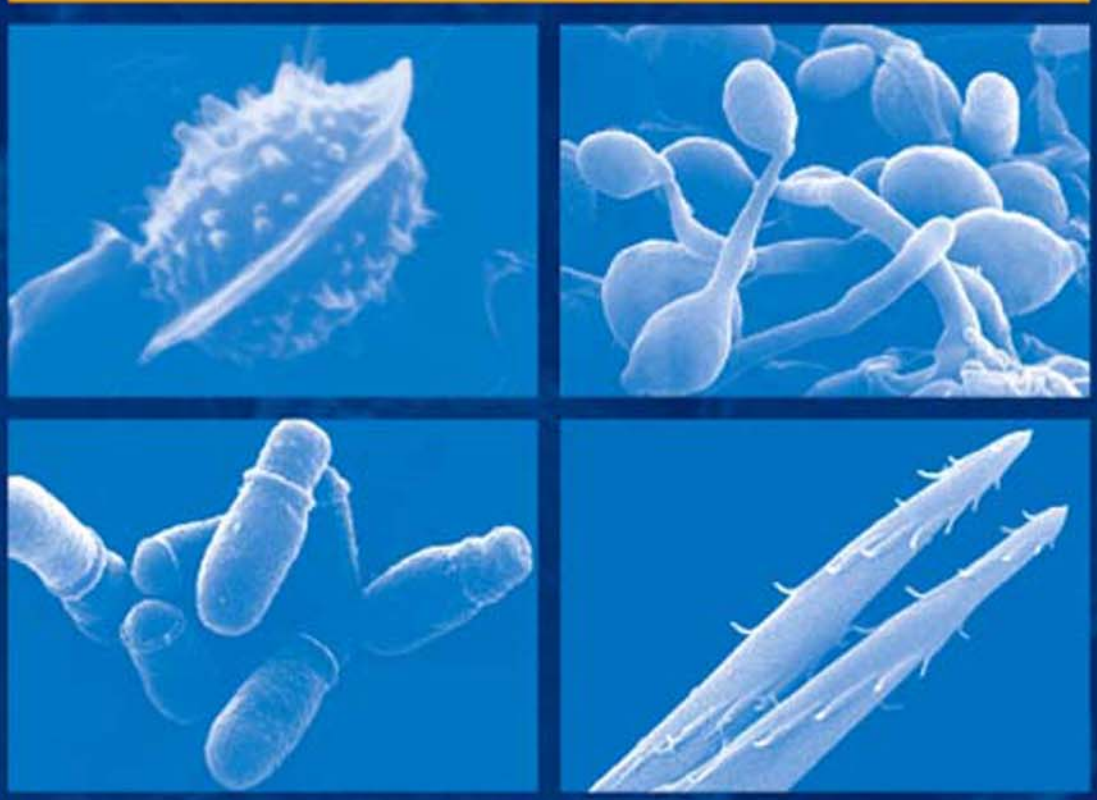
THE YEASTS

A TAXONOMIC STUDY

FIFTH EDITION



VOLUME 1

EDITED BY
C.P. KURTZMAN • J.W. FELL • T. BOEKHOUT

The Yeasts, a Taxonomic Study

Volume 1

This book is dedicated to the memory of

Robert J. Bandoni
Helen R. Buckley
Nellie J. W. Kreger-van Rij
Martin W. Miller
Herman J. Phaff
Wilhelmina Ch. Slooff
Isabel Spencer-Martins

The Yeasts, a Taxonomic Study

Volume 1

Fifth Edition

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Part Vb
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Preface

The importance of yeasts is underscored by our often daily consumption of bread and fermented beverages. Recent advances in biotechnology have increased our reliance on yeasts for pharmaceuticals and for bulk biochemicals such as citric acid. Furthermore, clinically important yeasts are commonplace, especially as numbers of immunosuppressed patients increase, and biologists are continuing to discover the importance of yeasts in the ecosystem and their application in the biocontrol of plant pests. All of these areas of science and technology have a common need: the rapid and accurate identification of yeasts. The goal of this book is to provide that information.

This book, the fifth edition of *The Yeasts*, *a Taxonomic Study*, represents a continuation of the monographic series begun by J. Lodder and N.J.W. Kreger-van Rij (1st edn, 1952), J. Lodder (2nd edn, 1970), N.J.W. Kreger-van Rij (3rd edn, 1984) and C.P. Kurtzman and J.W. Fell (4th edn, 1998). In the fourth edition (1998), 100 genera and over 700 species were described. In the present edition, there are 149 genera and nearly 1500 species. The application of gene sequence analysis is largely responsible for the increase in the number of taxa presented in this edition. In 1998 and in 2000, diagnostic gene sequences were published for essentially all known yeasts. This advance allowed rapid, accurate species identification for the first time, and the method has been widely adopted by the yeast community to catalogue new species. Sequence analysis has also demonstrated that genera were often polyphyletic, and from such analyses many genera are now phylogenetically circumscribed. There is still much to do to understand phylogenetic relationships among species and genera, but a good start has been made.

In this edition, a large array of fermentation and growth tests is reported for each species. These tests can be used for species identification, but as this is now commonly done from gene sequences, their major value is to provide information for the selection of biotechnologically important species, to understand how metabolism affects species ecology and for the selective isolation of taxa. Many of the species are illustrated by photographs or line drawings because it is important to know the species morphology and the method of growth.

This edition includes chapters on the importance of yeasts and the current methods used for their identification and classification. We hope readers will find these chapters useful, and that they will provide a starting point for more extensive studies with a fascinating group of fungi that we know as the yeasts.

Cletus P. Kurtzman
Jack W. Fell
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Use of this Book

1. INTRODUCTORY CHAPTERS

With the widespread use of gene sequences for yeast identification, the number of known species has doubled since publication of the fourth edition of this book in 1998. As a result, the book has grown from one to three volumes. As in previous editions, the book begins with introductory chapters that discuss the definition of yeasts, their importance and the means for their characterization and classification. Thus in Volume 1, Part I discusses the current definition of yeasts, their classification and the rules for their nomenclature. Part II focuses on yeasts that are human and plant pathogens, those that cause food and beverage spoilage, species used for biocontrol of plant pests, their applications in biotechnology, and an overview of yeast ecology. Part III provides chapters on phenotypic characterization, chemotaxonomy, ultrastructure and molecular biological characters that are used to identify yeasts and to develop a phylogenetic framework for their classification. Volume 2 is devoted to the ascomycetous yeasts, and Volume 3 includes the basidiomycetous yeasts, along with the genus *Prototheca*.

The format of this edition differs from previous editions in the placement of the all-species key, summary table of species characteristics, glossary, and indexes to taxa and literature references in Volume 1 with the introductory chapters, rather than at the back of the book as was done previously. Because we now have three volumes, we reasoned that it would be more convenient, while looking for taxa and their references or keying species, to have these sections in a separate volume from the species descriptions, thus avoiding the need to flip between the front to back of a single volume while looking for the information. We hope that readers will find this format to be a convenient choice for a multi-volume taxonomic work.

2. DESCRIPTIONS OF GENERA AND SPECIES

Genera are arranged alphabetically within four groups: teleomorphic ascomycetes, anamorphic ascomycetes, teleomorphic basidiomycetes and anamorphic basidiomycetes. The introductory chapters for both ascomycetes and basidiomycetes include discussions of genera and their phylogenetic placement. These discussions also note relationships between teleomorphic and anamorphic genera. Each chapter begins with a narrative description of the genus, a phylogenetic tree that depicts species relationships within the genus, and a key to species based on growth characteristics, which is followed by a table that includes the key characters. Each species description begins with a designation of the anamorph or teleomorph, where known, followed by a listing of synonyms. The characterization proceeds to morphological and physiological descriptions. Representative species for each genus are illustrated by either drawings or photographs, which include a scale bar and growth conditions. Noteworthy information for the species is given in sections entitled Systematics, Ecology, Biotechnology, Agriculture and food, and Clinical importance.

Abbreviations used throughout the text are standard. For mol% G+C of nuclear DNA, the method for determination is included and abbreviated as follows: T_m, thermal melt; BD, buoyant density; HPLC, high-pressure liquid chromatography.

The following symbols are used for the fermentation and assimilation reactions given with species descriptions:

+	positive
l	latent (rapid development of a positive reaction after a lag period)
+/l	positive or latent
s	positive but slow
w	weak
ws	weak and slow
+/w	positive or weak
w/−	weak or negative
lw	latent but weak (rapid development of a weak reaction after a lag period)
−/l	negative or latent
v	variable
−	negative
n	no data

3. YEAST-LIKE TAXA

Some microbial taxa that could be mistaken as yeasts are briefly discussed. For example, species of the dimorphic euascomycete genus *Aureobasidium* are commonly isolated, and often appear white to light pink in color and yeast-like on isolation plates. Consequently, *Aureobasidium pullulans* is included in the all-species key. Similarly, the achlorophyllic algal genus *Prototheca* is often misidentified as a yeast, and for this reason a chapter on the genus is included.

4. SPECIES SUMMARY TABLE AND KEY TO ALL TAXA

A summary table of fermentation and assimilation reactions and certain key biochemical characteristics is placed near the end of Volume 1. Taxa are listed alphabetically, first by genus and then by species. A key using the physiological data in the table includes all species of ascomycetes, basidiomycetes and the genus *Prototheca* for which data are available. The following abbreviated symbols are used in the table and for the key:

+	+, s, l, +/l, +/w , w, ws, lw
−	−
v	v, w/−, −/l
n	no data or not applicable

5. GLOSSARY

A glossary has been provided that includes morphological, genetic and molecular biological terms.

6. INDEXES TO TAXA

There are two indexes to taxa. The first lists genera followed by assigned species and their synonyms. Validly accepted combinations are in bold type. The second index alphabetically lists all species and variety names followed by all genera to which the species and varieties were assigned. Validly accepted genera are in bold type.

7. REFERENCES

The references for all chapters have been consolidated into a single list. This saved sufficient space to allow inclusion of titles which would have been omitted if each chapter had a reference list.

Part I

Classification of Yeasts

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Chapter 1

Definition, Classification and Nomenclature of the Yeasts

Cletus P. Kurtzman, Jack W. Fell and Teun Boekhout

1. DEFINITION AND CLASSIFICATION OF THE YEASTS

The English word yeast and its equivalents in many other languages are based on words meaning "foam" and "to rise", direct references to the fermentation processes that produce beer and bread. For this reason, yeasts are often thought of as fermentative ascomycetous fungi similar to *Saccharomyces cerevisiae*. In fact, it is not uncommon in some areas of molecular biology to treat the words "yeast" and "*Saccharomyces*" as synonyms. The discovery that some taxa are basidiomycetes has broadened our perception of the nature of yeasts. As a consequence, we have come to view yeasts as fungi that asexually reproduce by budding or fission, which results in growth that is comprised mainly of single cells.

An imprecise distinction has been made between yeasts and those dimorphic filamentous fungi that often produce abundant yeast-like growth. Yeasts can be defined as those fungi whose asexual growth predominantly results from budding or fission, and which do not form their sexual states within or upon a fruiting body. For ascomycetous yeasts, this distinction has been substantiated by molecular comparisons, which demonstrate that budding and fission yeasts are phylogenetically distinct from one another and from the euascomycetes (Pezizomycotina) (see Chapter 13). One exception is the genus *Eremascus*, which has unenclosed asci, but budding cells are not formed. A similar distinction can be made for basidiomycetous yeasts, which are often phylogenetically separate from the mushrooms and other taxa that form complex fruiting bodies (see Chapter 100). In summary, yeasts, whether ascomycetes or basidiomycetes, are generally characterized by budding or fission as the primary means of asexual reproduction, and have sexual states that are not enclosed in fruiting bodies.

2. TAXONOMY

Rules for taxonomy of the yeasts and other fungi fall under the authority of the International Code of Botanical Nomenclature. The most recent version of the Code (McNeill et al. 2006) was adopted at the Seventeenth International Botanical Congress, Vienna, Austria, 2005 (http://ibot.sav.sk/icbn/main.htm). The following is a brief discussion of the Botanical Code as it applies to yeasts.

2.1. Description of New Taxa

2.1.1. Species

Publication of new species must include a description of essential characters as well as a diagnosis that distinguishes the taxon from previously described species. Since January 1, 1935, the description and/or diagnosis must be given in Latin. Failure to comply with this requirement results in an invalidly described species termed a *nomen invalidum* (*nom. inval.*). A *nomen invalidum* also results if publication is not in a recognized scientific journal, e.g., as in a patent or a trade magazine. If the new species is designated without a description or a diagnosis, it is invalid and termed a *nomen nudum* (*nom. nud.*). Names of taxa must be given in Latin or modified in such a way that they follow the rules of Latin derivation including appropriate gender designations. If a name has been incorrectly crafted, it may be treated as an "orthographic error" and corrected. An example is *Pichia membranifaciens* for which the 1888 spelling "*membranaefaciens*" has been corrected. The authority name does not change due to the spelling correction. Other requirements for valid publication include deposition of type material in a publicly accessible herbarium. This material must be an original specimen of the organism, and it is to be dead and dried. The 1994 Code (Greuter et al. 1994) changed the requirements to allow lyophilized specimens to be valid type material (holotype) and that living cultures derived from the lyophilized material are considered *ex typo*, i.e., from the type. It seems that once the original material is exhausted, there is no longer type material available. A possible solution to this problem would be to lyophilize new material and designate it as a neotype, a convention permitted when the original type material is lost or destroyed and the species can be otherwise recognized. This discussion leads to the recognition that a majority of presently accepted yeast species are technically invalid because legitimate type material has not been preserved. A portion of presently lyophilized stocks of the holotype that are maintained in culture collections should be withheld from distribution and designated as type. Consequently, the designation of "Type strain" given for each cultivatable species described in this book can, at best, represent an ex-type. The 1994 Code recognized the need for living cultures in the practice of modern taxonomy and stated in Recommendation 8B.1:

> *"Whenever practicable a living culture should be prepared from the holotype material of the name of a newly described taxon of fungi or algae and*

deposited in at least two institutional culture or genetic resource collections. (Such action does not obviate the requirement for a holotype specimen under Art 8.2)."

The 1994 Code further states in Recommendation 8B.2:

"In cases where the nomenclatural type is a culture permanently preserved in a metabolically inactive state (see Art. 8 Ex. 1), any living isolates obtained from that should be referred to as 'ex-type' (ex typo)*, 'ex-holotype'* (ex holotypo)*, 'ex-isotype'* (ex isotypo)*, etc., in order to make it clear they are derived from the type but are not themselves the nomenclatural type."*

From these recommendations, it is clear that the Code strongly encourages scientific cooperation and communication through active sharing of published taxonomic specimens. A listing of commonly used yeast culture collections is given in Chapter 7.

From gene sequence comparisons, strains that represent new species are usually easily recognized (Chapter 10). Nonetheless, some would argue against description of a new species based on a single strain. The argument is that a single strain does not reflect the genetic variation that might be found in a species, and that little can be learned of the ecology of a species when only a single strain is available. However, nearly one-third of described yeast species are based on a single strain. If these species had not been described, much less would be known about the phylogenetic diversity of the yeasts. From the perspective of understanding diversity among the yeasts, description of single-strain species is to be supported, although descriptions based on multiple strains are preferred. Further, it is recommended that the description should be based on multigene analysis to lessen the possibility that the strain represents a hybrid of known species.

2.1.2. Genera, Families, Orders

The rules for describing new genera, families and orders are similar to those for describing new species. The taxa must be based on a validly described species and provided with a Latin description and diagnosis. The rules of priority are briefly described below, but one exception is that orders are exempt from priority usage.

2.2. Basionyms, Synonyms, Priority of Usage

Because of the inexact art of species characterization, as well as the occasional situation in which two independent investigators describe the same new species, the Botanical Code has provided a set of rules to reconcile resulting problems. The following example should prove helpful to the reader, but the Code needs to be consulted for full details.

1. *Saccharomycopsis fibuligera* (Lindner) Klöcker (1924)

   Synonyms:

2. *Endomyces fibuligera* Lindner (1907)
3. *Endomycopsis fibuligera* (Lindner) Dekker (Stelling-Dekker 1931)
4. *Pichia fibuligera* (Lindner) Boidin, Pignal, Lehodey, Vey & Abadie (1964)
5. *Endomyces lindneri* Saito (1913)
6. *Saccharomycopsis lindneri* (Saito) Klöcker (1924)
7. *Endomycopsis fibuligera* (Lindner) Dekker var. *lindneri* Dekker (Stelling-Dekker 1931)
8. *Endomyces hordei* Saito (1914)
9. *Saccharomycopsis hordei* (Saito) Klöcker (1924)
10. *Endomycopsis fibuligera* (Lindner) Dekker var. *hordei* (Saito) Dekker (Stelling-Dekker 1931)
11. *Candida lactosa* Dwidjoseputro & Wolf (1970)

In the example, *Saccharomycopsis fibuligera* (1) is the currently accepted name. The species was originally described by Lindner in 1907 as *Endomyces fibuligera* (2) and transferred to *Saccharomycopsis* by Klöcker in 1924. Other authors transferred the species to *Endomycopsis* (3) and to *Pichia* (4), but in all cases, the original author, Lindner, is listed in parentheses. The other species listed (5–11) are misidentified strains of *S. fibuligera*. The genus assignments for these "species" were also changed before it was recognized that they were conspecific with *S. fibuligera*. In the context of the Code, *Endomyces fibuligera* (2) is the basionym or basal name for the species. Synonyms 2, 3 and 4 are nomenclatural or obligate synonyms of *S. fibuligera* because they are all based on the same type strain. Synonyms 5–11 are termed taxonomic or facultative synonyms because they are based on different type strains. Species 6 and 7 represent obligate synonyms of *Endomyces lindneri*, hence the listing of synonyms is ordered by priority of publication date of obligate synonyms followed by publication date of facultative synonyms and their own obligate synonyms. The Code requires, with few exceptions, that the first described name has priority of usage. Thus, *Endomyces fibuligera* Lindner (1907) is the basionym, and personal preference for another name to serve as basionym, e.g., *Endomyces lindneri* Saito (1913), is generally not allowed. Arguments for exceptions might include uncertain names and the substitution of economically or medically important names for an older but obscure basionym.

The following publications provide examples of descriptions of new taxa and the conservation of older names, but the most recent edition of the Botanical Code should be consulted as well.

New species and genera

Fell, J.W., A.C. Statzell, I.L. Hunter and H.J. Phaff. 1969. *Leucosporidium* gen. n., the heterobasidiomycetous stage of several yeasts of the genus *Candida.* Antonie van Leeuwenhoek 35, 433–462.

New combinations

Kurtzman, C.P. 1995. Relationships among the genera *Ashbya, Eremothecium, Holleya* and *Nematospora* determined from rDNA sequence divergence. J. Ind. Microbiol. 14, 523–530.

Conservation of taxa

Fell, J.W., C.P. Kurtzman and K.J. Kwon-Chung. 1989. Proposal to conserve *Cryptococcus* (fungi). Taxon 38, 151–156.

2.3. Teleomorphs, Anamorphs and Holomorphs

Fungi are unique among living organisms because they may have two valid names. The primary name is based on the sexual state or teleomorph, but a second valid name may be based on the asexual state or anamorph. This redundancy of names developed because teleomorphs have not been found for many fungi, or it has not been clear that a particular teleomorph is the same species as a particular anamorph. If a teleomorph is unknown, a species can be described in an anamorphic genus, e.g., genera 86–99 and 135–162 in this book. Use of gene sequence comparisons has made the connection between teleomorphs and anamorphs for some species, but for many, the teleomorphic states are unknown. When both teleomorph and anamorph are known for a species and are part of the same type specimen, the combination is termed the holomorph. In the descriptions of teleomorphic yeasts presented in this book, the anamorph name is given if it exists.

Various possibilities have been offered to limit fungal species to just one name. Anamorphic species that are members of a teleomorph clade might be renamed to the teleomorph genus. For example, *Candida pseudolambica*, a member of the *Pichia* clade, might be renamed "*Pichia pseudolambica*" and the assignment to *Candida* would then be considered an invalid name. One disadvantage would be the incorrect implication that an ascosporic state has been found for *C. pseudolambica*.

Article 59.7 of the Vienna Code (2006) states

"Where a teleomorph has been discovered for a fungus previously known only as an anamorph and for which there is no existing legitimate name for the holomorph, an epitype exhibiting the teleomorph stage may be designated for the hitherto anamorphic name even when there is no hint of the teleomorph in the protologue of that name."

Guided by Article 59.7, use of an anamorphic genus name for a teleomorphic state would be straightforward if the particular clade had no teleomorphic species. For example, the anamorphic genus *Trichosporon* has no described teleomorphs, and if one were found, it could be named *Trichosporon*. However, it appears contrary to the Code to preferentially select an anamorph genus name and discard the teleomorphic genus name. An example of this would be to use *Cryptococcus neoformans* (anamorph) rather than *Filobasidiella neoformans* (teleomorph), because the name *Cryptococcus neoformans* is more often found in publications. In this book, both anamorphic and teleomorphic names have been maintained, but this may change in the future.

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Part II

Importance of Yeasts

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Chapter 2

Yeasts Pathogenic to Humans

Chester R. Cooper, Jr.

1. INTRODUCTION TO THE MEDICALLY IMPORTANT YEASTS

Prior to global emergence of the human immunodeficiency virus (HIV), which is the causative agent of the acquired immunodeficiency syndrome (AIDS), approximately 200 fungal pathogens were recognized from among the more than 100,000 then-known fungal species (Kwon-Chung and Bennett 1992, Rippon 1988). About 50 of these species were regularly associated with fungal disease (mycosis). Since then, there has been a concurrent dramatic increase in both the number of known fungal species and the incidence of mycoses that they cause. Moreover, the spectrum of pathogenic fungi has changed radically. Though HIV infection has been noted to be a significant factor in these changes, it is only one of many diseases that have encompassed the dynamic transformation of human affairs over the past several decades. The developments that have played noteworthy roles in the increased frequency of mycoses include the relative ease of international travel, an expanding and aging population, a breakdown in public health measures, rising immigration rates, heightened awareness of disease, industrialization, prolongation of life due to medical advances, and, sadly enough, politics (Cooper 2002, Lederberg et al. 1992, Smolinski et al. 2003). The collective result of these changes markedly influenced the diversification of etiological agents by permitting virtually any fungus capable of growing at or near body temperature (37°C) to eclipse a critical first hurdle in ascending to the level of pathogen.

Yeasts are among the most prominent of the disease-causing fungi, especially *Candida* spp., *Cryptococcus* (*Cr.*) *neoformans*, and *Cr. gattii*. There also exists a spectrum of unicellular fungi that have emerged as significant infectious agents (Table 2.1). Moreover, while the term "yeast infection" tends to elicit an image of an agent that exists mainly in a monomorphic state, other fungi also produce yeast cells or yeast-like forms *in vivo*. These include several thermally dimorphic fungi that exist as saprotrophic molds when grown at moderate temperatures (22°C–28°C), but convert to a yeast phase upon tissue invasion or *in vitro* culture at 37°C. In addition, a number of members grouped within the form-family Dematiaceae exhibit a yeast form both in culture and *in vivo*. Moreover, the enigmatic fungus *Lacazia loboi*, the agent of lobomycosis, presents chains of yeasts in tissue although this organism has yet to be isolated from nature. Hence, in medical mycology, the above examples support using the term "yeast" to strictly define a morphological feature without ascribing a taxonomic significance. However, many clinicians commonly use this term to refer mainly to the pathologies designated as candidiasis and cryptococcosis.

As an introduction to this text, the present review is meant to serve as a brief, descriptive compendium of mycoses caused by yeasts. Yet, it is not the author's intent to suggest that yeast biologists will regularly encounter the organisms described below. In fact, many medical mycologists spend entire careers without direct clinical exposure to many of these fungi. Rather, the purpose of this review is to enlighten the non-medical mycologist as to the diversity of yeast and mold species regularly associated with human and animal disease that also, at least in part, present a unicellular mode of growth *in vivo*.

The following descriptions present a concise overview of the key biological and clinical features of these fungi. Where appropriate, references to recent reviews of particular disease agents and their pathologies are provided. For a global perspective of fungal diseases, including in-depth clinical discussions of specific pathologies, diagnoses, and treatments, the reader is referred to several outstanding and recently published texts (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006a, Merz and Hay 2005). In addition, brief summaries of mycotic diseases and their etiological agents, including currently accepted binomials for these fungi, can be found on the Internet web page; Doctor Fungus (www.doctorfungus.com). Moreover, the narratives below provide brief taxonomic descriptions for each species and, if known, its teleomorph. In some instances, the particular classification of the sexual state was retrieved from the National Library of Medicine (www.ncbi.nlm.nih.gov/genomes/static/euk_o.html). Finally, of those fungi presented below, with the possible exception of *Histoplasma*, all can be handled using Biosafety Level 2 practices (www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s7b.htm).

2. ASCOMYCETOUS YEASTS OF CLINICAL SIGNIFICANCE

Overall, ascomycetous yeasts comprise the largest group of pathogenic fungi. Most of these pathogens are members of the anamorphic genus *Candida*. To a far lesser extent, fungi classified within the genera *Saccharomyces, Pichia* (including species formerly assigned to *Hansenula*), and *Magnusiomyces* (formerly *Dipodascus, Blastoschizomyces*) also cause human infections.

2.1. *Candida*

A number of *Candida* species exist in a commensal relationship with humans as normal residents of the gastrointestinal tract, mucocutaneous tissues, and skin. The most notable of these species is *Candida albicans*. However, being opportunistic pathogens, *Candida* spp. can exploit local or systemic weaknesses in host resistance, to cause disease in virtually any part of the body. Such infections, termed candidiasis, have become increasingly common during the past several decades for a number of reasons, which include the use of immunosuppressive therapies for other diseases as well as the unfortunate

TABLE 2.1 Selected Yeast and Yeast-Like Pathogens for Humans and Animals

Anamorph	Known Teleomorph
Ascomycetous Fungi	
Candida albicans	None
Candida dubliniensis	None
Candida glabrata	None
Candida nivariensis	None
Candida bracarensis	None
Candida guilliermondii	*Meyerozyma* (*Pichia*) *guilliermondii*
Candida krusei	*Pichia kudriavzeii*
Candida lusitaniae	*Clavispora lusitaniae*
Candida parapsilosis	None
Candida metapsiloisis	None
Candida orthopsilosis	None
Candida tropicalis	None
—	*Saccharomyces cerevisiae*
—	*Saccharomyces boulardii*
—	*Wickerhamomyces* (*Pichia*) *anomalus*
—	*Ogataea* (*Pichia*) *polymorpha*
Blastoschizomyces capitatus	*Dipodascus capitatus*
Basidiomycetous Fungi	
Cryptococcus neoformans var. *neoformans*	*Filobasidiella neoformans*
Cryptococcus neoformans var. *grubii*	*Filobasidiella neoformans*
Cryptococcus gattii	*Filobasidiella bacillispora*
Malassezia spp.	None
Trichosporon spp.	None
Rhodotorula spp.	None
Sporobolomyces spp.	None
Pseudozyma spp.	*Ustilago* spp.

prevalence of HIV infection. Other factors, such as increased awareness and improved diagnostic tools, have contributed to the sense that candidiasis has risen in importance as an affliction of humans and animals.

Evidence supporting the significance of *Candida* spp. as infectious agents might be readily noted by the number of publications cited in the medical literature that document case studies and research reports featuring these fungi. Using the words "*Candida albicans*", the present author searched for citations in the PubMed database supported by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). From 1970 through 1974, 1124 publications included the topic "*Candida albicans*". Over the next 30 years, this number rose by more than 334% with exponential increases from 1985 to 2004. Data for the past 2 years suggest that such publications will continue to proliferate exponentially over the next several years. Therefore, if the number of citations is indeed representative of the significance of *C. albicans* to public health, and by extrapolation to other *Candida* spp. as well, it is clear that these yeasts and the infections they cause have commanded much attention from clinical and experimental mycologists.

In addition to the human body, *Candida* spp. can be found in a wide variety of environmental habitats. Like those typically considered non-pathogenic, disease-causing species can be recovered from many of the same sources including air, water, foodstuffs, clothing, toothbrushes, etc. However, the environmental isolation of *Candida* spp. that are regularly associated with infections is often the result of contamination by humans or animals rather than a reflection of a true primary habitat for such fungi. This is a particularly disturbing fact that coincides with reports showing health care facilities to be a major location for contracting candidiasis (Jarvis 1995). Collectively, *Candida* spp. are the fourth most common cause of hospital-acquired (nosocomial) infections in North America.

A detailed description of the various clinical manifestations of candidiasis, as well as clinically relevant biological investigations of *Candida* spp., can fill entire books. Indeed, several books (Bodey 1993, Calderone 2002, Odds 1988, Segal and Baum 1994), chapters within medical mycology texts (Anaissie et al. 2003, Dismukes et al. 2003, Heitman 2006, Kauffmann 2006a, Kwon-Chung and Bennett 1992, Merz and Hay 2005), and numerous reviews (Pappas 2006, San-Blas and Calderone 2004, Sims et al. 2005, Spellberg et al. 2006) have been published over the past two decades covering these topics. As such, the reader is referred to these resources. The remainder of the present discussion will focus on brief portrayals of the more significant pathogenic species of *Candida*. Suffice to note, however, candidiasis encompasses a broad range of infections. Mucocutaneous forms of the disease (e.g., vaginitis) are very common and rarely life threatening to the immune competent host. Rather, such mucosal and superficial skin infections are typically troublesome in terms of physical comfort, cosmetic concern, and occasional relapse of the condition. In contrast, mucocutaneous candidiasis in immunocompromised hosts (e.g., HIV-infected individuals with esophageal candidiasis) is more serious and subject to frequent relapses. The more serious cases of systemic candidiasis, either in immune compromised persons or others with predisposing conditions (e.g., burn patients), can produce a multitude of symptoms and involved organ systems. Highly significant mortality rates accompany these forms of candidiasis.

As yeasts, *Candida* spp. are generally small, ovoid, thin-walled fungi that reproduce mainly by budding. Relatively few species are routinely isolated from human and animal infections (Table 2.1). Clinical isolates are often distinguished from one another by using one of several simple methods. For example, as discussed below, yeast cells of several etiological agents of candidiasis produce germ tubes in serum at 37°C, thereby reducing the number of possible identifications. Also, only a few species produce chlamydospores, again restricting identification to a few yeasts. Perhaps one of the quickest means to a presumptive identification is the use of CHROMagar *Candida* media. Based upon the color and morphology of colonies growing on this proprietary medium, the clinical mycologist can distinguish among yeasts of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. glabrata*, and other non-*C. albicans* species (Jabra-Rizk et al. 2001, Odds and Bernaerts 1994, Pfaller et al. 1996). However, the traditional method of employing commercially available carbohydrate fermentation and assimilation assays (e.g., API 20C, Vitek 2 ID-YST, etc.) remains key to making more precise identifications when ambiguous or equivocal phenotypic results are obtained. Hopefully, some of the ambiguity will be resolved as progress is made in the development and application of molecular methods in which DNA-based technology will provide the means to more rapidly and accurately identify pathogenic yeasts. For a review of traditional and commercial yeast identification methods, as well as a capsule summary of genotypic techniques, the reader is referred to a recent publication by Pincus et al. (Pincus et al. 2007).

The following discussions are limited to those species that are well-documented *Candida* pathogens. Regarding the taxonomy of these species, to note that this topic has been a source of tremendous study would be a gross understatement. For a comprehensive review of the taxonomic aspects of these fungi, the reader is referred to the pertinent sections of the present publication. To summarize, the following fungi are considered ascomycetes due to the teleomorphic state exhibited by some species as well as other cellular and molecular properties. Physiologically, these yeasts fall within Group VI

described by Meyer et al. (Meyer et al. 1998), in that all grow at 40°C, but they do not assimilate nitrate, erythritol, or *myo*-inositol.

2.1.1. Candida albicans

This fungus is the most common cause of candidiasis, but is not readily isolated from the environment. This apparent absence from the environment may be due to its adaptation to a parasitic life cycle with the concomitant loss of the properties permitting it to easily survive outside a host. In addition to a yeast phase, *C. albicans* produces true hyphae and pseudohyphae when it is cultured on the appropriate media and incubated under suitable environmental conditions. *In vivo*, both hyphal-like elements and yeast cells can be observed. The hyphae generate grape-like clusters of blastoconidia. The latter can grow as budding yeasts or germinate as hyphae. Also, *C. albicans* is well known for the ability to form thick-walled entities, termed chlamydospores, at the terminal ends of hyphae. This structure is often used as a diagnostic feature for this species, although *C. dubliniensis* also produces chlamydospores. An additional diagnostic feature of *C. albicans* is the ability of yeasts grown at 37°C in serum to form germ tubes. These hyphal initials differ from the outgrowth of other *Candida* spp. in that a constriction is absent at the germ tube base. Finally, on CHROMagar *Candida*, this species produces distinctive green colonies.

A true teleomorph of *C. albicans* has yet to be established although recent advances have demonstrated that this yeast possesses mating type genes (Hull and Johnson 1999). Genetically modified strains provided evidence of "mating" within a mammalian host (Hull et al. 2000). In the laboratory setting, appropriate strains can undergo a type of cytoplasmic fusion followed by karyogamy that strongly resembles that exhibited by *Saccharomyces cerevisiae* (Bennett and Johnson 2005, Miller and Johnson 2006, Soll 2006). A key difference is that the mating strains are naturally diploid and the daughter cell product of this mating is a tetraploid. Subsequent loss of chromosomes during asexual growth of the daughter cell eventually results in the re-establishment of a diploid state. This entire process is highly regulated and is associated with a genetically controlled morphological phenomenon known as phenotypic switching.

2.1.2. Candida dubliniensis

In 1995, a new species of *Candida* was isolated from the oral cavity of HIV-infected patients. This new species was designated as *C. dubliniensis*. Subsequently, like *C. albicans*, *C. dubliniensis* proved to be readily recovered from HIV-positive patients across the world. This yeast has also been isolated from bone marrow transplant patients and those persons on broad-spectrum antibiotics. A recent review highlights the status of this pathogen since its discovery (Sullivan et al. 2005).

Candida dubliniensis is morphologically similar to *C. albicans* in that it forms germ tubes and chlamydospores. These two properties are only exhibited by these two species among all members of the genus *Candida*. However, a published report suggests that Staib agar supports chlamydospore formation only by *C. dubliniensis*, thereby providing a possible diagnostic tool for differentiating these species (Staib and Morschhauser 1999). A more recent study suggests that the regulatory signals that control chlamydospore formation differ between *C. dubliniensis* and *C. albicans* (Staib and Morschhauser 2007). Such data may help decipher the biological function of this curious structure. In addition to the morphological and developmental resemblance of *C. dubliniensis* and *C. albicans*, both of these species are physiologically similar bearing only subtle differences. However, the two species can be distinguished from one another by incubating strains at 45°C. At this temperature, *C. dubliniensis* will not grow, whereas *C. albicans* readily forms colonies under the same conditions. On CHROMagar *Candida*, *C. dubliniensis* also forms green colonies like *C. albicans*, though on newer formulations of the medium the colonies are darker. There are differences in karyotypes and rDNA sequences, but the applications that would be employed to assess these characteristics are usually beyond the purview of a typical clinical laboratory.

2.1.3. Candida glabrata

From a medical viewpoint, the significance of this species of *Candida* can be found in its increased incidence worldwide, compounded by an apparent increase in its resistance to commonly applied antifungal agents. *Candida glabrata* is the second most common cause of bloodstream infections following *C. albicans*, with the upsurge in frequency being due to the larger population of immunocompromised individuals and the widespread use of antimycotics. This species is also one of the most prevalent isolates from cases of oral infections and vaginitis. For further information regarding the biological and clinical properties of this yeast, the reader is referred to recently published reviews (Bialkova and Subik 2006, Kaur et al. 2005).

Isolates of *C. glabrata* can be readily recovered from clinical specimens using routine medical mycological media. A presumptive identification can be made using CHROMagar *Candida* media upon which colonies of *C. glabrata* appear purple to pale pink. Recent clinical findings, however, suggest that some diagnoses of *C. glabrata* infections may be missed since the growth of particular isolates is dependent upon exogenously supplied cholesterol (Bard et al. 2005, Hazen et al. 2005, Rezusta et al. 2007). Routine isolation media that are not supplemented with a source of cholesterol will not support the growth of these strains, which appear to have arisen from lipid-enriched therapies associated with the antifungal treatment. Apparently, these isolates are able to scavenge and use exogenous cholesterol in place of ergosterol for their plasma membrane structure. Because the mode of action of certain antifungals used to treat *C. glabrata* infections is related to the production of ergosterol (e.g., terbinafine, fluconazole, etc.) or its presence in the plasma membrane (e.g., amphotericin B) (see selected chapters within Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005), the antifungal drug may have facilitated the selection of cholesterol-dependent strains following their acquisition of spontaneous mutations in ergosterol biosynthesis (Bard et al. 2005). Recently, an amphotericin B-resistant, cholesterol-dependent isolate of *C. glabrata* has been recovered (Rezusta et al. 2007). Isolating such strains might have been predicted based upon the mechanism of action of amphotericin B. Moreover, this observation further suggests that cholesterol-dependent isolates of *C. glabrata* that are simultaneously resistant to certain azole drugs might be encountered in the near future.

The taxonomy of this species had been one of controversy for a number of years. The primary argument focused on the inability of *C. glabrata*, previously designated as *Torulopsis glabrata*, to form pseudohyphae as opposed to *Candida* spp. that are characteristically noted to possess this property. However, the use of pseudohyphal formation as a diagnostic tool was demonstrated to be unreliable (Odds et al. 1997). Both molecular and phenotypic observations clearly support placing this species within the genus *Candida* (Fidel et al. 1999). In addition, laboratory investigations indicate that *C. glabrata* undergoes a mating process, similar to that recently discovered in *C. albicans*, and apparently analogous to that of *S. cerevisiae* (Brockert et al. 2003, Dodgson et al. 2005, Soll 2006, Srikantha et al. 2003). Recently, two additional species, *C. nivariensis*

and *C. bracarensis*, have been isolated from clinical specimens and these species are closely related to *C. glabrata*. However, analyses of 26S rRNA gene sequences support the differentiation of these two organisms as separate species (Alcoba-Flórez et al. 2005, Correia et al. 2006, Wahyuningsih et al. 2008).

2.1.4. Candida guilliermondii

This yeast species (teleomorph *Meyerozyma* (*Pichia*) *guilliermondii*) has been isolated from a wide number of environmental sources, e.g., fresh and salt water, soil, sand, amphibians, birds, and humans. It is also a noted source of nosocomial infections. Overall, the incidence of infections due to *C. guilliermondii* is low, but cases of candidemia, endocarditis, and invasive disease have been recorded (Girmenia et al. 2006).

Morphologically, pseudohyphal formation varies in abundance from strain to strain. True hyphae, however, are not produced by this species. The blastoconidia of *C. guilliermondii* may be found in short chains or clusters. Colonies of *C. guilliermondii* appear pink to lavender on CHROMagar *Candida*.

2.1.5. Candida krusei

This species (teleomorph *Pichia kudriavzevii*) is the fifth most common cause of candidemia, but probably is most noteworthy for its innate resistance to the antifungal agent fluconazole in addition to somewhat reduced susceptibility to other drugs (Pelletier et al. 2005). Most commonly isolated from neutropenic patients, *C. krusei* has sometimes been inadvertently selected as a pathogen in some patients receiving prophylactic fluconazole therapy. This yeast, which is commonly recovered from various environmental sources, is a significant etiological agent of vaginitis although it is not typically recovered from mucosal surfaces of healthy persons.

The blastoconidia of *C. krusei* are typically elongate reaching up to 25 μm in length. These cells often take on a "match-stick" like appearance. In stationary liquid cultures *C. krusei* forms a pellicle on the surface of the medium, and on agar media the colonies often appear wrinkled and flat. Physiologically, *C. krusei* can grow on vitamin-free media and differs from other *Candida* spp. in a number of properties. Colonies of *C. krusei* appear pink and have a rough texture on CHROMagar *Candida*.

2.1.6. Candida lusitaniae

Studies have shown *C. lusitaniae* (teleomorph *Clavispora lusitaniae*) to be part of the normal mycobiota of animals, though its prevalence among isolates from clinical samples is low. In health care settings, the possible transmission of this yeast from hospital personnel can lead to nosocomial colonization of the digestive and urinary systems. However, the medical importance of *C. lusitaniae* resides in the intrinsic resistance of some strains to the polyene antifungal agent, amphotericin B (Hawkins and Baddour 2003). Acquired resistance by *C. lusitaniae* to this drug has also been noted. Serious infections by *C. lusitaniae* typically involve patients with hematological malignancies as well as other types of individuals being treated in intensive care units.

Strains of *C. lusitaniae* produce pseudohyphae upon which chains of blastoconidia develop. Colonies of this species appear pink to lavender on CHROMagar *Candida* and some produce a waxy texture on this medium.

2.1.7. Candida parapsilosis

This yeast is one of the most common causes of candidemia, especially in neonatal intensive care units (Bendel 2003, Chapman 2003). Patients with intravenous catheters and prosthetic devices are frequently at risk of infection by *C. parapsilosis*. This fungus produces an adhesive slime layer that enables the transmission to patients from environmental sources and hospital personnel.

In culture, *C. parapsilosis* produces long, branching pseudohyphae that present a "pine forest" appearance. However, the degree of pseudohyphal formation varies among strains. On CHROMagar *Candida*, colonies of this species appear ivory to pink to lavender and some are wrinkled.

To date, no teleomorph for *C. parapsilosis* has been documented. Within this species, though, a variety of previous typing studies was able to discern three different groups of isolates, designated I, II, and III. Using a multilocus typing scheme, however, investigators established two new species, *C. orthopsilosis* and *C. metapsilosis*, to replace the existing designations of *C. parapsilosis* groups II and III, respectively (Tavanti et al. 2005a).

2.1.8. Candida tropicalis

As a frequent isolate from blood cultures, *C. tropicalis* mainly afflicts individuals suffering from leukemia, prolonged neutropenia, or extended hospitalization in intensive care units. This yeast is also found to be a frequent isolate from the oral cavities of asymptomatic persons. Like many other cases of candidiasis, infections due to *C. tropicalis* can be endogenous, i.e., from within the normal mycobiota of the patient, or transmitted from hospital personnel. On CHROMagar *Candida*, steel blue to dark gray colonies are formed by *C. tropicalis* that also often exhibit a brown to purple halo. No documented teleomorph has been observed for *C. tropicalis*.

2.2. *Saccharomyces*

This genus is comprised of eight species. One, *S. cerevisiae*, has been documented to cause human infection (Enache-Angoulvant and Hennequin 2005, Munoz et al. 2005). Strains of *S. cerevisiae* appear to be more pathogenic, especially when used as a probiotic preparation in immune compromised patients. However, with appropriate treatment, such individuals tend to have a better prognosis than persons infected by other routes.

Commonly known as baker's or brewer's yeast, *S. cerevisiae* may colonize the mucosal surfaces of persons with underlying illness. Such infections tend to be superficial (e.g., thrush, esophagitis, vaginitis). Conceivably, cases of vaginitis caused by *S. cerevisiae* may be mistakenly attributed to *C. albicans* due to their similar symptoms. This may result in empirical treatment without performing a culture. In addition, serious invasive infections and fungemia due to *S. cerevisiae* have been recorded. Typically, such patients possess profoundly compromised immunity and infections are often associated with risk factors such as surgery, burns, malignancies, central catheters, hyperalimentation, and broad-spectrum antibiotic use. Because *S. cerevisiae* is a common colonizing fungus, histopathological examination of tissues is necessary to diagnose and confirm infection.

Most individuals tend to think of *S. cerevisiae* as being solely monomorphic. However, it does form pseudohyphae and chains of budding yeasts under the appropriate conditions. For example, nitrogen-poor media induces pseudohyphal growth in *S. cerevisiae* (Gagiano et al. 2002). This alternate growth form of *S. cerevisiae* is the result of a well-developed nutritional sensing mechanism that impacts cellular morphogenic programs (Gimeno et al. 1992).

2.3. *Pichia* and Derived Genera

Many medical mycology texts and journal publications cite two species formerly classified in the genera *Hansenula (H.)* and *Pichia* as

causes of human infection. One, *Wickerhamomyces anomalus (H. anomala)*, is encountered more than the second, *Ogataea polymorpha (H. polymorpha*). The following brief description will employ the current binomials *W. anomalus* and *O. polymorpha*, respectively.

The incidence of mycoses due to *W. anomalus* and *O. polymorpha* has been relatively small. Documented infections include pediatric pneumonia, endocarditis, urinary tract infection, fungemia, and a "thrush-like" condition. One case of invasive disease was associated with chronic granulomatous disease in a child. In all cases, the predisposing factors to infection appear to coincide with those most often associated with encouraging colonization by opportunistic fungi. Clinical distinction between disease caused by *W. anomalus* and *O. polymorpha* is not well established. Isolates of these two species are distinguished from similar yeasts by their sugar fermentation patterns.

2.3.1. Magnusiomyces (Dipodascus) capitatus (Blastoschizomyces capitatus = Geotrichum capitatum)

Dipodascus capitatus is the teleomorph of the fungus commonly known as *Blastoschizomyces capitatus* and occasionally as *Geotrichum capitatum*. This species has been routinely isolated from the environment, particularly woody areas and from poultry feces. However, an environmental source is often not associated with infections. Infections can involve a single organ or multiple organs. Fungemia is common. Disseminated disease is similar in pathology to that evoked by infections due to *Candida* species. Most disease occurs in individuals with hemotologic abnormalities including leukemia and neutropenia (Anaissie et al. 2003, Christakis et al. 2005, Dismukes et al. 2003, Gadea et al. 2004, Levy et al. 2006, Martino et al. 2004, Merz and Hay 2005, Pimentel et al. 2005). Cultures of *D. capitatus* grow as hyaline mycelia that are septate and produce arthroconidia.

3. BASIDIOMYCETOUS YEASTS OF CLINICAL SIGNIFICANCE

The following section provides a brief review of those basidiomycetous yeasts that have been isolated from diseased individuals. The major pathogenic genera, *Cryptococcus* and *Malassezia*, afflict significantly more persons on a regular basis than those yeasts belonging to the genera *Sporobolomyces, Rhodotorula, Trichosporon*, and *Ustilago*. Nonetheless, the latter are included to provide a broad view of the spectrum of infectious fungi. For in-depth descriptions beyond that presented below, the reader is referred to several clinical mycology texts (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006a, Merz and Hay 2005).

3.1. *Cryptococcus*

The genus *Cryptococcus* is comprised of at least 70 species that have been isolated from various habitats and animals on every continent. Though the species *Cryptococcus (Cr.) laurentii, Cr. curvatus,* and *Cr. albidus* have caused occasional infections, termed cryptococcosis, only two species, *Cr. neoformans* and *Cr. gattii*, have been routinely documented as pathogenic for humans. The biology and clinical significance of these two species have been recently reviewed (Campbell and Carter 2006, Chayakulkeeree and Perfect 2006, Lin and Heitman 2006, Perfect 2006).

Cryptococcosis is caused by basidiomycetous yeasts within the "*Cr. neoformans* species complex" (Lin and Heitman 2006). Phenetic, biologic, and phylogenetic analyses suggest that this complex is comprised of two distinct species, *Cr. neoformans* and *Cr. gattii* (Kwon-Chung et al. 2002, Kwon-Chung and Varma 2006). Both are encapsulated yeasts, but *Cr. gattii* colonies on agar media tend to be more mucoid. Also, subtle morphological differences exist between the yeast cells of *Cr. neoformans* and *Cr. gattii*. For example, *Cr. neoformans* produces colonies of ovoid to spherical cells. In contrast, *Cr. gattii* yeasts tend to be more ellipsoid in appearance. Also, these two species can be differentiated via their biochemical differences. Most notable is that *Cr. gattii* reacts positively on CGB agar, whereas *Cr. neoformans* does not. Furthermore, *Cr. neoformans* appears to consist of two varietal states (var. *neoformans* and var. *grubii*), whereas *Cr. gattii* seems to be a distinct species possessing pronounced intraspecific genetic diversity. Not all taxonomists agree with this position, however. Different experts have championed the establishment of up to eight distinct species within the *Cr. neoformans* species complex (Coenjaerts 2006, Lin and Heitman 2006). Much of this debate has been prompted by advances in the development and application of molecular epidemiological methods as well as the phylogenetic and genotypic analyses of various clinical, environmental, and hybrid isolates (Boekhout et al. 1997, 2001, 2007, Bovers et al. 2006, 2007, Campbell et al. 2005, Diaz et al. 2000, Escandon et al. 2006, Kidd et al. 2004, Meyer et al. 2003, Meyer et al. 1999, Trilles et al. 2003). The collective data from these various studies show that the *Cr. neoformans* species complex is highly divergent at the genomic level and comprises at least nine molecular types. Taken alone, the genotypic variation supports the contention that the complex is in the process of evolving new species. However, what is not clear is at which point does this genotypic variation define the emergence of separate species, particularly if phenetic and biologic information is not considered. Hence, based upon a species concept involving phenetic and biologic evidence, in addition to cladistic data, Kwon-Chung and Varma (2006) argued for two distinct species within the *Cr. neoformans* species complex. The following is a brief summary of this argument.

Prior to 1950, cryptococcosis was considered to be a mycosis caused by the single, homogeneous species, *Cr. neoformans*. However, investigations of the polysaccharide capsule of this species revealed the existence of four capsular epitopes which were designated A, B, C, and D. Apparent hybrid strains possessing serotype AD were also noted. Subsequently, in 1975, heterothallism in *Cr. neoformans* was demonstrated in laboratory experiments (Kwon-Chung 1975, 1976a). To date, the teleomorph of *Cr. neoformans* has not been observed in nature. Laboratory crosses of appropriate mating type strains produce dikaryotic hyphae that form true clamp connections characteristic of basidiomycetous fungi. Some hyphal apices differentiate into basidia wherein meiosis occurs followed by the formation of uninucleate basidiospores. From matings attempted with the various different serotypes, two different biological species were defined. Both can be distinguished on the basis of basidiospore morphology. One species, designated *Filobasidiella neoformans*, produced spherical basidiospores. This teleomorph was initially observed between compatible serotype D strains and later demonstrated between appropriate strains bearing serotypes A and D. Correspondingly, the anamorphic strains comprising these serotypes were designated *Cr. neoformans*. In contrast, mating of suitable strains exhibiting serotypes B and C produced meiospores that are ovoid and bacilliform. Therefore, based upon the morphological differences, the teleomorph resulting from the serotype B and C crossings was defined as a second species, *Filobasidiella bacillispora*. Concomitantly, the anamorphic strains of the B and C serotypes were given a new epithet, *Cr. bacillisporus*, which was later changed to *Cr. gattii* (Kwon-Chung et al. 2002). Subsequent biochemical analyses, e.g., glycine metabolism, canavanine resistance, etc. (Kwon-Chung et al. 1982, Min and Kwon-Chung 1986, Polacheck and Kwon-Chung 1980, 1986)

further supported the phenetic distinction of *Cr. neoformans* and *Cr. gattii* as separate species. Moreover, with the development of molecular methodologies, phylogenetic analysis using nucleotide sequences from ribosomal RNA genes, various housekeeping genes, and virulence-associated genes demonstrated the genetic relatedness of all serotypes (Butler and Poulter 2005, Diaz et al. 2000, 2005, Diaz and Fell 2005a, Fan et al. 1995, Fell et al. 2000, Litvintseva et al. 2003, Xu et al. 2000). That is, serotypes A, D, and AD tended to cluster together as did serotypes B and C. Such observations are consistent with the phenetic and biological analyses that demonstrate the existence of two species.

With regard to pathogenesis, *Cr. neoformans* and *Cr. gattii* tend to afflict different types of individuals. Infections by *Cr. neoformans* are markedly more frequent in immunocompromised individuals, such as HIV-infected patients, with var. *grubii* (= serotype A) being the major etiological agent. In contrast, *Cr. gattii* appears to more commonly cause disease in persons with competent immune systems. Moreover, these species appear to differ in their ecological niches. Whereas *Cr. neoformans* appears to be distributed worldwide, *Cr. gattii* tends to be found mainly in tropical and subtropical regions. However, a recent and ongoing outbreak of *Cr. gattii* infections in Vancouver, Canada and the Northwestern region of the US suggests that this species has disseminated beyond those artificial boundaries (Hoang et al. 2004, Kidd et al. 2004, 2007a, 2007b, Lindberg et al. 2007, MacDougall and Fyfe 2006, MacDougall et al. 2007, Upton et al. 2007).

Cryptococcosis can present a number of clinical manifestations (Chayakulkeeree and Perfect 2006). Infection presumably begins with the inhalation of the fungus via the aerosolization of particles (e.g., basidiospores) from bird dung (Sukroongreung et al. 1998). Such infections may cause asymptomatic lung colonization or a range of symptomatic pulmonary afflictions up to and including fulminant disease resulting in respiratory failure. In immune competent individuals, a coordinated cellular immune response eliminates the fungus or induces a quiescent state within pulmonary foci and lymph nodes. Subsequent debilitation of the immune system may permit such latent organisms to spread to other body locations. In immune compromised persons, however, colonization of the airways by *Cr. neoformans* typically leads to dissemination from the lungs to the central nervous system causing subacute or chronic meningitis. In a significant number of infected individuals, especially HIV-infected patients, skin manifestations occur. Less common are infections of the bone and other organs.

Various attributes of *Cr. neoformans* and *Cr. gattii* contribute to their virulence, including the presence of a polysaccharide capsule, the ability to synthesize melanin, thermotolerance, and the secretion of various metabolites (e.g., mannitol) and enzymes (e.g., proteases). In addition, host response via cellular immunity plays a crucial role in determining if infection is limited to transient colonization of the airways or subsequent establishment of diverse clinical manifestations. Also, the capsule serves as a diagnostic feature that is readily visible when specimens are stained with India ink. The presence of encapsulated yeasts from normally sterile body locations (e.g., spinal fluid) is a presumptive identifying characteristic for cryptococcosis.

Determining the ecological niches of *Cr. neoformans* and *Cr. gattii* may help to identify crucial factors involved in virulence as well as in the dissemination of cryptococcosis. The source of *Cr. neoformans* in the environment has often been associated with pigeon droppings and infected soils. However, guano from other types of birds (e.g., chickens, parrots, turkeys, and canaries), and the soils contaminated by it, has also yielded *Cr. neoformans* upon culture. Both var. *neoformans* and var. *grubii* have been isolated from these sources worldwide. Prior speculation had suggested that bird (pigeon) guano actually selected for the growth *of Cr. neoformans* var. *neoformans* and var. *grubii* since *Cr. gattii* has never been cultured from this source. Nonetheless, it was recently demonstrated that media prepared from pigeon guano does support the growth of both *Cr. neoformans* and *Cr. gattii*, but only *Cr. neoformans* exhibits robust sexual reproduction and basidiospore formation under the same culture conditions (Nielsen et al. 2007). The results of these investigations help explain the cosmopolitan distribution of cryptococcosis due to *Cr. neoformans*, given pigeon migratory patterns and evidence that infection begins via the inhalation of basidiospores. In contrast, *Cr. gattii* is most often found in a unique and restricted habitat. This species has been regularly isolated from gum trees (*Eucalyptus spp*.) located in numerous tropical and subtropical areas across the world. Correspondingly, a significant number of cryptococcosis cases in areas harboring these trees have been due to *Cr. gattii* strains. Other trees in tropical areas may also be niches for this species. Hence, the localized nature of the ecological niche provided by eucalypts and other types of vegetation, which grow mainly in tropical and subtropical regions, helps to clarify the restricted endemicity of infections due to *Cr. gattii*. Curiously, however, the ongoing outbreak of cryptococcosis in the temperate zones of Vancouver, Canada and the Northwestern US appears to be caused by a genetic subset of *Cr. gattii* strains (Kidd et al. 2004, 2007b, MacDougall et al. 2007). These strains appear to have developed the ability to grow in soil and thereby have expanded or altered the ecological niche in which they exist. Subsequently, dispersal of the organism was shown to be anthropogenic (via footwear and the wheels of vehicles) (Kidd et al. 2007a) suggesting that *Cr. gattii* infections will continue to reach beyond this geographical area.

3.2. *Malassezia*

Malassezia spp. are considered part of the normal skin mycobiota of humans and animals (Ashbee 2006, Batra et al. 2005, Chen and Hill 2005, Crespo-Erchiga and Florencio 2006). They have, however, garnered a great deal of attention over the years mainly for their association with various dermatological afflictions. Among these conditions are pityriasis versicolor, seborrhoeic dermatitis, atopic eczema/dermatitis syndrome, psoriasis, dandruff, folliculitis, and otitis. However, members of this genus have also been associated with invasive disease. The following discussion briefly highlights some of the salient features of this fungus.

The taxonomy of the genus *Malassezia* has been, and continues to be, an area of intense investigation. No teleomorph is known for this genus. However, the basidiomycetous affinity of the genus *Malassezia* has been demonstrated phylogenetically using ribosomal RNA gene sequence analysis (Begerow et al. 2000, Fell et al. 2000). Collectively, the species used in these studies formed a separate clade (Malasseziales) within the class Ustilaginomycetes. Presently, physiologic and molecular methods have established the 13 species listed in the following studies (Ashbee 2006, Batra et al. 2005, Cabañes et al. 2007). All species of *Malassezia*, except *M. pachydermatis*, require an exogenous source of lipids for growth. The particular lipid requirements appear to vary among species. However, all grow as yeasts that bud in a repetitive, unipolar fashion. The buds are generated enteroblastically, usually forming a wide base between parent cell and buds. *Malassezia* species can also produce a mycelial form.

Malassezia species are known to produce a diverse range of metabolites, including lactones that give rise to the fruity smell when the fungus is cultured (Ashbee 2006, Labows et al. 1979). These organisms also synthesize compounds (e.g., pityriarubins) that interfere with the respiratory burst of host immune cells (Kramer et al. 2005a). In addition, *Malassezia* species generate azelaic acid

that inhibits reactive oxygen species (Akamatsu et al. 1991). These latter two compounds may be factors that contribute to the survival of the fungus within the harsh environment of the host skin. Moreover, melanin production by *Malassezia* species has been documented *in vivo* (Gaitanis et al. 2005a). Melanin is a known fungal virulence factor that enhances survival of the invading organism on or within the host (Nosanchuk and Casadevall 2003). Hence, melanin formation may be another attribute that contributes to the ability of *Malassezia* species to colonize and survive on the skin.

Although considered to be generally benign members of the normal skin mycobiota, *Malassezia* spp. can pose clinical problems (Ashbee 2006, Batra et al. 2005). Under certain conditions, they initiate a superficial skin infection that, due to their lipid requirements, most often occurs in the sebum-rich areas of the body, i.e., face, forehead, scalp, back and trunk. The major clinical conditions arising from *Malassezia* infections include pityriasis versicolor, seborrhoeic dermatitis, and dandruff. Additional types of superficial pathologies attributed to *Malassezia* include atopic eczema/dermatitis syndrome, otitis, folliculitis, and psoriasis among others. Traditionally, diagnosis of infection was based upon histopathological examination as well as biochemical and phenotypic characterization of clinical isolates. However, the advent of molecular biology has prompted the development of a number of nucleic acid-based methods for the diagnosis of *Malassezia* infection as well as the identification of the specific species involved, including techniques that are culture independent (Boekhout et al. 1998a, Cafarchia et al. 2007, Diaz et al. 2006a, Gaitanis et al. 2002, Gemmer et al. 2002, Guillot and Guého 1995, Guillot et al. 2000, Morishita et al. 2006, Sugita et al. 2001e, Takahata et al. 2007, Theelen et al. 2001).

Perhaps one of the more notable clinical conditions caused by *Malassezia* species is pityriasis versicolor. This dermatologic manifestation is a chronic superficial infection characterized by round to oval lesions on the arms, trunk, and back. The lesions can be hypo- or hyperpigmented. Presumably, pityriasis versicolor occurs when the *Malassezia* species colonizing the skin converts to a mycelial form that subsequently invades the stratum corneum. Hypopigmentation of the lesions may result from the destruction of skin melanocytes caused by the release of malassezin by the invading fungus (Kramer et al. 2005b). Histopathological examination will confirm infection by noting the presence of characteristic yeast and mycelial forms in what is commonly referred to as "spaghetti and meatballs". Studies have suggested that *M. globosa* is the major pathogen causing this condition (Crespo-Erchiga et al. 2000, Crespo-Erchiga and Florencio 2006), although *M. sympodialis* may also be a significant causative agent of this condition (Gupta et al. 2001a, 2004a,b). Two other conditions noted above, seborrhoeic dermatitis and dandruff, are frequently associated with *Malassezia* spp. It has been suggested that seborrhoeic dermatitis, and likely dandruff as well, are caused by an abnormal host response to the yeasts on the skin rather than overgrowth of the pathogen. The major species involved in these conditions appear to be *M. restricta* and *M. globosa*. Similarly, atopic eczema/dermatitis syndrome appears to result from the chronic inflammation of the skin due to various allergens released by *Malassezia*, perhaps in conjunction with other microbiota.

Finally, *Malassezia* spp. have been isolated as agents of deep-seated and systemic infections, including abscesses, mastitis, and peritonitis. Usually, solid organ involvement does not occur. However, the most commonly reported systemic infection is fungemia, particularly in those patients receiving lipid infusions via a catheter. These cases have most often occurred in neonatal units in hospitals, but adult infections have been recorded (Ashbee 2006, Batra et al. 2005, Cannizzo et al. 2007, Chryssanthou et al. 2001, Curvale-Fauchet et al. 2004, Devlin 2006, Giusiano et al. 2006, Rosales et al. 2004). Left untreated, the fungus can disseminate to the lungs and brain. If the infection appears catheter related, simply removing this line helps alleviate the condition although antifungal treatment should still be considered in certain situations.

3.3. Less Common Basidiomycetous Yeast Pathogens

A number of other yeasts with basidiomycetous affinities have been observed to cause mycoses. These include *Trichosporon* spp., *Rhodotorula* spp., and *Sporobolomyces* spp. In addition, two cases of infection due to the corn smut fungus, *Ustilago*, have been recorded (Patel et al. 1995, Teo and Tay 2006). Collectively, these fungi do not cause large numbers of infections, yet they are often difficult to treat due to the condition of the patient or the low susceptibility of the etiological agent to antifungal regimens. A brief review of these fungi is provided below. For further details, the reader is referred to various clinical mycology texts and reviews (Anaissie et al. 2003, Boekhout and Guého 2003, Bouza and Munoz 2004, Dismukes et al. 2003, Girmenia et al. 2005, Groll and Walsh 2001, Kauffmann 2006a, Kiken et al. 2006, Martino et al. 2004, Merz and Hay 2005).

Infections caused by members of the genus *Trichosporon* can be either superficial or deep. There is a variety of species within this genus, but the most well known pathogens are *T. cutaneum* and *T. asahii*. The superficial colonization of hair shafts, termed "white piedra", is caused by several species of *Trichosporon*, but is primarily due to *T. cutaneum* (Anaissie et al. 2003, Dismukes et al. 2003, Kiken et al. 2006, Merz and Hay 2005). Deep-seated infections caused by *Trichosporon* are being reported with increasing frequency (Chowdhary et al. 2004, Girmenia et al. 2005, Rodrigues Gda et al. 2006, Tokimatsu and Kadota 2006). The most common etiological agent is *T. asahii*. Most afflicted patients possess predisposing conditions such as catheterization, steroid use, immunosuppressive therapy, chemotherapy, granulocytopenia, surgical procedures, continuous ambulatory peritoneal dialysis, and HIV infection. Hypersensitivity pneumonitis due to *T. asahii* and *T. mucoides* has also been recorded (Ono et al. 2007, Sugiyama et al. 2005, Tokimatsu and Kadota 2006). The genus is characterized by septate hyphae that produce abundant arthroconidia. Budding cells are also produced but are less common.

The genus *Sporobolomyces* encompasses several species of yeast-like fungi commonly found in various environments. In culture, colonies are pink-orange in color much like *Rhodotorula rubra*. However, *Sporobolomyces* often can be discerned from other yeasts by formation of reproductive ballistoconidia. To date, only seven cases of disease have been recorded due to *Sporobolomyces* spp. (Anaissie et al. 2003, Bergman and Kauffman 1984, Dismukes et al. 2003, Merz and Hay 2005, Morris et al. 1991, Morrow 1994, Plazas et al. 1994, Sharma et al. 2006). These cases included dermatitis, formation of nasal polyps, skin blisters, eumycetoma, and endophthalmitis. Two others involved disseminated disease in two HIV-infected patients indicating the potential of this genus to produce invasive infection in immune compromised hosts.

Finally, *Rhodotorula* is another common environmental yeast that has been documented to cause infection (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005). However, like *Sporobolomyces*, the number of cases is small. The most common condition is fungemia, but other conditions have been described, including peritonitis, meningitis, endocarditis, and eye infections. A number of cases have occurred in HIV-infected patients (Kaur et al. 2007, Merkur and Hodge 2002). *Rhodotorula* grows as orange-pink colonies in culture and characteristically does not produce hyphae. Among the various species, some represent the anamorph of the teleomorphic genus *Rhodosporidium*.

4. MEDICALLY-IMPORTANT DIMORPHIC FUNGI

The dimorphic fungi that cause disease in humans typically exist as saprotrophic molds in nature (Anaissie et al. 2003, Dismukes et al. 2003, Kauffmann 2006b, Merz and Hay 2005) (Table 2.2). Upon tissue invasion, many, but not all, undergo a morphological transition to a yeast form. To the non-medical mycologist this can lead to confusion, particularly when culture of clinical specimens results in growth of a mold form.

Another curious feature common to many of the clinically significant dimorphic fungi is their endemic nature. As described below, most of the infections caused by a particular species are geographically restricted. Nonetheless, diseases by these fungi are regularly diagnosed outside their endemic area. In some cases, infections are diagnosed in indigenous people from the region who have traveled outside its boundaries, whereas others have developed in non-native persons subsequent to visiting a particular endemic area.

The descriptions of the dimorphic fungi presented below mainly focus on their anamorphic state. In some cases, the teleomorphs of particular fungi have been demonstrated and each falls within the Ascomycota. For the remaining dimorphic fungi described below, despite the absence of a teleomorph, genetic and morphological evidence strongly suggests that all possess an ascomycetous nature.

4.1. *Histoplasma capsulatum*

The most common endemic disease in the United States is histoplasmosis caused by the fungus *Histoplasma (Histo.) capsulatum* var. *capsulatum*. A second variety, *Histo. capsulatum* var. *duboisii*, is restricted to portions of the African continent. A third variety, *Histo. capsulatum* var. *farciminosum*, is a pathogen of mules and horses in parts of Asia and Africa. Although the latter variety is also thermally dimorphic, it will not be included in the present discussion. For more detailed information regarding histoplasmosis and the biology of *Histoplasma*, including virulence attributes, the reader is referred to recent reviews (Couppie et al. 2006, Wheat 2006, Woods 2006).

Briefly, *Histo. capsulatum* var. *capsulatum* mainly resides in the Mississippi and Ohio River valleys of the United States and in portions of Central and South America, whereas *Histo. capsulatum* var. *duboisii* is most often found between the Tropic of Cancer and the Tropic of Capricorn in Africa. Sources of exposure for both varieties include caves, decaying and rotting organic matter, and bird roosts or chicken coops. Moreover, the pathologies of these two etiologic agents are distinct. For further details, see Couppie et al. (2006), Wheat (2006), and Woods (2006). Morphologically, both varieties are indistinguishable in their mold phase. Yet the yeast forms *of Histo. capsulatum* var. *capsulatum* and *Histo. capsulatum* var. *duboisii* are different. The former species appears as tiny budding yeasts (2–4 μm) and are typically found residing within macrophages *in vivo*. By comparison, the budding yeast form of *Histo. capsulatum var. duboisii* is significantly larger (8–15 μm), having thick walls and a prominent bud scar. In addition, this latter variety often appears as short chains of yeasts within infected tissue. The ascosporic state of *Histo. capsulatum* var. *capsulatum* is *Ajellomyces capsulatus* (Class Eurotiomycetes, Order Onygenales, Family Ajellomycetaceae). Interestingly, although an ascosporic state for var. *duboisii* has not been observed, strains of this variety will mate with the *capsulatum* variety and form cleistothecia that are indistinguishable from those produced by *A. capsulatus*. The ascospores in this cross-variety mating do not germinate *in vitro*, but they will grow and cause disease in mice.

4.2. *Blastomyces dermatitidis*

Blastomyces dermatitidis is the causative agent of the fungal disease termed blastomycosis (Bradsher et al. 2003, Bromel and Sykes 2005, Kauffman 2006b). This mycosis is endemic to the eastern half of North America, thus earning it the ethnocentric name of North American blastomycosis. However, the disease has been documented in the continent of Africa as well as portions of central India.

Infection by *B. dermatitidis* is initiated via the inhalation of conidia. If suspected, diagnosis of blastomycosis can be readily made due to the relative ease of culture. The mold phase that grows at 25°C readily produces macroconidia, but these are quite similar to those formed by many other types of fungi. Instead, observation of the yeast phase of *B. dermatitidis*, either *in vitro* or *in vivo*, is a key diagnostic feature. Microscopically, yeast cells of this fungus are large (8–12 μm in diameter), multinucleate, and have a thick wall, often described as a "double wall". The most prominent attribute of the yeast form is that budding cells exhibit a wide base of attachment between a cell and its bud, i.e., broad-based budding. The ascosporic state of *B. dermatitidis* is *Ajellomyces dermatitidis* (Class Eurotiomycetes, Order Onygenales, Family Ajellomycetaceae).

4.3. *Paracoccidioides brasiliensis*

Paracoccidioidomycosis is the clinical pathology caused by the dimorphic fungus, *Paracoccidioides (Para.) brasiliensis* (San-Blas et al. 2002, Visbal et al. 2005). This fungus has exhibited no known teleomorph. However, based upon phylogenetic comparisons of rRNA gene sequences, *Para. brasiliensis* belongs to the order Onygenales, family Onygenaceae. The geographic distribution of *Para. brasiliensis* ranges from Mexico to South America, but it is more prevalent mainly on the southern continent rather than Central America.

Like other endemic fungi, infection begins through breathing in an infectious propagule that may or may not result in a symptomatic

TABLE 2.2 Selected Dimorphic Fungi Pathogenic for Humans and Animals

Anamorph	Known Teleomorph	*In Vivo* Morphology
Histoplasma capsulatum var. *capsulatum*	*Ajellomyces capsulatus*	Small (2–4 μm) ovoid, budding yeasts
var. *duboisii*	*Ajellomyces capsulatus*[a]	Larger (8–15 μm), thick-walled budding yeasts with a prominent bud/birth scar
Blastomyces dermatitidis	*Ajellomyces dermatitidis*	Large (8–15 μm), broad-based budding yeasts
Paracoccidioides brasiliensis	None	Multiply budding yeasts ("Pilot's Wheel", 15–30 μm)
Sporothrix schenckii	None	Round to ovoid yeasts (4–6 μm, cigar-shaped)
Penicillium marneffei	None	Small (3–5 μm), globose to elongated fission yeast

[a]*Variety* duboisii *will mate with an appropriate strain from var.* capsulatum *and form a teleomorph indistinguishable from* A. capsulatus.

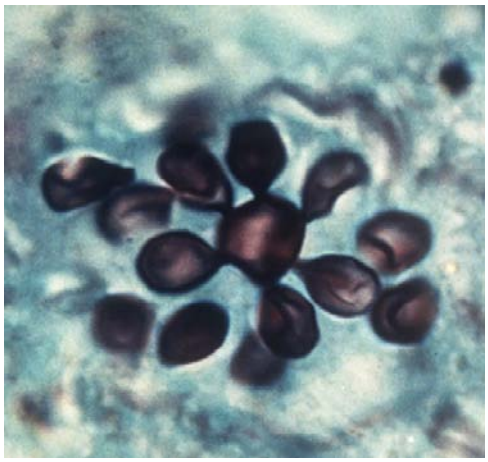

FIGURE 2.1 Multiple budding yeast form of *Paracoccidioides brasiliensis* in tissue. Gomori Methenamine Silver stain. Bar = 10 μm. Figure adapted from image provided through the courtesy of www .doctorfungus.org © 2007.

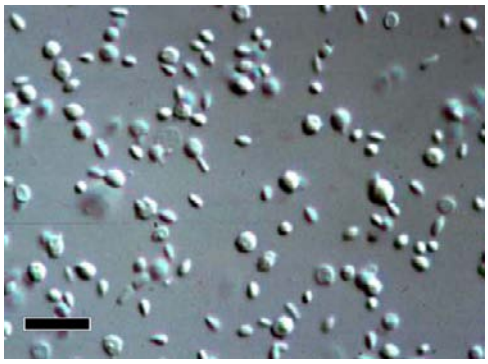

FIGURE 2.2 Budding yeast cells of *Sporothrix schenckii* grown in a 37°C broth culture. Differential interference contrast optics. Bar = 12 μm. Figure adapted from image provided through the courtesy of www.doctorfungus.org © 2007.

response. Once established in the lungs, however, *Para. brasiliensis* undergoes a morphological transformation that gives rise to the yeast phase that is decidedly characteristic for the mycosis caused by this fungus. At 37°C or *in vivo*, spherical yeast cells develop over a wide range of sizes (3–30 μm in diameter). From attachment points all along the surface of a central yeast cell, lemon-shaped buds (2–10 μm in diameter) develop that are connected to the parent cell through a narrow-based, isthmus-like connection. In simple terms, this entity is often referred to as a "pilot's wheel" or "mariner's wheel" (Fig. 2.1).

4.4. *Sporothrix schenckii*

The etiological agent of sporotrichosis, *Sporothrix schenckii*, causes a cosmopolitan disease that probably ranks as the world's most frequent subcutaneous mycosis (Bustamante and Campos 2001, Kauffman 2006b, Pang et al. 2004). Sporotrichosis occurs in many areas of the world, but is most frequently encountered in the United States, Central and South America, Africa, and Japan. Most cases are sporadic, although rare epidemics have been well documented.

Transmission of *S. schenckii* typically occurs through traumatic implantation, such as a prick from a rose thorn or pine needle. In several documented epidemics, implantation was directly related to small skin injuries due to handling pine seedlings, shrubs, or other types of vegetation. However, several cases of sporotrichosis have been reported that resulted from non-implantation means of infection. Many of these were laboratory-acquired infections (Cooper et al. 1992). In addition, pulmonary disease has been traced to the inhalation *of S. schenckii* conidia.

Like the other dimorphic fungi described above, *S. schenckii* grows as a mold at room temperature forming unicellular, tear-shaped to clavate conidia that are darkly pigmented. When incubated at 37°C, though, this fungus undergoes mold-to-yeast conversion. The yeast phase is distinctive in that the budding yeast cells (4–6 μm in diameter) tend to exhibit a "cigar shaped" appearance (Fig. 2.2). Curiously, however, the yeast phase of this fungus is rarely seen in clinical specimens. Finally, a few sporotrichosis infections have been caused by *S. schenckii* var. *luriei*. This variety differs from *S. schenckii* in that the yeast form, which can exist at 25°C, is large and thick-walled.

No known sexual phase has been observed for *S. schenckii* though evidence suggests it may be closely related to the genus *Ophiostoma*. In addition to *S. schenckii*, which is classified within the ascomycetous family Ophiostomataceae (Class Sordariomycetes, Order Ophiostomatales), cases of sporotrichosis have been ascribed to a far less common species, *S. cyanescens*. This species does not readily convert to a yeast phase *in vitro* and possesses septal structures consistent with basidiomycetous fungi. Recently, this species was transferred to the basidiomycetous genus *Fugomyces* (Sigler and Verweij 2003).

4.5. *Penicillium marneffei*

Infections by *Penicillium s*pp. (penicilliosis) are rare with one exception – those caused by *Penicillium (Pen.) marneffei* (Cooper 1998, Cooper and Haycocks 2000, Cooper and Vanittanakom 2008, Vanittanakom et al. 2006, Viviani and Vanittanakom 2005). The ability of this fungus to cause disease is directly associated with its formation of a yeast phase *in vivo*. This species emerged in the early 1980s as a significant pathogen of HIV-infected individuals residing or having traveled in Southeast Asia, the endemic region of disease caused by this fungus. Prior to this time, infections by *Pen. marneffei* were relatively rare. Penicilliosis due to *Pen. marneffei* typically disseminates systemically in immunocompromised patients and, left untreated, is universally fatal.

Infection by *Pen. marneffei* is presumably initiated by the inhalation of conidia that are subsequently phagocytized by pulmonary histiocytes. There, the fungus grows as a small yeast ($2–3 \times 2–7$ μm) and is approximately the same size as yeast cells of *Histo. capsulatum* (Fig. 2.3). This may have been the factor by which many of the early cases were misdiagnosed as histoplasmosis. The distinguishing feature of *Pen. marneffei* yeast cells is that they divide by fission as opposed to the budding yeasts of *Histo. capsulatum*. Cultures of *Pen. marneffei* incubated at 37°C also reproduce as fission yeasts.

Taxonomic studies have placed this fungus within the Phylum Ascomycota (Class Eurotiomycetes, Order Eurotiales, Family Trichocomaceae) (LoBuglio and Taylor 1995, Vanittanakom et al. 2006, Woo et al. 2003). Although no ascosporic state has been observed for *Pen. marneffei*, experimental studies have noted potential heterothallism in this fungus by documenting the existence of mating-type-like genes in its genome (Woo et al. 2006). Other studies have suggested that the profound asexual nature of this fungus has led it to develop a niche-adapted genotype, thus perhaps explaining its endemic nature (Fisher et al. 2005).

FIGURE 2.3 *Penicillium marneffei* growing as a fission yeast in a broth culture incubated at 37°C. Differential interference contrast optics. Bar = 5 μm.

5. OTHER YEAST-LIKE MYCOTIC AGENTS

The following brief descriptions cover three distinct types of fungi that are not often given consideration as medically important yeasts. One, the dematiaceous (phaeoid) fungi, can exhibit growth as budding yeasts both *in vitro* and *in vivo*. A second, *Lacazia loboi*, is a non-cultureable fungus that elicits keloidal lesions containing yeast cells often in chains. The remaining organism, *Pneumocystis*, is a major opportunistic pathogen of HIV-infected patients and for years was considered to be protozoan-like in nature, but is presently considered to belong to the Ascomycota (see Chapter 58, *Pneumocystis*).

5.1. Dematiaceous (Phaeoid) Fungi

The dematiaceous fungi represent a heterogeneous collection of darkly pigmented organisms (Cooper 2005). The term "dematiaceous", though not an appropriate description based upon its Greek root, has been used for so many years that it has found nearly complete acceptance in the mycological literature. Still, a better term to describe these pigmented fungi is "phaeoid". Both terms will be used interchangeably in the following discussion.

The dematiaceous fungi exist either as monomorphic or pleomorphic organisms capable of causing a wide range of mycoses (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005). It is the pleomorphic nature of some of these fungi that presents many challenges to the clinical mycologist. Some species can grow in a variety of forms, among which includes mold, yeast, and pseudohyphal phases. To further complicate this issue, some species growing as molds can exhibit more than one type of conidium generated by different modes of conidiogenesis. In essence, a significant number of the phaeoid fungi express multiple synanamorphs. Of the most intriguing synanamorphs are those designated "black yeasts".

The phaeoid yeasts are anamorphs of certain darkly pigmented molds. They mainly reproduce by budding. However, not all dematiaceous molds produce a yeast phase. Of those that do, most exhibit ascomycetous characteristics, but a few appear more basidiomycetous in nature. Moreover, not all black yeasts are associated with human disease. Those that do cause infection represent two distantly related phylogenetic groups within the Ascomycota (de Hoog 1993, de Hoog et al. 2003, Matos et al. 2003, Spatafora et al. 1995, Uijthof 1996). Most of the infections caused by these fungi can be grouped into the pathological condition known as phaeohyphomycosis, but they can also elicit other conditions (Anaissie et al. 2003, Dismukes et al. 2003, Merz and Hay 2005).

Clinical specimens of black yeasts can present a challenge in their identification (Dixon and Polak-Wyss 1991). Complicating this effort is that taxonomy of the phaeoid fungi appears to be in continual flux, thereby prompting seemingly constant nomenclatural changes. For an up-to-date synopsis of current nomenclature and obsolete synonyms of medically relevant fungi, including the black yeasts, the reader is urged to access the excellent web site, Doctor Fungus (www.doctorfungus.com). Despite these difficulties, however, Sanche et al. (2003) presented a clear description of those factors that differentiate between the two groups of black yeasts previously defined by phylogenetic methods as well as the individual species comprising these groups. The reader is referred to this reference for details.

Within the scheme of Sanche et al. (2003), Group 1 consists of *Exophiala* species and *Wangiella dermatitidis*, both of which exhibit early growth as yeasts, but frequently convert to a mycelial phase in culture. There is a great deal of controversy regarding the correct taxonomy of *Wangiella dermatitidis* and its alternate binomial *Exophiala dermatitidis*. The issue basically comes down to two different interpretations on the mode of conidiogenesis in this fungus. Perhaps future genetic examination of the genomes of these putatively different fungi will settle the matter. Regardless of specific epithet, different species of these fungi are responsible for infections resulting in various forms of phaeohyphomycosis, eumycetoma, endocarditis, and chromoblastomycosis. However, it is primarily in phaeohyphomycosis that the yeast phase of these species is noted *in vivo*.

Group 2 of the black yeasts encompasses *Aureobasidium pullulans, Hormonema dematoides,* and *Phaeoannellomyces werneckii (= Hortaea werneckii)*. Cases of phaeohyphomycosis, peritonitis, onychomycosis, and keratitis have been attributed to *A. pullulans* and *H. dematoides*. Both of these fungi are characteristically found in soils, waters, and fruits. Again, the yeast phase is primarily observed in phaeohyphomycosis. Infection in all these pathologies usually occurs via traumatic implantation of the fungus. In contrast, *P. werneckii* is the etiological agent of a superficial type of phaeohyphomycosis designated tinea nigra. This pathology is characterized by darkened areas of the palm or sole of the foot, in which yeast-like cells can be observed. The condition is strictly cosmetic and infection presumably occurs via skin abrasion in the presence of a suitable environmental source of the fungus.

5.2. *Lacazia loboi*

Lobomycosis is a rare, chronic subcutaneous infection of the skin. The infection is marked by the formation of keloidal, ulcerated, or verrucose lesions that contain the etiological agent, *Lacazia loboi* (Fonseca 2007, Talhari and Pradinaud 2005). The disease is confined to parts of Central and South America and also appears to be associated with seawater off the coasts of these areas. It is there and in other offshore tropical waters that infected dolphins have been documented (Haubold et al. 2000). However, the exact source of the organism is unknown, particularly since *L. loboi* has never been cultured from nature. Some evidence exists that the organism has been cultured in experimental animals using infected tissue (Talhari and Pradinaud 2005).

Diagnosis of infection is made by the histopathological observation of *L. loboi* in tissue. This fungus is present in large numbers mainly as chains of yeast cells connected by a small isthmus. Solitary yeast cells are also present, but hyphae have never been noted.

Despite the absence of an *in vitro* culture system for *L. loboi*, advances have been made to phylogenetically assess the lineage of

this fungus. Using novel methods to extract DNA from infected lesions, various gene fragments were amplified, cloned, and sequenced (Haubold et al. 1998, Herr et al. 2001, Mendoza et al. 2005, Vilela et al. 2005). The resulting data clearly show that a close relationship exists between *L. loboi* and *Paracoccidioides brasiliensis*, thereby placing the former species within the ascomycetous order Onygenales.

5.3. *Pneumocystis*

Species of *Pneumocystis* are known pathogens of humans and animals. However, when first isolated in 1909, *Pneumocystis* was mistakenly identified as part of the life cycle of the protozoan parasite, *Trypanosoma cruzii*. This association relegated *Pneumocystis* to the protozoan realm, and virtual obscurity, until the 1950s when it was shown to be a cause of epidemic pneumonia. This prompted renewed interest in the pathogenicity of *Pneumocystis* as well as evoking studies directed towards its proper classification. Subsequent morphological and biochemical studies of this organism yielded some clues that suggested an affinity to the fungi, but these data were far from conclusive. Then, in 1988, Edman et al. (1988) demonstrated the relationship of *Pneumocystis* to the fungi based upon comparisons of ribosomal RNA gene sequences. Thereafter, a number of phylogenetic studies have been performed which include trees based upon SSU RNA gene sequences that infer a relationship with ascomyceteous yeasts (Gargas et al. 1995). For a more in-depth historical perspective of the taxonomy and phylogeny of *Pneumocystis*, the reader is referred to an excellent review by Cushion (2005), as well as Chapter 58 in this book.

A complete description of the biology of this fungus, its host range, pathogenicity, and diagnosis are beyond the scope of this work. Therefore, the reader is directed to several reviews that address these and other topics (Cushion 2005, Frenkel 1999, Hui and Kwok 2006, Morris et al. 2004a, Peterson and Cushion 2005, Redhead et al. 2006, Thomas and Limper 2007, Wazir and Ansari 2004). To summarize, however, there are currently four species of *Pneumocystis* recognized. *Pneumocystis jiroveci*, previously known as *P. carinii*, is a pathogen of humans, particularly those that are immunocompromised. Such conditions include HIV infection, leukemia, renal disease, co-infection by other viruses, and carcinoma. Moreover, *P. jiroveci* has been detected as a pathogen of neonates and pregnant women. Not all infections by *P. jiroveci* result in the pneumonia typically associated with HIV-infected patients. Extrapulmonary infections do occur in a variety of organ systems, though this is rare. The remaining species of *Pneumocystis* afflict other mammals, particularly rodents. Rats are infected by *P. carinii* and *P. wakefieldiae*, whereas mice are hosts for *P. murina*. Diagnosis of infection by culture is not possible given the absence of a continuous *in vitro* system for *Pneumocystis*. Instead, diagnosis relies on microscopic observations of stained specimens, serological tests, and molecular methods involving DNA amplification techniques.

6. SUMMARY

The medically significant yeasts comprise more genera than the traditionally held pathogens in the genera *Candida* and *Cryptococcus*. Clinically important yeast forms can also be found among the endemic dimorphic fungi, the dematiaceous molds, and even a noncultureable fungus. Collectively, these fungal pathogens exhibit either teleomorphs of the Ascomycota or Basidiomycota, or their anamorphic forms express morphological and genetic features consistent with one of these taxa. They comprise a diverse spectrum of fungi that have found the means, primarily by exploiting a host organism's weakened immune system, to become noteworthy mycotic agents. Furthermore, they portend the potential for the spectrum of fungal pathogens to become wider and deeper in the number of etiological agents derived from normally benign species. Vigilance by the general as well as the medical mycologist must be maintained so as to better understand how to address the future challenges likely to be posed to public health by fungi, particularly the yeasts.

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Chapter 3

Yeast Biotechnology

Eric A. Johnson and Carlos Echavarri-Erasun

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The following genus abbreviations are used in this chapter: *Bla., Blastobotrys; C., Candida; Crypt., Cryptococcus; D., Debaryomyces; E., Eremothecium; Geo., Geotrichum; K., Kluyveromyces; Kom., Komagataella; O., Ogataea; Ph., Phaffia; P., Pichia; Pseud., Pseudozyma; Rhspor., Rhodosporidium; Rh., Rhodotorula; S. Saccharomyces; Scopsis, Saccharomycopsis; Schef., Scheffersomyces; Schiz., Schizosaccharomyces; Schwan., Schwanniomyces; Tor., Torulaspora; Tr., Trichosporon; X., Xanthophyllomyces; Y., Yarrowia; Z., Zygosaccharomyces.*

1. INTRODUCTION

Yeasts have benefitted humankind for millenia. They have wide-ranging fundamental and industrial importance in scientific, food, medical, and agricultural disciplines (Fig. 3.1). Traditional industrial attributes of yeasts include their primary roles in many food fermentations such as beers, cider, wines, sake, distilled spirits, bakery products, cheese, sausages, and other fermented foods (Table 3.1). Other long-standing industrial processes that involve yeasts are the production of fuel ethanol, single cell protein (SCP), feeds and fodder, industrial enzymes, and small molecular weight metabolites. More recently, *Komagataella* (*Pichia) pastoris*, *Saccharomyces cerevisiae*, *Ogataea* (*Hansenula) polymorpha*, and certain other yeast species have been developed as industrial organisms for the heterologous production of enzymes and proteins, including protein pharmaceuticals. Yeasts, especially *S. cerevisiae*, are increasingly being used as hosts for expression of protein biocatalysts and multi-enzyme pathways for the synthesis of fine chemicals and small molecular weight compounds of medicinal and nutritional importance. Yeasts have important roles in agriculture as agents of biocontrol, bioremediation, and as indicators of environmental quality. Several of these processes and products have reached commercial utility, while others are in development. Owing to advances in functional genomics and systems biology, *S. cerevisiae* is presently the primary model organism for study of eukaryotic biology and human disease. The objective of this chapter is to describe yeast processes currently used by industry and those in developmental stages and close to commercialization. Emphasis will be given to new developments and opportunities in industrial applications of yeasts since traditional processes have been thoroughly described in the existing literature.

2. HISTORICAL HIGHLIGHTS

Since ancient times, fermented beverages and foods produced through the activities of yeasts have contributed prominently to the worldwide advancement and sustainability of human societies (Legras et al. 2007, Ulber and Soyez 2004). The domestication of *Saccharomyces cerevisiae* can be considered a pivotal event in human history. Archaeologists found evidence that alcoholic fermented beverages produced by yeasts were consumed in Neolithic times (8500–4000 BC) in China, Iran and Egypt, and other areas of the world (Cavalieri et al. 2003, McGovern 2003, McGovern et al. 1997, 2004, Ulber and Soyez 2004). Babylonian stone tablets more than 6,000 years old illustrated beer and wine recipes (Legras et al. 2007, Ulber and Soyez 2004). Many other fermented foods also originated in Neolithic times, such as African kafir and sorghum beer, pulque in Mexico, leavened breads in various regions, and several fermented foods and beverages from soy, rice, and vegetables (Aidoo et al. 2006, Beuchat 1995, Boekhout and Robert 2003, Dirar 1994,

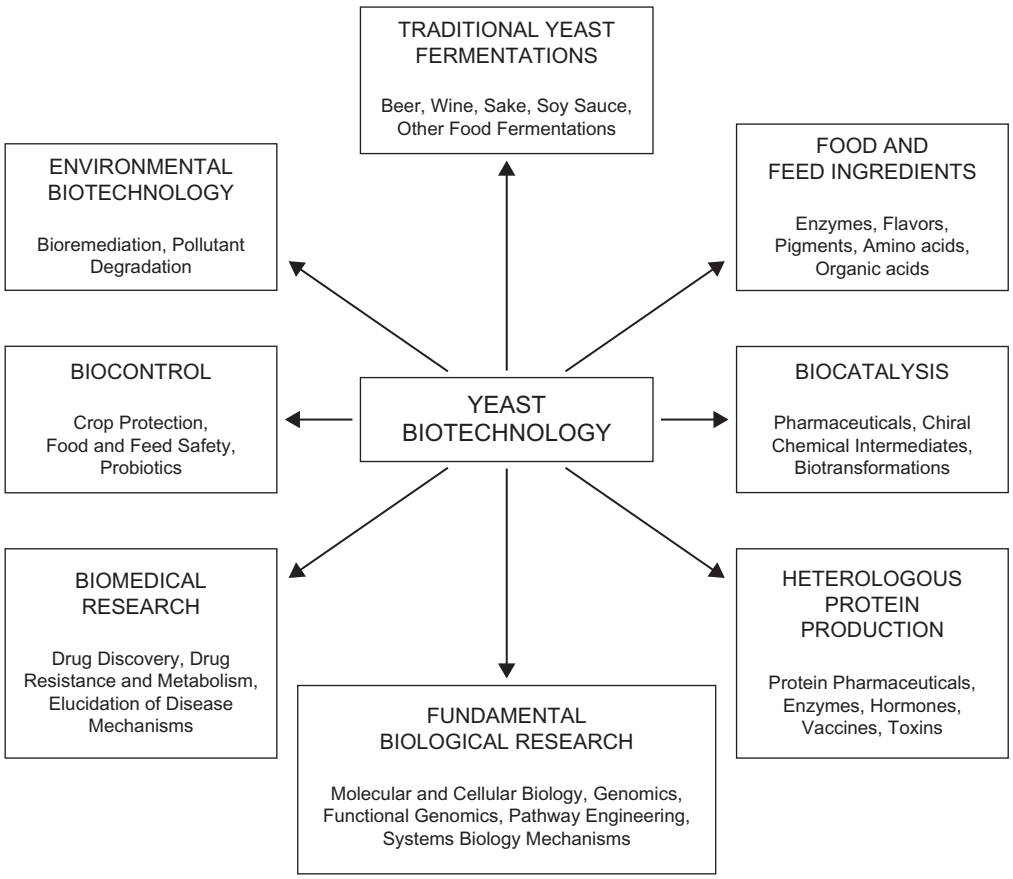

FIGURE 3.1 Various disciplines in yeast biotechnology (adapted and expanded from Walker 1998).