediated predominantly by a polysaccharide capsule that surrounds the cell wall and has multiple effects on the host immune system. Capsule provides a physical barrier that interferes with the normal phagocytosis and clearance by the immune system. Capsule components inhibit the production of proinflammatory cytokines, deplete complement components, and reduce leukocyte migration to sites of inflammation (Buchanan and Murphy, 1998). The capsule also constitutes the major diagnostic feature of cryptococcosis, because its components can be detected in the bloodstream and can be visualized with light microscopy by using India ink staining. The capsule excludes the ink particles and forms characteristic halos, whose diameters are often several times that of the cell. The elaborate structure of the capsule can be appreciated by electron microscopy (Fig. 2.8).

Genus *Trichosporon* is characterized by the production of arthroconidia and ballistoconidia and its species are able to assimilate many C compounds and degrade urea (Gueho et al., 1994a,b). The clinical manifestation caused by

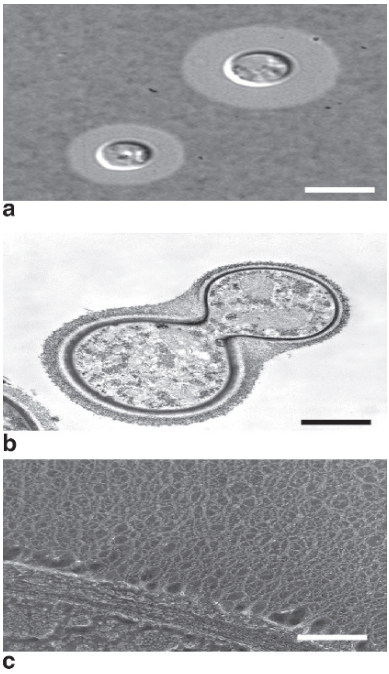

Fig. 2.8 Views of the cryptococcal capsule. (**a**) Differential interference contrast micrograph of cells that were mixed with India ink after induction of capsule formation by growth in low-iron medium. Scale bar, 3 μm. (**b**) Thin-section micrograph of a budding cell fixed in the presence of ruthenium red dye. Scale bar, 1 μm. (**c**) Quick-freeze, deep-etch image of the edge of a cell, with an arc of cell wall separating the cell interior (lower left) from the abundant capsule fibers emanating upwards. Scale bar, 0.15 μm. Pierini and Doering 2001. *Mol. Microbiol.* **41:** 105–115. Copyright Blackwell Publishing. Reproduced with permission

Trichosporon spp. vary widely from mild superficial infections such as 'white piedra' to localized or disseminated infections in patients with hematological malignancies (Herbrechet et al., 1993; Tashiro et al., 1995). Six species, *T. asahii, T. inkin, T. mucoides, T. cutaneum., T. ovoides* and *T. asteroids* are of clinical significance. *Rhodotorula, Sporobolomyces* and *Tilletiopsis* are anamorphic genera with pathogenic species assigned to the order Ustilaginales. *Rhodotorula* is characterized by pink colonies and budding cells with narrow bud scars. The teleomorphs of the type species, *R. mucilaginosa,* produce dominantly ballistoconidia but also produces teliospores in culture after mating with compatible strains. Kurtzman and Fell (1998) recovered 34 species of *Rhodotorula*, of which only three, *R. glutinis, R.mucilaginosa,* and *R. minuta* were found to be pathogenic (Gyaurgieva et al., 1996; Nucci et al., 1995). These three species can be distinguished from each other by the assimilation or lack of nitrate and raffinose. *Sporobolomyces salmonicolor*, the anamorph of *S. johnsonii*, also develops salmon pink colonies but they are

differentiated from *Rhodotorula* spp. by the production of ballistoconidia on large sterigmata. This agent has been reported as a cause of infection in AIDS patient (Plazas et al., 1994).

Phaffia rhodozyma has the ability to secrete β-carotene and astaxanthin; 'white-haze' symptoms occur on apple as a result of colonization by ballistoconidium forming fungi (Hui et al., 2005). It occurs in mild form in the field and subsequently it becomes problematic after ultra-low oxygen storage and, is considered as a post harvest disorder. *Rhodosporidium toruloides* (anamorph, *R. glutinis*) is a common phylloplane epiphyte which has biocontrol potential (Buck and Andrews, 1999).

Buzzuni and Martini (2002) recovered 155 strains of basidiomycetous yeasts from extreme environments to assess their extracellular enzymatic activity profiles. Approximately one-third basidiomycetes exhibited caseinolytic activity; this character was expressed almost exclusively by isolates of *Cryptococcus* sp. and *Pseudozyma* sp. In particular, 83% of *Pseudozyma antarctica* strains were caseinolytic and only half of caseinolytic basidiomycetes were able to hydrolyze gelatine whereas a few strains were chitinolytic.

Thirty-seven basidiomycetous yeasts belonging to 30 different species within seven genera were grown on media containing L-cysteine or L-methionine as sole nitrogen sources with the objective of evaluating production of volatile organic sulfur compounds (VOSC). The headspace of yeast cultures was analyzed by the solid-phase microextraction (SPME) sampling method, and volatile compounds were quantified and identified by GC-MS techniques. Ten strains assimilating L-methionine produced the following VOSCs: 3-(methylthio)-1-propanol, methanethiol, S-methyl thioacetate, dimethyl disulfide, dimethyl trisulfide, allyl methyl sulphide and 4,5-dihydro-3(2H)-thiophenone. The production level was usually small (<1 mg l^{-1}) except for 3-(methylthio)-1-propanol (40 and 400 mg l^{-1}). Higher alcohols (isobutyl alcohol, isoamyl alcohol and active amyl alcohol) and esters (ethyl acetate, ethyl propionate, n-propyl acetate, isobutyl acetate, n-propyl propionate, n-butyl acetate, isoamyl acetate, amyl acetate, isoamyl propionate, amyl propionate and 2-phenylmethyl acetate) were also sporadically produced. This is the first report of production of VOSCs by basidiomycetous yeasts. Consequently, basidiomycetous yeasts may be considered as an interesting new group of microbial production of VOSCs for the flavour industry (Buzzuni et al., 2005).

A cold-active polygalacturonase (PG) has been characterized from the extracellular fraction of *Cystofilobasidium capitatum* strain PPY-1. The purified PG from strain PPY-1 has a molecular mass of about 44 kDa, and exhibits high activity at 0°C, with optimum of 45°C. Although the Km value for polygalacturonate as a substrate at 45°C is 11.2 mg ml^{-}, it decreases gradually with decreasing temperature; it was 0.66 mg ml^{-} at 0°C. Moreover, the cleavage pattern of this enzyme is endo-type. These findings suggest that this PG from strain PPY-1 is a novel type of cold-active endo-PG that is able to degrade pectin compounds at low temperatures (Nakagawa et al., 2005).

2.10 Future Perspectives

Since the release of the yeast genome DNA sequence, there has been an expected change in the technology of yeast research as well as a rather surprising change in its goals. Most of the new understanding of individual yeast gene functions has come from comparative genomics and relatively little from the high-throughput genomic technologies. The latter have, however, fueled the changes in goals, from a focus on individual genes and their interactions to a focus on the system-level transactions that make the robustly functioning organisms as pioneer find in the nature.

The future of genome-scale technologies is, indeed, very promising. It is not clear whether the slow rate at which new annotations are verified is caused by problems in data analysis and representation, or by a more simple lack of focus on the need for such verification. Some methods, now in early stages of development, will no doubt help: among these are methods based on natural variation (examples include Brem et al., 2002; Steinmetz et al., 2002; Fay et al., 2004), methods that are not limited to nonessential genes (e.g. synthetic lethality with conditional alleles) (Tong et al., 2004) or titratable promoter alleles (Mnaimneh et al., 2004), methods that study the locations and movements of intracellular molecules (Ghaemmaghami et al., 2003; Huh et al., 2003), and methods that use more biological information from other species (for example, Harbison et al., 2004). Fortunately, in the post–genome-sequence era, it is much easier to acquire this kind of information on a comprehensive scale, and this appears to be the path forward. Another challenge of this nature is to understand the basis on which selection acts on the ensemble of genes, proteins, networks, and systems to produce organisms capable of surviving in new environments.

Finally, there remains the eternal issue of verification. One expects that the need for tests of hypotheses generated by genome-scale experiments and quantitative models will persist for a very long time. As has always been the case, every model (and the data used to generate it) must be tested, and to be tested, it must be specified in full and available to the public. The yeast community has an excellent record in this regard, one that we believe is a major reason that yeast continues to be the very model of a model organism.

Keeping in mind that fungi are significant and increasingly cause morbidity and mortality in immunocompromised patients and that the opportunistic infections they cause are severe and life threatening, rapid diagnosis and efficient therapeutic measures are essential. Of course, collaboration between the clinicians and the laboratory is absolutely necessary. Additionally, the precise diagnosis is based on the morphologic identification of the tissue forms in biopsy material or pus by microscopy and culture. The final diagnosis of the agent is achieved only after culture of material is obtained from the lesions. Detection of specific antibodies and specific antigens and/or metabolites in body fluids or tissues may be of great value in the future, especially molecular diagnostic techniques.

In conclusion, the interaction of *C. elegans* with the yeast *C. neoformans* involves a number of genes that are also important during the host pathogen interaction during mammalian infection. Identification of new *C. neoformans* virulence factors using this model may lead to new targets for antifungal therapies as well as a deeper understanding of the host–fungus interaction.

A new taxon in the genus *Sporidiobolus* has been described as *Sporidiobolus metaroseus* sp. nov. (type strain CBS 7683^T) (Valerio et al., 2008). Margesin et al. (2007) reported three novel psychrophilic species of the genus *Rhodotorula viz., R. psychrophila* sp. nov. (type strain PB19^T=CBS 10440^T=DSM 18768^T), *R. psychrophenolica* sp. nov. (type strain AG21^T=CBS 10438^T=DSM 18767^T) and *R. glacialis* sp. nov. (type strain A19^T=CBS 10436^T=DSM 18766^T). In addition, a novel species *viz., Rhodotorula subericola* has been isolated from bark of *Quercus suber* (cork oak) (Belloch et al., 2007). Besides, a novel species *Rhodotorula pacifica*, was isolated from sediments collected on the deep-sea floor in the northwest Pacific Ocean (Nagahama et al., 2006). Shin et al. (2006) described two novel basidiomycetous yeasts species *viz., Cryptococcus mujuensis* sp. nov. (type strain KCTC 17231^T=CBS 10308^T) and *Cryptococcus cuniculi* sp. nov. (type strain KCTC 17232^T=CBS 10309^T). In addition, Wang et al. (2006) reported two novel ustilaginomycetous anamorphic yeast species *viz., Pseudozyma hubeiensis* and *Pseudozyma shanxiensis*, recovered from wilting leaves of various plants in China. Pohl et al. (2006) reported a novel anamorphic basidiomycetous yeast, *Cryptococcus anemochoreius* sp. nov. (type strain CBS 10258^T).

In addition, a novel anamorphic genus, *Farysizyma*, is created in the *Ustilaginales* to accomodate three novel epiphytic basidiomycetous yeast species *viz., F. itapuensis, F. setubalensis* and *F. taiwaniana,* based on nucleotide sequences of the D1/D2 domains of the 26S rRNA gene and the ITS region (Inacio et al., 2008). Statzell-Tallman et al. (2008) reported a novel teleomorphic yeast species, *Kwoniella mangroviensis,* from mangrove habitats in the Florida Everglades and Bahamas. Two new *Cryptococcus* species *viz., C. bestiolae* and *C. dejecticola*, were isolated from litchi fruit borer *Conopomorpha sinensis* Bradley (Thanh et al., 2006). *Pseudozyma jejuensis* sp. nov., a novel cutinolytic ustilaginomycetous yeast species (Seo et al., 2007), isolated from orange leaves on Jeju island in South Korea, and two new taxa, *Malassezia caprae* sp. nov. (type strain MA 383=CBS 10434), isolated mainly from goat, and *M. equina* sp. nov. (type strain MA146=CBS 9969), isolated from horses (Cabanes et al. (2007), using analysis of the of the D1/D2 regions of the 26S rRNA gene and the ITS1+2 regions. Cafarchia et al. (2008) described genetic variants of *Malassezia pachydermatis*, isolated from canine skin. In addition, Paulina et al. (2008) compared the *Malassezia* microbiota from six healthy body locations and two psoriatic lesions, and evaluated its stability over time using multiplex real-time PCR. *Malassezia restricta* was the most abundant species in the majority of samples, and high amounts of *Malassezia globosa* were also reported.

Eigenheer et al. (2007) conducted a proteomic analysis of secreted and cell wall-bound proteins with an acapsular strain of *C. neoformans*, to study the extracellular proteome of the human fungal pathogen *C. neoformans*. Different isolates of *C. neoformans* express ectophosphatase activity, which is not influenced by capsule

size or serotype, suggesting that ectoenzyme expression can contribute to the pathogenesis of *C. neoformans* (Collopy-Junior et al., 2006). Extracellular phospholipase B (PLB) is a virulence determinant of *C. neoformans* and *C. gattii*, that causes cryptococcosis (Wu et al., 2007). van Staden et al. (2007) investigaated phytase activity in ten *Cryptococcus* strains, of which the *Cryptococcus laurentii* ABO 510 strain showed the highest level of activity. Dunlap et al. (2007) determined the effect of cold adaptation on the physicochemical properties of *C. flavescens* that may be responsible for its improved desiccation tolerance. A combination of *C. laurentii* with indole-3-acetic acid (IAA) at 0.1 mg ml^{-1} was more effective in suppressing blue and gray mold diseases (*Penicillium expansum* and *Botrytis cinerea*) on pea fruit than application of *C. laurentii* alone (Yu and Zheng, 2007). In addition, *Pseudozyma antarctica* is one of the best producer of the glycolipid biosurfactants known as mannosylerythritol lipids (MELs), which show not only excellent surface-active properties but also versatile biochemical actions (Morita et al., 2007).

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