

6th EDITION

Larone's MEDICALLY IMPORTANT FUNGI

A GUIDE TO
IDENTIFICATION



Thomas H. Walsh
Randall T. Hayden
Davise H. Larone



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Cover: *Aspergillus fumigatus* on Sabouraud dextrose agar at 30°C for 4 days. Green velvety colony with a narrow white border. Microscopic structures consisting of septate hyphae and unbranched conidiphores with enlarged vesicle at the top. Compact uniseriate phialides bearing chains of round conidia only on the upper two-thirds of the vesicle. See [pp. 295](#) and [450](#).

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*Dedicated with love
To Ronit
Jessie
Beth
and with loving memory
to John D. Lawrence*

*Dedicated with love
To Laura, Emma, John, Frances, and Margaret Walsh*

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Preface to the Sixth Edition

Each edition of this book has been written with the intention of making the identification of clinically encountered fungi a more logical, understandable, and enjoyable endeavor for the personnel of clinical mycology laboratories as well as for others with an interest in the field. The accompanying goal is to broaden the reader's knowledge and provide current information regarding emerging and established fungal pathogens as well as new methods that can be applied in clinical laboratories. To those ends, the original format that has proved to be so successful is carefully maintained in this edition while additions and updates have been made throughout the book.

This new edition represents a passing of the torch of writing this esteemed, beloved, and time-honored book by Dr. Davise H. Larone to Dr. Thomas J. Walsh and Dr. Randall T. Hayden. Tom, Randy, and Davise worked with extraordinarily close synergy in providing seamless continuity in order to update this important laboratory diagnostic resource in medical mycology.

The majority of recent advances in our field have been based on molecular taxonomic studies applied to the medically important fungi. This continues to result in a molecular labyrinth of taxonomy and nomenclature adjustments. Phylogenetic (evolutionary development) studies continue to discover that organisms which may appear morphologically identical and that have been thought to be a single species are instead a species complex (a collection of related but distinct species). The "new" individual species may possess unfamiliar names. Moreover, these studies also are revealing that organisms thought to be within one genus or order now need to be transferred to another phylogenetic category that allows for a more accurate classification but leads to a change in nomenclature.

Since these changes may be confusing, we have endeavored to provide continuity with earlier names and nomenclatural stability

in areas of uncertainty. Nonetheless, over time, eventual accumulation of data regarding the distribution, clinical relevance, and antifungal susceptibility of newly described fungal species may lead to improved diagnosis and treatment of our patients. Recognizing the rapid changes that are occurring in terminology, we have introduced a discussion on taxonomy and nomenclature in order to help guide the reader through the substantial changes that are occurring in this area of medical mycology. The “Taxonomy and Nomenclature” essay in the Basics section outlines the principle of “one fungus/one name” and the consequences of simplifying, or in some instances complicating, the nomenclature. We note the transition of some common *Candida* species to the less commonly known teleomorph names, and exemplify cases of “cryptic species” that may only be identified by molecular tools.

The companion offshoot of molecular studies is the expanded variety of methods developed for the identification of fungi. As these methods are escalating in availability and usage, especially in reference laboratories, Part III, “Basics of Molecular Methods for Fungal Identification,” has been updated in this edition. This section is not an instruction on performing molecular assays; instead, its aim is to provide basic information to increase familiarity, comprehension, and comfort with the terminology, principles, and literature involved. It is written with the goal of increasing familiarity with the methods of molecular identification, especially for those readers who have traditionally relied on morphology and biochemicals to determine the identification of clinical isolates. Availability of morphological assessment, biochemical reactions, and molecular methods will allow the use of whichever systems are appropriate under the particular circumstance. We discuss several new technological advances that have become available since the last edition. Several commercial systems have incorporated real-time PCR and melt curve analysis in an integrated platform. Next-generation sequencing (NGS) is being incorporated into several new platforms that will likely be used increasingly in laboratory diagnostic mycology. The T2 system, which employs PCR and magnetic resonance technology, has the ability to rapidly detect five leading *Candida* spp. directly from blood.

This edition also includes descriptions of new emerging pathogens, such as *Candida auris* and *Aspergillus tanneri*. Detailed footnotes of nomenclatural changes that may be ongoing, but are not fully validated or routinely used in clinical laboratories, are now provided. Of particular note is the substantial increase in detailed descriptions of the epidemiological, clinical, and antifungal susceptibility characteristics of each organism. We also have revised the references throughout the book, adding many more primary references as well as updated atlases for resources. The section on reagents and biochemicals has been extensively reviewed to assure that all contact information, including websites, is most current.

Suggested readings for further information on each organism are still a standard component of each page in Part II. As new books and many valuable journal articles have been published in recent years, they have replaced some of the old, standard texts that many of us have used for a long time. In some instances, the older texts are still listed; this is because they contain a wealth of classic information that is not often covered as completely in the newer texts. However, the old nomenclature in these classic texts needs to be evaluated carefully to ensure correct interpretation relative to the more recent nomenclature.

Each edition of this book has been written with the needs of the reader foremost in mind. We strive to serve the clinical mycology community and their patients with this book as a key resource for laboratory diagnosis of medically important fungi. Throughout the years, many readers have offered helpful suggestions and requests that have been taken to heart and implemented toward the enhancement of the book. Such input plays a large role in ensuring that the goals of the book will be met; it is therefore most sincerely appreciated and we hope that it will continue.

March, 2018

Preface to the First Edition

More than ever, clinical laboratory personnel with limited experience in mycology must culture and identify fungi isolated from clinical specimens. Even after attending a course in the subject, technologists often need guidance in identifying the great variety of organisms encountered in the lab. With the advent of proficiency testing by local and national organizations, technologists have a need and opportunity to practice and increase their skills in the medical mycology laboratory.

Most classic texts, though rich in information, are arranged according to the clinical description of the infection; the textual discussion of any particular fungus can be located only from the index or table of contents. Since the technologist doesn't know the name of an unidentified fungus and usually has little or no knowledge of the clinical picture, these texts are at best difficult to use effectively. The unfortunate result is the all-too-common practice of flipping through an entire mycology textbook in search of a picture that resembles the organism under examination. Such a practice may make the more accomplished mycologist's hair stand on end, but it is a fact to be acknowledged.

This guide is not meant to compete with these large texts, but to complement them. The material here is so arranged that the technician can systematically reach a possible identification knowing only the macro- and microscopic morphology of an isolated organism. Reference can then be made to one of the classic texts for confirmation and detailed information.

Many possible variants of organisms are found under several categories of morphology and pigment. The outstanding characteristics are listed on the page(s) apportioned to each organism, and references are suggested for further information and confirmation (see [How To Use the Guide](#)).

Medically Important Fungi avoids the jargon so commonly and confusingly used in most mycology books. Drawings are used wherever possible to illustrate organisms described in the text. To ensure clarity, a glossary of terms is included, as well as a section on laboratory techniques for observing proper morphology. Another section includes use of the various media, stains, and tests mentioned in the book.

The actinomycetes, although now known to be bacteria rather than fungi, are included because they are frequently handled in the mycology section of the clinical laboratory.

It is believed that this guide will enable students and medical technologists to culture and identify fungi with greater ease and competency and in so doing to develop an appreciation of the truly beautiful microscopic forms encountered.

I wish to acknowledge with gratitude the encouragement and advice received from my co-workers at Lenox Hill Hospital, and Dr. Norman Goodman, Mr. Gerald Krefetz, Mr. Bill Rosenzweig, Ms. Eve Rothenberg, Dr. Guenther Stotzky, Mr. Martin Weisburd, Dr. Irene Weitzman, and Dr. Marion E. Wilson.

New York
December, 1975

Acknowledgments

With the writing of this new edition, we are grateful for the willingness of so many in our field to help in numerous ways. Our everlasting gratitude is also extended to the many colleagues who assisted in the preparation of previous editions; most of their contributions are now substantive and integral parts of the ongoing Guide.

Dr. Sanchita Das from North Shore University Hospital, Evanston, IL, generously contributed her time and expertise to update the section entitled “Basics of Molecular Methods for Fungal Identification” (Part III) which she had originally written for the 5th edition.

Our colleagues in Dr. Francis Barany’s molecular microbiology research laboratory at Weill Cornell Medicine have been extremely helpful and supportive during the writing of this edition as well as the previous edition.

Dr. Stephen Jenkins, Director of the Clinical Microbiology Laboratory of the NewYork–Presbyterian Hospital/Weill Cornell Medicine (NYPH/WCM) has always readily offered valuable advice and been very supportive. Dr. Lars Westblade, Associate Director of the Clinical Microbiology Laboratory, contributed numerous helpful suggestions for additions to this edition. As noted in previous editions, almost all the organisms shown microscopically and/or as cultured colonies were prepared in the Mycology Laboratory of NYPH/WCM. We will forever be indebted to the staff of the mycology lab for their enormously significant contributions over the years.

Pat Kuharic of the Photography Department of Weill Cornell Medicine has given us the benefit of her outstanding expertise in preparing the excellent color photographs of fungal colonies as well as the black and white photomicrographs. With her talent and professional passion to get it “just right,” she is a great asset to the book.

Aleina Zehra, administrative assistant to Dr. Walsh, meticulously reviewed and updated the many Internet websites and suppliers’

locations mentioned throughout the book, as well as assisting in the preparation of working manuscript drafts of various sections.

We thank the staff of the St. Jude Children's Research Hospital, Department of Environmental Health and Safety, for their assistance in reviewing the section related to packaging and shipping of hazardous materials.

Dr. Sybren de Hoog kindly provided a 2-year subscription to the *Atlas of Clinical Fungi*. Drs. David Warnock and Michael Pfaller discussed perspectives with us on fungal taxonomy and nomenclature.

Last, but definitely not least, we extend our deep appreciation to the members of ASM Press. Christine Charlip, Director, has been highly supportive of this 6th edition as an important contribution to ASM Press's library of clinical microbiological resources. Kudos also goes to Larry Klein, Production Manager; Ellie Tupper, Senior Production Editor; and the marvelous associates who contributed to the production of this edition: Mark Via, copyeditor; Susan Schmidler, interior design; and Debra Naylor, cover design. They have been extremely helpful, creative, flexible, and patient and are much appreciated.

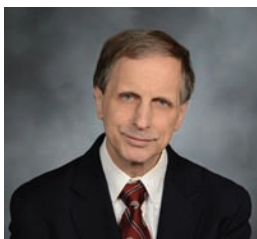
A NOTE FROM DR. LARONE

It has been my great honor, and a source of enormous satisfaction, to have written the first five editions of *Medically Important Fungi: A Guide to Identification* as sole author. I realized, after the 5th edition, that it would be wise to have some colleagues join me in writing the next editions. I can't thank Tom Walsh and Randy Hayden enough for agreeing to take on that endeavor. Enormous appreciation also goes to Sanchita Das for working so closely with me to create the Molecular section in the 5th edition and updating it with us in the 6th edition.

My greatest continuous long-standing appreciation goes to Ellie Tupper, who for the 4th through 6th editions has been production editor and watchful overseer, always working very closely with me and ensuring the beautiful production and high quality of the book. I have often said, and most truly mean it, that her name, along with ours, deserves to be on the cover of the book, not just on this page.

Ellie: I, the coauthors, and all the readers owe you an enormous "Thank You" and look forward to your remaining our very talented and essential partner and guide.

About the Authors



Thomas J. Walsh, MD, PhD (hon), FIDSA, FAAM, FECMM, serves as Professor of Medicine, Pediatrics, and Microbiology & Immunology at Weill Cornell Medicine of Cornell University; founding Director of the Transplantation-Oncology Infectious Diseases Program and the Infectious Diseases Translational Research Laboratory, Henry

Schueler Foundation Scholar in Mucormycosis; Investigator of Emerging Infectious Diseases of the Save Our Sick Kids Foundation; and Attending Physician at the NewYork–Presbyterian Hospital and Hospital for Special Surgery. Dr. Walsh directs a combined clinical and laboratory research program dedicated to improving the lives and care of immunocompromised children and adults with invasive mycoses and other life-threatening infections. The objective of the Program's translational research is to develop new strategies for laboratory diagnosis, treatment, and prevention of life-threatening invasive mycoses in immunocompromised patients. Dr. Walsh brings to this book more than three decades of experience in the field of medical mycology, with clinical and laboratory expertise across a wide spectrum of medically important fungi and mycoses. In addition to patient care and translational research, Dr. Walsh has also mentored more than 180 students, fellows, and faculty, many of whom are distinguished leaders in the field of medical mycology throughout the world.



Randall T. Hayden, MD, is Director of Clinical Pathology Laboratories and Director of Clinical and Molecular Microbiology and Member in the Department of Pathology at St. Jude Children's Research Hospital in Memphis, Tennessee. He joined the faculty there in 2000, following postdoctoral training in microbiology and molecular microbiology at the Mayo Clinic and in surgical pathology at the MD Anderson Cancer Center. He is board certified in Anatomic and Clinical Pathology with sub-specialty certification in Medical Microbiology. His research interests focus on the application of molecular

methods to diagnostic challenges in clinical microbiology, with particular emphasis on the diagnosis of infections in the immunocompromised host. He is editor-in-chief of *Diagnostic Microbiology of the Immunocompromised Host*, 2nd Edition; co-editor of *Molecular Microbiology, Diagnostic Principles and Practice*, 3rd Edition; and section editor for the *Manual of Clinical Microbiology*, 12th Edition, all from ASM Press.



Davise H. Larone is Professor Emerita at Weill Cornell Medicine in the Department of Pathology and Laboratory Medicine and the Department of Clinical Microbiology and Immunology. From 1997 to 2008, she served as Director of the Clinical Microbiology Laboratories of The New York–Presbyterian Hospital, Weill Cornell Center. Prior to that, she was for many years at Lenox Hill Hospital, New York, rising from technologist to Chief of Microbiology. During that period, in 1985, she received her PhD in Biology/Microbiology from New York University. Her interest in clinical mycology dates from the

1970s. Her undergraduate degree was in Medical Technology from the University of Louisville, but her love for drawing led her to study art on the side. The combination of her organizational skills and her art background resulted in the first edition of this book in 1976. The subsequent editions in 1987, 1995, 2002, and 2011 all feature Dr. Larone's elegant drawings. Dr. Larone has served on numerous standards, advisory, editorial, educational, and examination boards/committees. Over the years, she has presented more than 100 workshops and lectures in 52 cities in the United States and in 14 cities in nine other countries. She has received numerous awards for teaching and for contributions to clinical mycology.

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How To Use the Guide

Before beginning to use the guide, the reader should understand several points.

Fungi often appear different in living hosts than they do in cultures. [Part I](#) (pp. 17–72) is designed as a guide for preliminary identification of fungi seen on direct microscopic examination of clinical specimens.

In [Part II](#) (pp. 73–331), the descriptions of the macroscopic and microscopic morphologies of the cultured fungi pertain to those on Sabouraud dextrose agar (SDA) unless otherwise specified. SDA may not be as regularly used for primary isolation of fungi directly from specimens as it was in the past, due to evidence that it is not as supportive as once believed (Scognamiglio et al., 2010). Fortunately, the descriptions can also be applied to growth on alternative media.

Many moulds begin as white mycelial growths, and coloration occurs at the time of conidiation or sporulation. Hence, organisms are listed under their most likely color(s) at maturity, when the typical microscopic reproductive formations are more readily observed.

In [Parts I](#) and [II](#), when feasible, organisms are arranged in an order based on morphologic similarities (rather than alphabetical order) to facilitate convenient comparison.

This book is a *guide to identification*. Standard texts and our suggested references should be used for additional information concerning clinical disease, history, ecology, immunology, and therapy.

As molecular assays are increasingly being employed for identification of fungi, the basics of these methods and their utility in the clinical mycology laboratory are discussed in [Part III](#) (pp. 333–357).

Instructions for general laboratory procedures, i.e., collection of specimens, direct microscopic preparations, primary isolation, slide cultures, special tests, maintenance of stock cultures, and the like, are

given in [Part IV](#) (pp. 359–384). Staining methods are described on pp. 385–394; the preparation and use of media are on pp. 395–425.

Any terms used that may not be familiar to the reader can most likely be found in the Glossary on pp. 457–468.

Once the organism has been properly collected, cultured, isolated, and observed microscopically, use of the guide is quite simple.

- 1.** Note the morphology of the unknown fungus.
 - a.** Is it a filamentous bacterium, yeastlike, thermally dimorphic, or a thermally monomorphic mould?
 - b.** Record color of surface and reverse (underside) of colony.
- 2.** Using the initial “Guide to the Identification of Fungi in Culture” on pp. 75–107, refer to a page that shows drawings of the microscopic morphologies of organisms having the appropriate macroscopic appearance. Here one may see either the exact organism under examination or several possibilities.
- 3.** Proceed to the page given in parentheses under the likely organism(s) to find more detailed information, including pathogenicity, rate of growth, colony morphology, an enlarged drawing of the microscopic appearance, a photomicrograph, and references for additional information. Where applicable, there will be reference to tables and color plates* and discussions of tests or characteristics that may help to differentiate extremely similar organisms.
- 4.** Ordinarily, the identification will be quite certain. If, however, any doubt remains, the organism should be sent to a reference laboratory for confirmation of identification as discussed in the following section.

* The Color Plates are found on pp. 427–455.

Use of Reference Laboratories and Regulations for Transport

Rare or atypical fungi can be difficult to identify even for a very experienced microbiologist or medical technologist. After a possible identification of an isolated organism is reached, confirmation is often necessary.

When the identification of an isolated fungus is dubious or when the fungus appears to be one that the laboratory worker has never before encountered, a reliable reference laboratory should be asked to confirm the identification. A reference laboratory should also be utilized if identification to species level is needed and requires molecular methods that are not available in the source laboratory. Because of the toxicity and known spectrum of activity of various antifungal medications, it is especially important to confirm the identification of organisms suspected of causing mycoses. Ordinarily, the state health department acts as a reference laboratory; otherwise, a reputable commercial laboratory of proven competency should be chosen to provide the service.

Cultures sent to reference laboratories should be pure, young, and actively growing on agar slants. Petri plates should not be transported. For details on the labeling, mailing, and delivering of potentially pathogenic isolates, one should consult the reference laboratory for specific requirements and comply with the shipping codes. Personnel involved in packing and shipping infectious substances must receive training in the proper procedures, and the training must be maintained and documented.

The regulations for transport of infectious substances and biological substances are developed and issued by three major authorities: (i) the International Civil Aviation Organization (ICAO), a United Nations agency; (ii) the International Air Transport Association (IATA); and (iii) the U.S. Department of Transportation (DOT). Thanks to a concerted effort made during the past few years, their

regulations are now in substantial agreement with one another. All of the required supplies are commercially available.

Infectious substances are now classified as either Category A or Category B. The definition of each category and the instructions for packing and shipping isolates from humans follow.

A **Category A Infectious Substance** is an infectious substance in a form capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. If there is doubt as to whether or not an isolate meets the criteria for Category A, it must be handled as a Category A organism. At the time of this writing, the lone fungus expressly listed in Category A is *Coccidioides immitis* (includes *Coccidioides posadasii*), in cultured form only. The list is not all-inclusive, so other fungi may, with careful consideration, qualify as Category A.

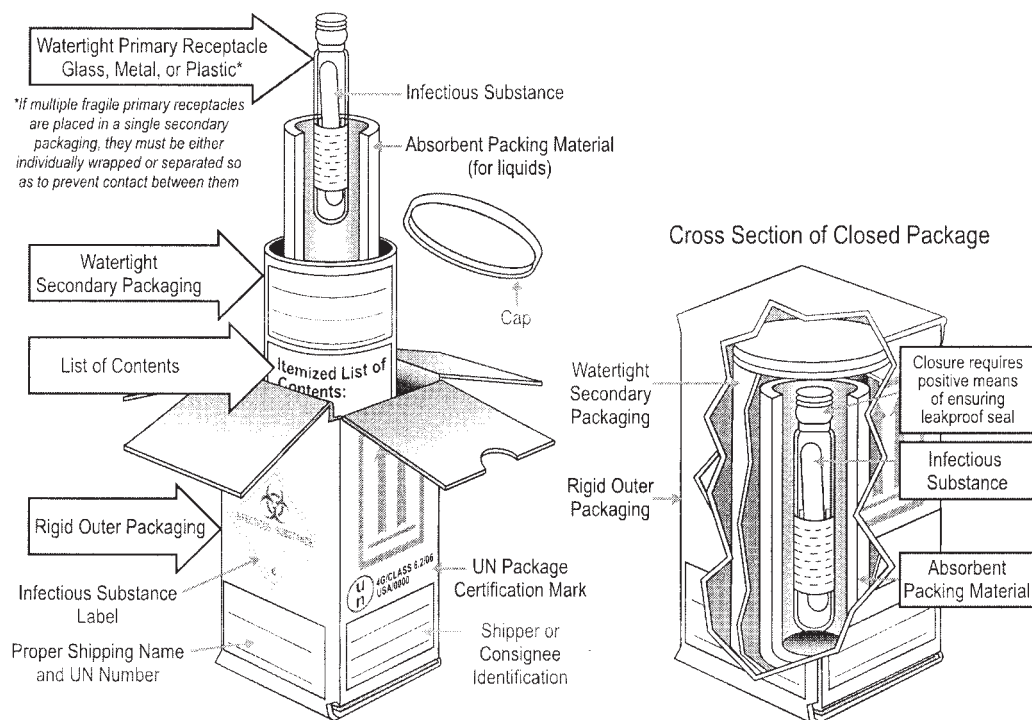
The instructions for packing Category A organisms are as follows.

1. The tube containing the slanted culture must be made of glass, metal, or plastic and should be labeled with the organism identification (in general terms if the identification is unknown). It must be securely closed and rendered leakproof by positive means (such as heat seal or metal crimp); if a screw cap is used, it must be secured with adhesive tape, paraffin sealing tape, or manufactured locking closure. The maximum allowable volume per tube is 50 ml (50 g). The culture tube is considered the “primary receptacle.”
2. Position enough packing and absorbent material at the top, bottom, and sides of the culture tube to prevent breakage and to absorb the entire volume of the culture in case of leakage. If more than one tube is being transported, each one must be wrapped individually to ensure that contact between them, and consequent breakage, is prevented.
3. Insert the wrapped culture tube(s) into durable, watertight “secondary packaging” (it must be made of material that is certified for ability to withstand an increased amount of pressure if it is to be shipped by air). Although several culture tubes may be placed in a single secondary container, the total contents cannot exceed 50 ml (50 g).
4. Attach an itemized list of the contents and quantities of the primary receptacle(s) to the outside of the secondary packaging.
5. Place the secondary package(s) in an outer shipping container that measures at least 4 × 4 inches on at least one surface and is constructed of sturdy material that meets the strict U.N. specifications for shipping Category A substances.

The outer shipping container must show (i) a “Class 6 Infectious Substance—In Case of Leakage” label; (ii) a label showing the proper shipping name, “Infectious Substance Affecting Humans,” and the UN2814 marking; (iii) the U.N. package certification mark; and (iv) shipper (or consignee) identification.

A Shipper’s Declaration must accompany shipment of a Category A substance. The necessary form has vertical red “candy stripes” along the left and right edges. Most carriers require that the form be typed (not handwritten). FedEx provides online software to generate the form (http://images.fedex.com/us/services/pdf/DiscColumnsColor_PDF.pdf). The form can also be found at <http://www.econotech.com/shipping/index.php>. Note that a color printer is required. A 24/7 emergency

response telephone number must be supplied; it must be monitored by a person (not an answering machine or message service) who has access to information concerning the hazards, and actions required, in case of human exposure to the contents of the shipment. This role can be filled by an agency or commercial company that provides this service.

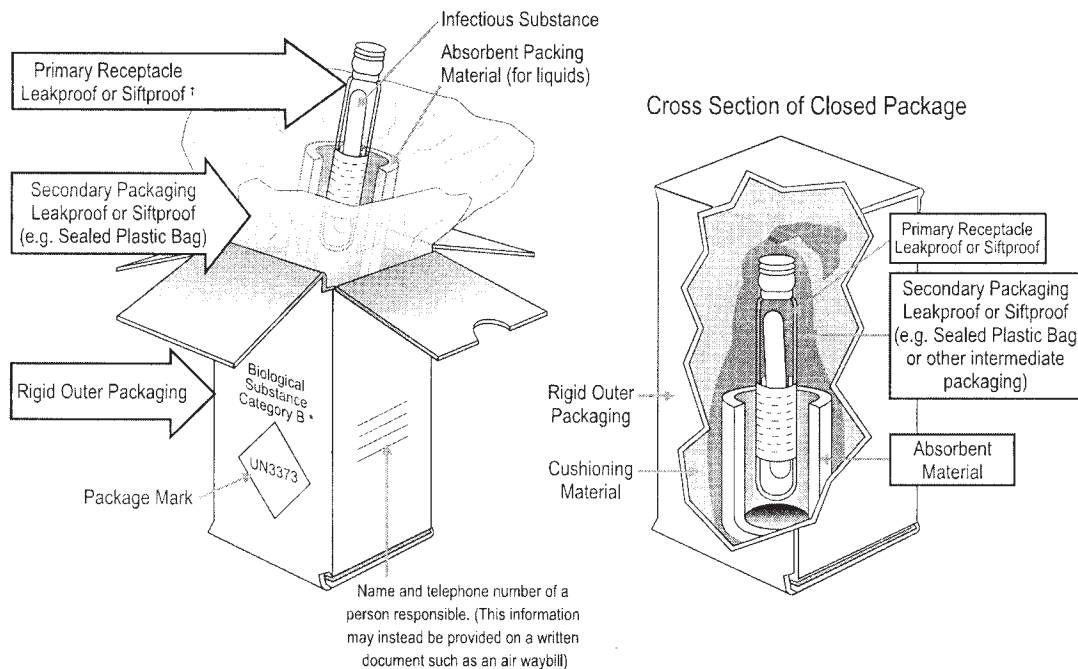


Category A packaging

A **Category B Infectious Substance** is an infectious substance that does not meet Category A criteria. The proper, complete designation for this type of organism is "Biological Substance, Category B."

The instructions for packing Category B organisms are very similar to those for Category A, but differ in the following ways.

- The primary receptacle does not require sealing by a positive means.
- The maximum allowable volume per primary receptacle is 1 liter (4 kg).
- The secondary packaging must be leakproof and sift-proof, but may be a sealed plastic bag or other intermediate packaging.
- Either the primary or secondary container must be pressure resistant for transport by air.
- There are no U.N. manufacturing specifications for the outer packaging, but it must be rigid and strong enough for its intended use and able to pass a 3.9-foot drop test.



Category B packaging

- The outer shipping container must show a label showing the proper shipping name, “Biological Substance, Category B,” and the UN3373 marking. The name and telephone number of a responsible person may be placed on the outer packaging or on a document such as an air waybill.
- The Shipper’s Declaration for Dangerous Goods is not required.
- An emergency response telephone number is not required.

For updates on the regulations, consult the Federal Motor Carrier Safety Administration site <https://www.fmcsa.dot.gov/regulations/hazardous-materials/how-comply-federal-hazardous-materials-regulations>.

Following are suppliers of packaging materials.

- Saf-T-Pak (<http://www.saftpak.com/>; the only one the author has used; can highly recommend)
- CARGOpak (<http://www.cargopak.com/>)
- EXAKT-PAK (<http://www.exaktpak.com/>)

Safety Precautions

Since many fungi produce conidia or spores that easily become airborne, precautions are essential to prevent contamination of the laboratory environment and infection of personnel.

- If at all possible, laboratories culturing filamentous fungi should ideally be in a separate room with negative air pressure; the minimum alternative is a designated separate space in the open microbiology laboratory.
- A suitable biological safety cabinet (Class II BSC) must be used when dealing with moulds. Yeast cultures can be handled on the bench in the same manner that bacterial cultures are routinely handled.
- Care must be taken not to spatter infectious material by careless flaming of wire needles or loops. Use of a benchtop microbiology incinerator is one way to avoid this hazard. Sterile disposable implements provide the best solution by also eliminating the need to flame.
- All used implements and any supernatants should be placed in a vessel containing disinfectant.
- Screw-cap tubes of slanted media are safer to handle than are petri plates. If plates are used, shrink seals should be employed. Petri plates should NEVER be used if *Coccidioides immitis* is suspected or if a culture of any fungus is to be mailed or otherwise transported to another laboratory.
- Plates and slants should be observed for growth before opening.
- A wet preparation should be made of all moulds before setting up a slide culture; DO NOT make slide cultures of isolates that may be *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Coccidioides posadasii*, *Paracoccidioides brasiliensis*, *Talaromyces marneffeii*, or *Cladophialophora bantiana*. These organisms

can usually be identified on well-made wet preps. DNA probes are commercially available for confirmation of *Histoplasma*, *Blastomyces*, and *Coccidioides*.

- All contaminated materials must be autoclaved before being discarded.
- Laboratory coats must be worn at all times in the work area.
- Gloves should be worn when manipulating a mould culture that is possibly a dermatophyte; when the task is completed, hands and wrists must be washed well (removing watches and bracelets).
- Personnel must wash their hands thoroughly with a disinfectant soap after handling any mycology cultures.
- The work area must be cleaned with disinfectant at least daily.
- Mouth pipetting must NOT be done.
- There can be no smoking, drinking, eating, gum chewing, application of cosmetics, or insertion of contact lenses in the laboratory.
- A culture must NEVER be sniffed to determine whether it has an odor; the typical *Streptomyces* sp. can be smelled from afar.

For further information, see

Centers for Disease Control and Prevention/National Institutes of Health, 2009, Biosafety in Microbiology and Biomedical Laboratories, 5th ed.

Miller, J. M. (ed.), 2011, Guidelines for Safe Work Practices in Human and Animal Diagnostic Laboratories. MMWR Supplement.

Taxonomy and Nomenclature

In 2011, a landmark development in the field of fungal taxonomy occurred: the concept of “One Fungus/One Name.” Throughout the past century, pleomorphic fungi had been defined with two different names based upon the phenotypic identification of the sexual stage (teleomorph) or the asexual form (anamorph). For example, the anamorph form *Blastomyces dermatitidis* was known as *Ajellomyces dermatitidis* in the teleomorph form. With the advent of numerous advances in molecular taxonomy, the teleomorphic and anamorphic stages of several medically important fungi were confirmed to be the same organism.

Recognizing the unique dilemma posed by the identification of pleomorphic fungi having two names, the Nomenclature Section meeting of the International Botanical Congress in Melbourne, Australia, in July 2011 recommended the discontinuation of the dual nomenclature system for fungi with anamorphic and teleomorphic forms (Hawksworth, 2011). Known as the “Amsterdam Declaration on Fungal Nomenclature,” this recommendation resulted in a historic revision of the International Code of Botanical Nomenclature. Becoming effective on January 1, 2013, the principle of “one fungus/one name” assigned priority to the oldest genus/species name independently of whether the organism was originally described as the anamorph or the teleomorph (de Hoog et al., 2013).

The field of medical mycology has witnessed a rapid expansion of knowledge in the molecular taxonomy of medically important fungi. These developments have enabled clinical microbiologists to better understand the relatedness of fungal pathogens and to more accurately communicate their identification, particularly at the genus/species level. In parallel with these advances, the International Society for Human and Animal Mycology (ISHAM) Working Group on Fungal Taxonomy issued a report advocating for nomenclatural stability among the medically important fungi, while enthusiastically

supporting continued research in fungal taxonomy (de Hoog et al., 2013; de Hoog et al., 2015). Underscoring that a given genus/species name conveys critical information on epidemiology, clinical manifestations, treatment, and prognosis for management of a patient, clear communication of a microbiological diagnosis is essential. In this book, we have addressed these concerns in three ways: (i) included the most recent name, while also parenthetically noting recent earlier names of the organism; (ii) added footnotes, where applicable, to explain the rationale for the change in genus and/or species; and (iii) retained the more established name of an organism, with further explanation in a footnote, when the field appears to be unresolved regarding proposals for a revision in nomenclature.

There have been multiple revisions in fungal nomenclature since the 5th Edition of *Medically Important Fungi* that may impact upon laboratory identification of medically important fungi (see the Index Fungorum [<http://www.indexfungorum.org/names/names.asp>] and de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4 [<http://www.clinicalfungi.org/>]). These revisions include the naming of newly identified taxa at the genus/species level (*nomen novum*) (de Hoog et al., 2013). As many of these names have not been validated for general clinical usage, we have elected not to include some of the more recently identified organisms within this text, pending further confirmatory studies of their clinical significance and nomenclature. For example, a proposal has been made by Manamgoda and colleagues to transfer *Bipolaris hawaiiensis* and *Bipolaris australiensis*, the two most frequently recovered human pathogens of the genus *Bipolaris*, into the genus *Curvularia* to create the new names of *Curvularia hawaiiensis* and *Curvularia australiensis* (Manamgoda et al., 2014). Pending further confirmation and general acceptance of this proposed revision, we retain here the use of *Bipolaris* for these medically important fungi.

Another change in nomenclature has arisen in the naming of medically important *Candida* species. Under the Amsterdam Declaration on Fungal Nomenclature, the commonly used names of several *Candida* spp., including *C. lusitaniae*, *C. krusei*, *C. kefyr*, *C. guilliermondii*, and *C. lipolytica*, would be changed to the name of the teleomorphs *Clavispora lusitaniae*, *Pichia kudriavzevii*, *Kluveromyces marxianus*, *Meyerozyma guilliermondii*, and *Yarrowia lipolytica*, respectively. For the purposes of maintaining clarity of communication among clinical microbiology laboratories and health care professionals involved in the management of fungal infections in patients, we have retained the original names of these *Candida* spp. while including many of the names of teleomorphs within parentheses.

Some newly identified organisms, such as *Aspergillus tanneri*, may be identified through morphological features (Sugui et al., 2012). However, “cryptic” species have also been identified recently. A cryptic species is morphologically identical to another species but can be distinguished by molecular methods. For example, *Aspergillus lentulus*—which phenotypically resembles *Aspergillus fumigatus*, but occurs less frequently and is more resistant to antifungal agents—is best identified by molecular methods.

On the other hand, some cryptic species may be the most commonly recovered among those causing disease. For example, a recent study of 114 children with

blastomycosis found that *Blastomyces gilchristii*, a relatively new cryptic species of genus *Blastomyces*, greatly exceeded *Blastomyces dermatitidis* (92% versus 8%) as the major cause of pediatric blastomycosis (Frost et al., 2017). While the clinical significance of these two species remains to be further studied, preliminary data suggest that *B. gilchristii* may be more prevalent in northern Wisconsin than is *B. dermatitidis*.

Another important advance in fungal taxonomy and nomenclature has emerged from a revision of the class of Zygomycetes (Kwon-Chung, 2012). The polyphyletic (containing diverse organisms without a close ancestor) class of Zygomycetes included the widely divergent orders of the Mucorales and Entomophthorales. Extensive studies now place the order Mucorales within the class Mucormycetes while placing the order Entomophthorales within the class Entomophthoromycetes (Kwon-Chung, 2012; Shaikh et al., 2016). Thus, the broader term of “zygomycosis” for the diseases caused by these organisms has been supplanted by the more specific terms “mucormycosis” and “entomophthoromycosis.”

As the fundamental purpose of this book is to provide a practical resource for clinical laboratory diagnosis, it will likely have an important role in proficiency testing of the College of American Pathologists (CAP). In reviewing the 2018 CAP list of acceptable names of medically important fungi (Master List of Fungi and Aerobic Actinomycetes), we found that the nomenclature used in this book correlated strongly with that used in the listing. Nonetheless, recognizing that names of fungi may change, we provide links to the following resources:

<http://www.mycobank.org/>
<https://mycology.adelaide.edu.au/>
<http://www.fungaltaxonomy.org/>
<http://www.clinicalfungi.org/>
<http://www.indexfungorum.org/names/names.asp>

In summary, the advances made in the fields of fungal taxonomy and nomenclature will help to further augment our understanding of the relatedness and identification of medically important fungi (Brandt and Lockhart, 2012; Warnock, 2016). As with all medical science, confirmation of the observations and validation of the epidemiological, clinical, pathogenetic, and therapeutic implications are important criteria for acceptance into the lexicon of medical mycology.

For further information, see
Brandt and Lockhart, 2012
de Hoog et al., 2013
de Hoog et al., 2015
Frost et al., 2017
Hawksworth, 2011
Kwon-Chung, 2012
Manamgoda et al., 2014
Shaikh et al., 2016
Sugui et al., 2012
Warnock, 2016

PART I

Direct Microscopic Examination of Clinical Specimens

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Introduction

Specimens submitted to the mycology laboratory from skin, tissue, or normally sterile body fluids, as well as any specimen from a patient strongly suspected of having a fungal infection, should be examined microscopically for the presence of fungal elements. This examination is important for several reasons.

- It may provide the physician with a rapid diagnosis and information regarding the possible need for treatment.
- It is helpful in determining the significance of an organism that may later be definitively identified on culture. If the cultured organism is one that is only sometimes pathogenic and it is seen on direct microscopic examination in appreciable numbers, it is more likely to be involved in a disease process and not merely present as a contaminant.
- Observation of unique fungal elements may indicate the need for special media, additional specimens from other sites, and/or serological or other tests.
- All too often, tissue sections are submitted to the anatomic pathology laboratory in fixative, rendering the specimens unsuitable for culture. In these situations, the direct microscopic examination of the tissue plays the principal role in diagnosis.
- Some organisms do not grow *in vitro*, rendering direct microscopic examination the only method of detection and identification.

It is not uncommon for microbiologists to be requested to examine tissue sections from the anatomic pathology laboratory when fungal infection is suspected or apparent. To attain an acceptable level of comfort and expertise in this responsibility, it is imperative to have at least a cursory knowledge of tissue reactions to mycoses. To familiarize the microbiologist with the histologic

responses to fungal infection, the following pages present definitions of pertinent terms, descriptions of the various tissue reactions, and a discussion and table of the stains employed.

The Guide to Interpretation of Direct Microscopic Examination is intended for use as a quick guide in reading direct smears as well as tissue sections. It is intended to allow rapid comparative viewing of the possible interpretations of the structures in the specimen and to direct the reader to the appropriate pages for more detailed descriptions of the direct microscopic appearance of each of the mycoses.

The detailed description of each mycosis includes (i) the etiologic agent(s), (ii) the likely anatomic site(s) of infection, (iii) the tissue reactions most commonly produced, (iv) the microscopic morphology of the implicated organism, and (v) a line drawing and photomicrograph of the organism on direct microscopic examination. In some cases, the direct microscopic examination yields a relatively certain identification of the organism (yet presumptive, to be confirmed by culture); in many other instances, culture is the only means of identification. **Every effort should be made to train physicians (especially surgeons) to submit specimens for culture whenever an infectious process is in the differential diagnosis.** The most beneficial specimens obviously are obtained prior to initiation of antimicrobial therapy.

As the direct microscopic examination often does not provide definitive identification of the fungal organism observed, it is of utmost importance to render a report that reflects the level of uncertainty, offers probabilities and possibilities for consideration, and thus increases the opportunity for appropriate treatment. A battery of standardized templates to attain that goal has been designed for anatomic pathology reports and can be modified for use in the clinical mycology laboratory (Sangoi et al., 2009). Such reports should include comments on the type of fungal elements, such as yeasts or hyphae, with a detailed description of their characteristics. For yeasts, comments should include an approximation of size, presence of budding, encapsulation, thickness of walls, and breadth of the base of budding. For hyphae, the description should include an estimate of width, presence of septation, and type of branching. The presence of pigment in the cell wall should also be noted. Organisms should be noted to be intracellular or extracellular. Reproductive structures, if present, should also be described.

Characteristics of various stains are summarized in [Table 1](#) (p. 28). Methods for direct microscopic examination of specimens in the mycology laboratory are outlined in [Part IV](#), pp. 366–368. Staining methods are described in [Part IV](#), pp. 385–394.

For further information and excellent photomicrographs of fungi in tissue, see
Chandler and Watts, 1987
Connor et al., 1997
Schnadig and Woods, 2009

Histological Terminology

Abscess Localized collection of suppurative inflammatory cells (polymorphonuclear neutrophils). In some instances, neutrophils and necrotic tissue are surrounded by a zone of granulation tissue. In time, the abscess may become walled off by connective tissue that limits further spread. ([Color Plate 1.](#))

Acanthosis Increase in the thickness of the epidermis.

Calcification Deposition of calcium in tissue; with H&E, calcium stains as an amorphous purple deposit. ([Color Plates 14 and 15.](#))

Caseous necrosis Histologically, eosinophilic amorphous, granular debris of fragmented, coagulated cells ([Color Plates 12 and 13](#)); on gross examination, it is white to yellowish or grayish and has a “cottage cheese” consistency.

Collagen Structural fibrous protein that provides support; collagen first appears as pink fibrils (with H&E) in early wound healing; later coalesces into dense organized bands in mature scars. ([Color Plate 7.](#))

Connective tissue Tissue that binds together and supports various structures of the body. It is made up of fibroblasts, collagen, elastin, and blood vessels.

Cyst Cavity or sac lined by epithelial cells; it usually contains liquid or semisolid material.

Dermis Broad, dense connective tissue layer of the skin located under the epidermis. ([Color Plate 19.](#))

Eosinophil A white blood cell containing a bilobulated nucleus and eosinophilic cytoplasmic granules. It is an acute inflammatory cell. ([Color Plate 4.](#))

Eosinophilic Easily stained with eosin dyes; eosinophilic structures stain deep pink with H&E stain.

Epidermis The outer, thinner layer of the skin; it consists of layers of stratified squamous epithelium. (Color Plate 19.)

Epithelioid histiocyte A cell of monocyte or macrophage origin having characteristics resembling those of an epithelial cell. A relatively large, polygonal or elongated cell with a pale nucleus, pale pink granular cytoplasm (H&E stain), and indistinct cell boundaries. The ratio of the size of the nucleus to total cell size is $\sim 1:4$. (Color Plate 5.)

Fibroblast An elongated, flattened, spindle-shaped cell with cytoplasmic projections at each end; common in connective tissue that is growing; gives rise to collagen fibers. (Color Plate 7.)

Fibrosis Mature, organized bands of collagen; scar tissue. It appears as pink, smooth collagen bundles. (Color Plates 8 and 15.)

Giant cell Multinucleated cell apparently created by fusion of macrophages. (Color Plates 9, 10, and 13.)

GMS Gomori methenamine silver (stain).

Granulation tissue Early reparative tissue composed of capillaries, fibroblasts, young collagen, and leukocytes (mostly mononuclear). As healing continues, granulation tissue becomes organized to form fibrous tissue (fibrosis). *Note:* This term should not be confused with “granuloma.” (Color Plate 6.)

Granuloma Distinct circumscribed pattern of chronic inflammation in which the predominant cell is the macrophage with epithelioid appearance, with or without the formation of giant cells. A granuloma generally has a pink center (H&E) with epithelioid cells or necrosis surrounded by a rim of mononuclear leukocytes (predominantly lymphocytes, possibly giant cells, and occasionally plasma cells); older granulomas may have an outer rim of fibroblasts, connective tissue, and fibrosis. (Color Plates 10, 11, 12, and 13.)

H&E Hematoxylin and eosin (stain).

Histiocyte A large, mononuclear phagocyte; an activated macrophage. (Color Plate 4.)

Hyperkeratosis Increase in the thickness of the stratum corneum.

Hyperplasia Abnormal increase in the number of cells in the tissue.

Infarct An area of tissue that has undergone necrosis as a result of obstruction of blood supply. (Color Plate 18.)

Lymphocyte A leukocyte that is relatively small and has scant cytoplasm, yielding a higher ratio of the size of the nucleus to total cell size ($\sim 1:1.2$) than that of a monocyte. It is characteristic of chronic inflammation. (Color Plates 2 and 4.)

Macrophage Term applied to a monocyte when it is seen in tissue; a large, mononuclear, phagocytic cell.

Monocyte White cell (leukocyte) found in the blood. A large, round to oval cell with a large, curved or horseshoe-shaped nucleus and a moderate amount of cytoplasm. The ratio of the size of the nucleus to total cell size is $\sim 1:3$.

Necrosis Spectrum of morphologic changes that follow cell death; results from progressive degenerative action of enzymes on the lethally injured cell. Histologically, there is increased eosinophilia (pink with H&E) of the cell; the cytoplasm may appear vacuolated and moth-eaten, while the nucleus shows shrinkage and fragmentation. The overall resulting image is one of amorphous cellular debris. (Color Plates 12, 13, 15, 16, and 17.)

Neutrophil White blood cell (WBC) with a multilobulated nucleus that appears as two to four small, round to ovoid nuclei connected by thin filaments of nuclear material; a polymorphonuclear leukocyte, commonly called a “poly.” It is a granulocytic WBC that is neither eosinophilic nor basophilic. This cell is characteristic of acute inflammation. (Color Plates 1 and 2.)

Parakeratosis Persistence of nuclei in the cells of the stratum corneum of the skin, which is normally devoid of nucleated cells.

Parenchyma The essential functional tissue of an organ, as distinguished from its supporting connective tissue and nutritive framework.

PAS Periodic acid-Schiff (stain).

Plasma cell A type of lymphocyte that is specialized for production of antibodies. It has an eccentric nucleus near one edge of the cytoplasm. The nucleus has a speckled chromatin pattern that has been likened to a clock face. The Golgi complex, next to the nucleus, produces a clear, pale-stained area. The ratio of the size of the nucleus to total cell size is $\sim 1:2$. (Color Plate 4.)

Pseudoepitheliomatous hyperplasia Extensive increase in the thickness of the epidermis that is benign but may resemble squamous cell carcinoma microscopically.

Splendore-Hoepli phenomenon The development of eosinophilic material around certain infectious agents in tissue, usually in the setting of chronic inflammation. (Color Plate 20.)

Stratum corneum The outermost layer of the epidermis; it consists of flat, denucleated cells that are composed of keratin. (Color Plate 19.)

Suppurative inflammation An acute reaction characterized by a large number of polymorphonuclear leukocytes and necrotic cells and a large amount of edema fluid. (Color Plates 1 and 2.)

Tissue Reactions to Fungal Infection

After damage of any kind, including infection, the body responds in a fairly predictable manner consisting of three major steps: (i) inflammatory cells (white blood cells) migrate to the damaged area in order to remove dead tissue and the etiologic agent; (ii) damaged areas are initially repaired by the production of granulation tissue, which is composed of macrophages, fibroblasts, collagen, and capillaries; and (iii) the granulation tissue is then replaced by fibrous tissue, commonly known as scar tissue.

While no tissue reactions during this process of inflammation and repair are absolutely diagnostic of a specific organism, they can be highly suggestive of the general category of fungal infection and in some instances can narrow the spectrum of possible fungal pathogens. Among the most common tissue findings consistent with invasive fungal infection are granulomas (necrotizing/nonnecrotizing/caseating) and suppurative reactions with polymorphonuclear leukocytes predominating.

Usually, the infectious agent is present where the inflammation is the most pronounced. If a tissue has many inflammatory cells or necrosis, it is more likely that the etiologic agent can be observed; if the tissue is healed with little inflammatory component remaining, the organisms are less likely to be detected. In patients with infections caused by angioinvasive moulds, such as *Aspergillus* spp., the organisms are often best located at the advancing border of the infarction or devitalized tissue.

The following are the major tissue reactions observed with fungal infections. Patients who are immunocompromised, due to immunosuppressive drugs or an immune deficiency disease, often do not produce a pronounced cellular response to fungal infections.

Acute Suppurative Inflammation and Microabscesses

Suppurative inflammation is an acute reaction and is characterized by the predominance of polymorphonuclear neutrophilic leukocytes. The suppurative area may be walled off by repair tissue, forming an abscess cavity. (Color Plate 1.)

Chronic Inflammation

Areas of acute inflammation that do not heal in a relatively short time will be replaced by a predominance of mononuclear cells (lymphocytes, plasma cells, and histiocytes). This reaction is nonspecific; it is seen in a variety of fungal infections, as well as in inflammatory reactions caused by other agents. (Color Plates 3 and 4.)

Granulomatous Inflammation (Granulomas)

The term “granulomatous” refers to a type of chronic inflammation characterized by aggregates of large mononuclear cells (macrophages or histiocytes) that often fuse to form multinucleated **giant cells**. In fungal diseases (as well as in tuberculosis), the nuclei are arranged along the inner periphery of the giant cell (known as a Langhans giant cell) (Color Plate 9); in foreign body or tumor giant cells, the nuclei are irregularly placed throughout the cytoplasm. Granulomatous reactions are characteristically observed in cases of histoplasmosis and tuberculosis.

The **tuberculoid granuloma** is commonly associated with mycobacterial infections but is also seen with some fungal diseases. It is a relatively well circumscribed accumulation of epithelioid macrophages surrounded by a border of lymphocytes. Varying numbers of giant cells and caseating necrosis may occur. (Color Plates 10 and 11.)

A **necrotic granuloma** is composed of a central area of dead tissue or necrotic debris surrounded by inflammatory cells (mainly epithelioid macrophages) that are in turn surrounded by a layer of fibrous tissue. (Color Plates 12 and 13.)

Pyogranulomatous inflammation is a combination of suppurative and granulomatous reactions. Neutrophils typical of acute suppurative inflammation are present, as well as the mononuclear cells typical of chronic granulomatous reactions.

Necrosis

Following cell death, there is a spectrum of morphologic changes due to progressive degenerative enzymatic action on the dead cells; the sum of these changes is known as necrosis. Histologically, the cytoplasm of the cell shows increased eosinophilia (pink with H&E stain) and may appear vacuolated and moth-eaten; nuclear changes include nuclear shrinkage (pyknosis), increased blueness (basophilia) with H&E stain, breaking up of the nuclear chromatin (karyolysis), and fragmentation of the shrunken nucleus (karyorrhexis). (Color Plate 16.)

Caseous necrosis is a form of necrosis that upon gross examination has a cottage cheese-like consistency. It is devoid of viable cells and microscopically appears as homogeneously staining, amorphous, finely granular material within which giant cells may be seen; caseous necrosis often accompanies granulomatous inflammation. (Color Plates 12 and 13.)

Angioinvasion, Infarction, and Necrosis

The Mucorales, *Aspergillus* spp., and several other fungi, such as *Scedosporium* spp. and *Fusarium* spp., have a tendency to invade blood vessels, blocking the lumen of the vessel, stopping the flow of blood, and causing death of the tissue deprived of blood supply. This process is known as infarction. Histologically, angioinvasion appears as infected thrombotic material blocking the lumen of arteries or veins with the surrounding tissue being hemorrhagic and necrotic; polymorphonuclear neutrophils and monocytic cells, as well as a large amount of degenerated cellular debris, are usually present. (Color Plates 17 and 18.)

Splendore-Hoepli Phenomenon

The Splendore-Hoepli reaction is indicative of a localized immunologic host response to antigens of a variety of infectious agents, including some fungi and bacteria; it is characterized by Splendore-Hoepli material coating or bordering the microorganisms. Histologically, it appears as radiating homogeneous, refractile, eosinophilic (pink with H&E), clublike material surrounding a central eosinophilic focus. (Color Plate 20.) Fungi associated with the phenomenon include *Coccidioides* spp., *Sporothrix schenckii*, and Entomophthorales, as well as those causing mycetoma and some forms of dermatophytosis. The actinomycetes and the nonfilamentous bacteria causing botryomycosis also generate the phenomenon.

Fungus Ball

A fungus ball results when filamentous fungi (most commonly, but not exclusively, *Aspergillus* spp.) colonize a previously formed cavity that has access to oxygen. There is minimal to no invasion of the tissues unless the host is immunocompromised. Hyphae and sometimes conidial heads, complete with phialides and conidia, develop. Fungal masses are seen lying freely within the cavity; the hyphae are not usually attached to the cavity wall. Surrounding tissue reaction may be minimal, or marked, potentially including granulation tissue with or without fibrosis and chronic inflammation composed of lymphocytes and other mononuclear inflammatory cells.

Stains

Although fungi may be seen in tissue with the routinely used hematoxylin and eosin (H&E) stain, there are special stains that enhance their detection. The most commonly used stains are summarized in [Table 1](#) (p. 28). The Gomori methenamine silver (GMS) stain is generally the preferred method for tissue sections and also for smeared material that requires a permanent stain; it is routinely performed in the histology laboratory. If there is debris in the tissue background that, when stained with GMS, mimics fungi (e.g., yeastlike black pigment in the lung or hyphalike elastic fibers in skin or lymph node), an alternative stain, such as periodic acid-Schiff (PAS), can be useful.

On wet preparations of specimens or on smeared and dried material, the fluorescent calcofluor white (CFW) stain ([p. 388](#)), with or without potassium hydroxide (KOH), is far superior to the traditional KOH alone. CFW is also useful in detecting sparse amounts of fungi in deparaffinized tissue sections.

Some stains are used as supplements to aid in more specific identification of an organism after detection with a preliminary fungal stain. For example, GMS readily detects yeast in tissue, but the mucicarmine stain or alcian blue can subsequently be performed on the tissue to determine whether the yeast has a polysaccharide capsule, typical of *Cryptococcus* spp. Similarly, GMS will detect the yeast cells of *Blastomyces* but it will not reliably demonstrate the doubly refractile wall, which can be better seen with a PAS or H&E stain.

The Fontana-Masson (FM) stain is used for detecting melanin, or its precursors, in cell walls. It can be helpful in the rare case of a capsule-deficient strain of *Cryptococcus neoformans* or *Cryptococcus gattii*, as the melanin in the cell wall will stain dark brown. FM is known to intensely stain dematiaceous fungi in tissue sections;

however, other fungi may have a variable but smaller amount of stain uptake (Kimura and McGinnis, 1998; Segal et al., 1998). Notably, *Candida*, *Scedosporium* species complex, and most *Fusarium* spp. are consistently negative.

See [Part IV, pp. 385–394](#), for further information on the stains most commonly used in the clinical mycology laboratory.

TABLE 1 Stains for direct microscopic observation of fungi and/or filamentous bacteria in tissue

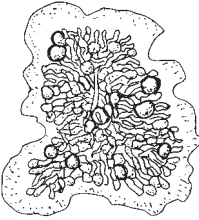
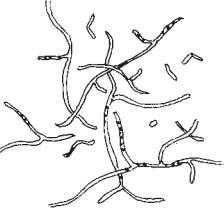
Stain (abbreviation)	Characteristics
Alcian blue	The polysaccharide capsule of <i>Cryptococcus neoformans</i> stains turquoise blue; the finer details of the capsule may be better preserved in some specimens when compared with mucicarmine.
Brown and Brenn (B&B)	Gram stain for bacteria; demonstrates the bacterial filaments of the actinomycetes, e.g., <i>Nocardia</i> , <i>Actinomadura</i> , etc.
Calcofluor white (CFW)	Stains the chitin in fungal cell walls; extremely useful for smeared preparations; fluorescence microscope required.
Fontana-Masson (FM)	Stains select fungi brown to black. It was originally thought to stain only dematiaceous fungi and <i>Cryptococcus neoformans</i> (organisms known to contain melanin or melanin precursors), but it has now been shown to stain variably, but less intensely, <i>Aspergillus fumigatus</i> , <i>Aspergillus flavus</i> , <i>Trichosporon</i> spp., <i>Fusarium chlamydosporum</i> , and some Mucorales. ^a
Giemsa	Most organisms (fungi and also bacteria) stain blue-purple. It enables visualization of <i>Histoplasma capsulatum</i> and <i>Talaromyces marneffei</i> in bone marrow and blood smears.
Gomori methenamine silver (GMS)	Fungi, including <i>Pneumocystis jirovecii</i> , are gray to black; background is green. It also stains <i>Nocardia</i> and other actinomycetes. Its drawback is that it often stains fungi too densely to observe structural details.
Hematoxylin and eosin (H&E)	Stains some fungal elements violet to bluish purple in mild to moderate contrast to the lighter background tissue. Has advantage of allowing observation of natural pigments of fungi. It is the best stain to demonstrate host tissue reaction.
Modified acid-fast	Filaments of <i>Nocardia</i> stain at least partially acid-fast (pink); <i>Actinomyces</i> and other actinomycetes are negative.
Mucicarmine	Stains the capsule of <i>Cryptococcus</i> pinkish red and may also stain the cell walls of <i>Blastomyces dermatitidis</i> and <i>Rhinosporidium seeberi</i> .
Periodic acid-Schiff (PAS)	Stains fungi pinkish red. Demonstrates the double-contoured refractile walls of <i>Blastomyces dermatitidis</i> that may not be visible with GMS stain. It is the fungal stain of choice of many dermatopathologists.

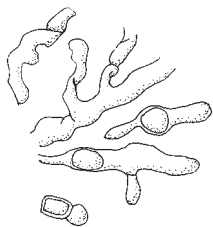
^aSource: Kimura and McGinnis, 1998.

Guide to Interpretation of Direct Microscopic Examination

Abbreviations: GF, Gridley fungus stain; GMS, Gomori methenamine silver stain; H&E, hematoxylin and eosin stain; PAS, periodic acid-Schiff stain.

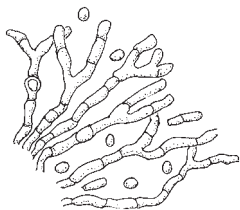
For further information and excellent photomicrographs, see Chandler and Watts (1987) and Connor et al. (1997).

Observation	Brief description	Interpretation
	• "Sulfur granules" (30–3,000 μm or more in diameter) • Delicate (<1-μm diameter), branched filaments • Gram positive and non-acid-fast • Stain with GMS and Giemsa stains but not with H&E, GF, and PAS	ACTINOMYCOSIS (p. 39)
	• Granule (white, yellow, or red) • Narrow (0.5–1 μm in diameter), intertwined filaments	ACTINOMYCOTIC MYCETOMA (p. 40)
	• Granules (white, yellow, brown, or black) • Hyphae (2–6 μm in diameter) • Often numerous chlamydospores and swollen cells, especially at the periphery of the granule	EUMYCOTIC MYCETOMA (e.g., <i>Madurella mycetomatis</i> , <i>Madurella grisea</i> , <i>Cladophialophora bantiana</i> , <i>Scedosporium</i> spp., <i>Acremonium strictum</i> , <i>Phaeoacremonium kraidenii</i> , <i>Actinomadura madurae</i> , <i>Fusarium falciforme</i> , and <i>Aspergillus nidulans</i>) (p. 40)
	• Delicate, narrow (0.5–1.0 μm in diameter) filaments • Tendency to branch at right angles • Frequently appear beaded or granular • Partially acid-fast	NOCARDIOSIS (p. 42)



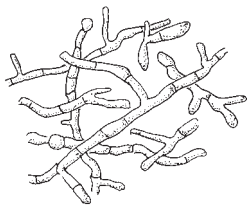
- Hyphae broad (3–25 μm in diameter; average, 12 μm)
- Pauciseptate (coenocytic) with nonparallel sides
- Branching is nondichotomous, irregular, sometimes at right angles

MUCORMYCOSIS (p. 43)



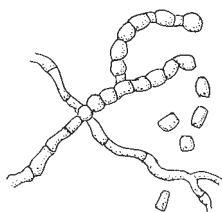
- Septate hyphae (3–12 μm in diameter)
- Dichotomous branching at acute angles
- Tendency to grow in radial pattern
- Hyphae nearly parallel to one another

ASPERGILLOSIS (p. 44)



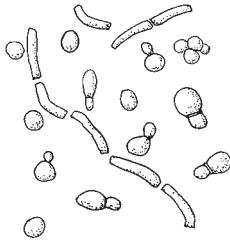
- Septate hyphae (2–8 μm in diameter)
- Hyphae irregular and haphazardly arranged
- Branching at 45 and 90° angles
- Phialides and conidia may form in closed lesions

HYALOHYPHOMYCOSES (e.g., *Paecilomyces* spp., *Purpureocillium lilacinum*, and *Fusarium* spp.) (p. 46)



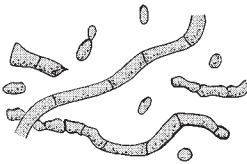
- Colorless, branched, septate hyphae
- Hyphae often break up into chains of arthroconidia
- Other conidia do not form in tissue

DERMATOPHYTOSIS (tinea, ringworm) (p. 48)



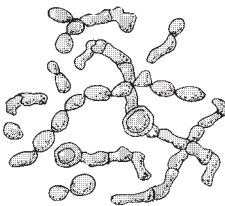
- Septate hyphal elements (2.5–4.0 μm in diameter)
- Hyphal elements short, slightly curved, form short chains
- Oval to round, thick-walled, yeastlike cells (3–8 μm in diameter), often in clusters
- Produce buds through small, phialidic collarettes
- "Spaghetti and meatball" appearance (clusters of yeastlike cells)

TINEA VERSICOLOR
(e.g., *Malassezia* spp.) (p. 49)



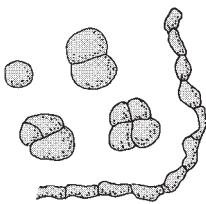
- Darkly pigmented, septate hyphae (1.5–3.0 μm in diameter)
- Sometimes elongated budding cells (1.5–5.0 μm in diameter)
- Occasionally chlamydoconidia

TINEA NIGRA
(*Hortaea werneckii*
(*Phaeoanellomyces werneckii*)) (p. 50)



- Brown-pigmented, septate hyphae (2–6 μm in diameter)
- Dark, budding, yeastlike forms may also occur

PHAEOHYPHOMYCOSIS
(e.g., *Bipolaris* spp., *Exophiala* spp., and *Cladophialophora bantiana*) (p. 51)



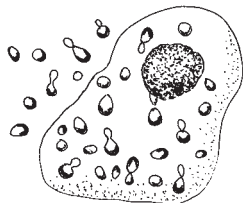
- Sclerotic bodies (5–12 μm in diameter):
Brown pigmented
Thick walled
Have horizontal and vertical septations
- Occasionally brown, septate hyphae (3–8 μm in diameter)

CHROMOBLASTOMYCOSIS
(e.g., *Fonsecaea pedrosoi*, *Phialophora verrucosa*, *Cladosporium carrionii*, and *Fonsecaea compacta*) (p. 52)



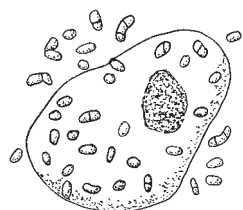
- Yeastlike cells:
Various sizes and shapes
Round to oval (2–6 μm in diameter); bud on narrow base
Some characteristically elongated "cigar bodies" (2 $\times$ 3 to 3 $\times$ 10 μm)

SPOROTRICHOSIS (p. 53)



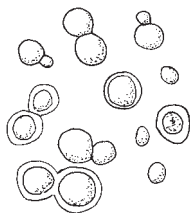
- Yeastlike cells:
Small (2–4 μm in diameter), usually ovoid
Budding on a narrow base at the smaller end
Characteristically within macrophages or monocytes
Commonly remain clustered when extracellular

HISTOPLASMOSIS (*Histoplasma capsulatum*) (p. 54)



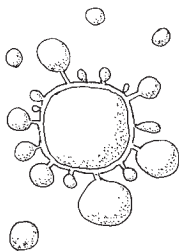
- Yeastlike cells:
Round or oval (~3 μm in diameter)
Characteristically within macrophages or monocytes
May elongate somewhat when extracellular
Have central septum; do not bud

TALAROMYCOSIS (formerly penicilliosis) (*Talaromyces marneffe*) (formerly *Penicillium marneffe*) (p. 56)



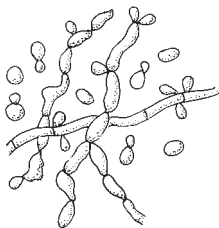
- Yeastlike cells:
Round to oval (3–30 μm in diameter; usually 8–15 μm)
Thick walled
Budding on a broad base

BLASTOMYCOSIS (p. 58)



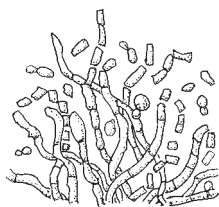
- Yeastlike cells:
Round to oval, large
(3–30 μm in diameter)
Multiple budding
Buds are attached to the
parent cell by narrow
connections

PARACOCCIDIOIDOMYCOSIS
(p. 59)



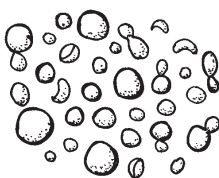
- Round to oval, budding yeast
cells (3–6 μm in diameter)
- Branching, septate hyphae
and pseudohyphae
- Chains of budding cells

CANDIDIASIS (CANDIDOSIS)
(p. 61)



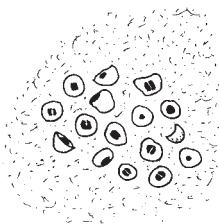
- Round to oval, budding yeast
cells (3–6 μm in diameter)
- Branching, septate hyphae
and pseudohyphae
- Arthroconidia

TRICHOSPORONOSIS (p. 62)



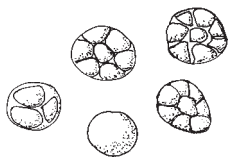
- Yeastlike cells:
Mostly round; some oval
(2–20 μm in diameter)
Encapsulated
Thin, dark walls
Budding on a narrow base

CRYPTOCOCCOSIS (p. 63)



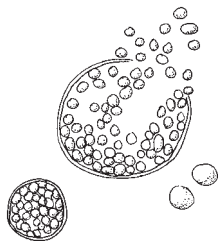
- Round, ovoid, or collapsed
crescent cyst forms (3–5 μm in
diameter) on silver stain
- Nonbudding
- Appear in small clusters on a
thick, foamy background
- Comma- or parenthesis-shaped
focal thickenings in cell wall

PNEUMOCYSTOSIS (p. 65)



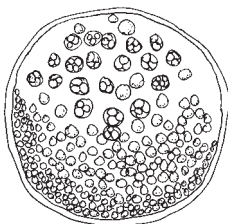
- Round or oval sporangia (range, 2–25 μm in diameter)
- Round or polyhedral endospores within sporangia
- No budding

PROTOTHECOSIS (p. 66)



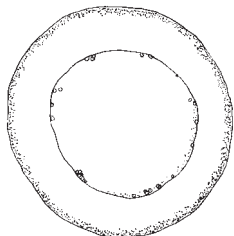
- Spherules (10–100 μm or more in diameter)
- Mature spherule relatively thin walled (1–2 μm thick)
- Round endospores (2–5 μm in diameter) in mature spherules
- No budding

COCCIDIOIDOMYCOSIS (p. 67)



- Large, round sporangia (100–350 μm in diameter when mature)
- Sporangia thick walled (~5 μm)
- Endospores vary in size (1–10 μm in diameter)
- Endospores are arranged in zonal pattern in mature sporangia
- Inner surface of sporangial wall and walls of endospores stain with mucicarmine
- No budding

RHINOSPORIDIOSIS (p. 68)



- Large, round adiaconidia (200–400 μm in diameter)
- Adiaconidia very thick walled (20–70 μm)
- Interior of adiaconidium usually appears empty
- No budding

ADIASPIROMYCOSIS (*Emmonsia parva*) (p. 70)

Detailed Descriptions

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Actinomycosis

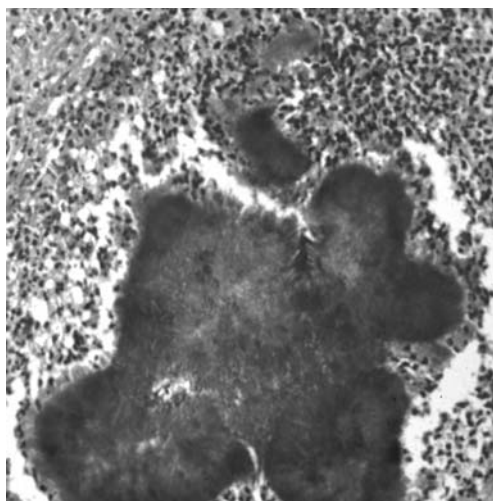
ETIOLOGIC AGENTS: *Actinomyces israelii* (in humans), *Actinomyces bovis* (in animals), and other *Actinomyces* spp.; occasionally *Arachnia*, *Rothia*, and *Eubacterium* spp.

SITES OF INFECTION: Neck and face area, lung, thoracic cavity, abdomen, pelvis, multiple systemic sites.

TISSUE REACTION: Typically suppuration with abscesses containing granules composed of the bacterial filaments. The innermost portion of the wall of the abscess sometimes contains foamy macrophages. Palisading epithelioid macrophages and giant cells often surround the abscess and may be encapsulated by fibrosing granulation tissue. Splendore-Hoeppli material may radiate around the abscesses. Sinus tracts connecting the abscesses or opening to the body surface are common.

MORPHOLOGY OF ORGANISM: Granules from an abscess or draining sinus tract are 30–3,000 μm or more in diameter and are commonly called “sulfur granules” (due to their yellow color, which resembles elemental sulfur, but they do not contain sulfur). When crushed, the granules appear microscopically as opaque masses with peripheral, gelatinous, club-shaped bodies. The granules are composed of numerous delicate ($<1\ \mu\text{m}$ in diameter) bacterial filaments that branch (often at right angles) and may exhibit beading (but not as commonly as do *Nocardia* spp.). In some instances, only small groups of branching filaments form instead of the characteristic granules. (Color Plate 21.) The organisms are Gram positive and non-acid-fast; they stain well with GMS and Giemsa stains, but the individual filaments may not stain as well with H&E and PAS. Other anaerobic bacteria may also be present.

Branching filaments of *Nocardia* spp. are morphologically similar, but most (although not all) *Nocardia* are partially acid-fast. Culture is required for definitive identification of the etiologic agent.



Courtesy of Joan Barenfanger.

Mycetoma (Actinomycotic or Eumycotic)

ETIOLOGIC AGENTS:

Actinomycotic: *Nocardia* spp., *Actinomadura* spp., and *Streptomyces* spp.

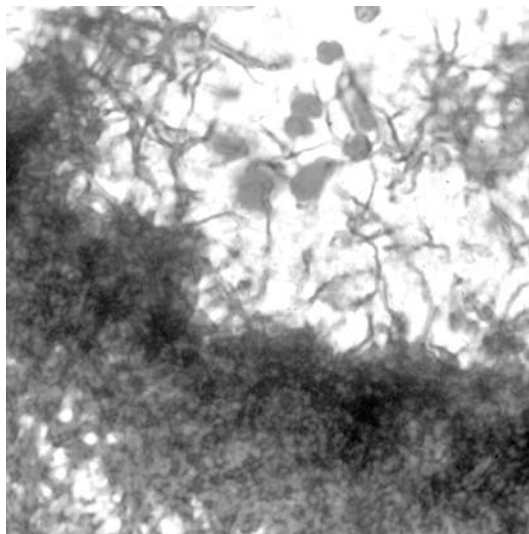
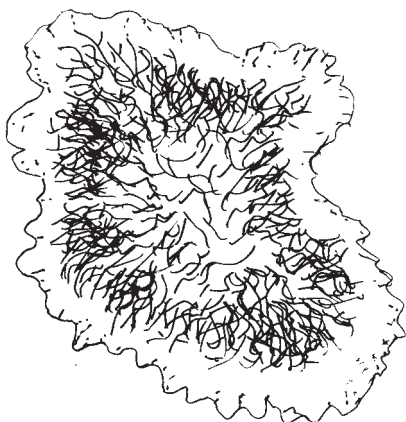
Eumycotic: *Pseudallescheria* spp., *Scedosporium* spp., *Rhinocladiella* spp., *Madurella* spp., *Exophiala jeanselmei* complex, *Acremonium* spp., *Fusarium* spp., *Curvularia* spp., and occasionally other moulds.

SITES OF INFECTION: Subcutaneous tissue and skin; long-standing infections may involve muscle, fascia, and bone. The infection is most commonly on lower leg or foot, rarely disseminated.

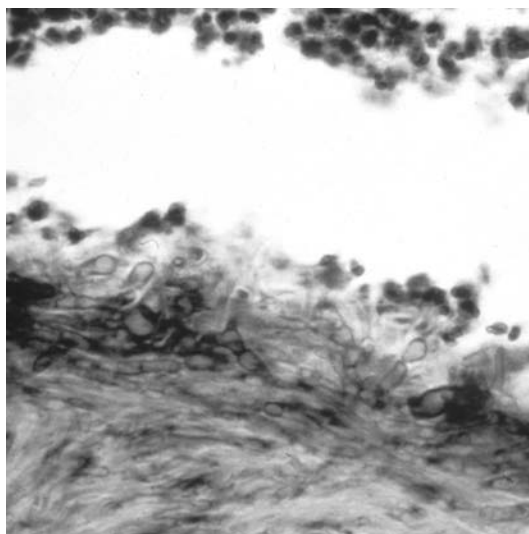
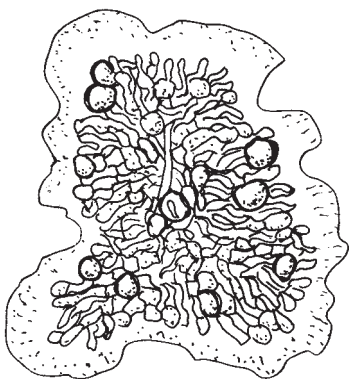
TISSUE REACTION: Similar reactions are seen with all mycetomas, i.e., both actinomycotic and eumycotic. Multiple draining sinus tracts with neutrophilic abscesses containing granules (composed of the etiologic agent) and necrotic debris are characteristic. The abscesses and sinus tracts are surrounded by chronic inflammation consisting of palisading epithelioid macrophages, multinucleated giant cells, lymphocytes, and plasma cells. Between the abscesses is granulation tissue that is usually vascular and contains many inflammatory cells. Splendore-Hoeppli material very often surrounds the granules. Fibrosis may occur in long-standing infections.

MORPHOLOGY OF ORGANISM: The etiologic agent typically organizes into aggregates in infected tissue to form granules ranging in size from 25 μm to 5 mm.

- Actinomycotic Mycetoma: Granules (white, yellow, or red) are composed of narrow (1 μm or less in diameter), intertwined filaments that are radially oriented and most numerous at the edge of the granule. *Nocardia* spp. are usually at least partially acid-fast, while *Actinomadura* and *Streptomyces* spp. are not acid-fast. All are Gram positive and stain well with GMS and Giemsa, but individual filaments may not stain as reliably with H&E or PAS.
- Eumycotic Mycetoma: Granules (white, yellow, brown, or black) contain septate, variously shaped, somewhat distorted hyphae (2–6 μm in diameter) that are often accompanied by numerous chlamydoconidia and swollen cells; the fungal forms are most commonly visible at the periphery of the granule. (Color Plate 22.)



Edge of actinomycotic granule.



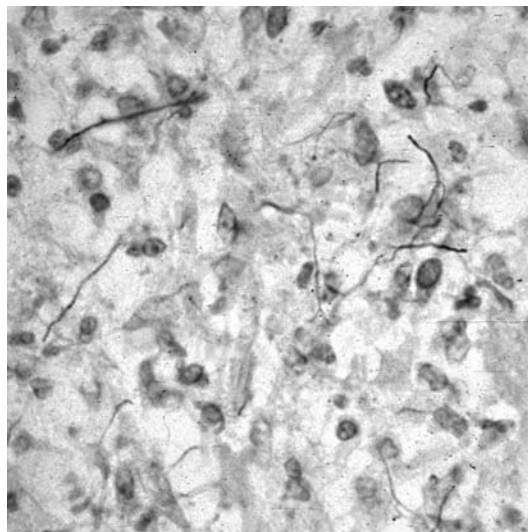
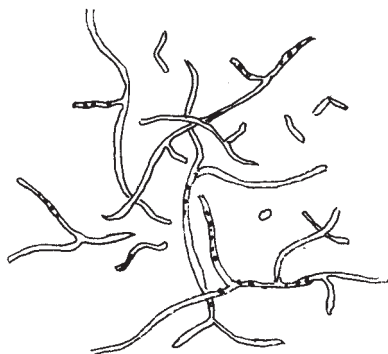
Edge of eumycotic granule.

ETIOLOGIC AGENTS: *Nocardia farcinica*, *Nocardia cyriacigeorgica*, *Nocardia nova*, *Nocardia brasiliensis*, *Nocardia abscessus*, *Nocardia otitidiscaviarum*, and occasionally other *Nocardia* spp.

SITES OF INFECTION: Lung, central nervous system, skin and subcutaneous tissue, multiple systemic sites.

TISSUE REACTION: Generally, the reaction is suppurative, sometimes necrotizing. Poorly defined, variably encapsulated abscesses may form. Occasionally, granulomatous tissue is seen in chronic infections. The Splendore-Hoepli phenomenon is rare in *Nocardia* infections.

MORPHOLOGY OF ORGANISM: Delicate, narrow (0.5–1.0 μm in diameter) filaments that tend to branch at right angles; coccobacillary elements may also form. Organisms characteristically appear beaded and may be numerous and highly branched. They are partially acid-fast when stained with the modified Kinyoun stain (p. 387) or other acid-fast stain using a weak acid solution for decolorization. (Color Plate 23.) The organisms are reliably Gram positive and stain well with GMS, especially when the staining time in the silver nitrate solution is increased; they do not reliably stain with H&E or PAS.



Courtesy of Ron C. Neafie.

Mucormycosis (Zygomycosis)

ETIOLOGIC AGENTS: *Rhizopus* spp., *Mucor* spp., *Rhizomucor* sp., *Lichtheimia corymbifera*, *Apophysomyces elegans*, *Cunninghamella bertholletiae*, and *Saksenaia vasiformis*.

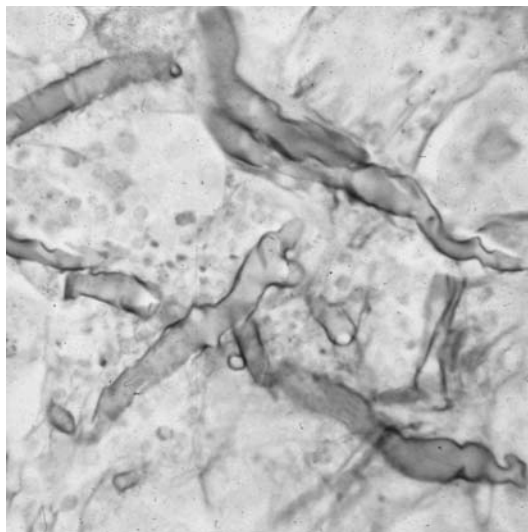
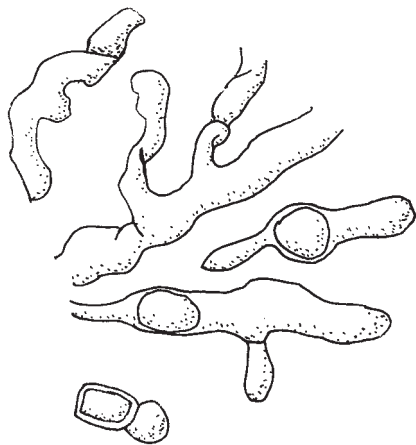
SITES OF INFECTION: Lung, nasal sinus, brain, eye, skin, multiple systemic sites.

TISSUE REACTION: Suppurative necrosis and occasionally granulomatous reactions occur. Members of the order Mucorales (p. 185) have a predilection for invasion of blood vessels, causing thrombosis and infarction. These agents must be differentiated from *Aspergillus* spp. and other hyphomycetes that also commonly invade blood vessels.

MORPHOLOGY OF ORGANISM: The key features are (i) broad hyphae (5–25 μm in diameter; average, 12 μm) with nonparallel walls; (ii) hyphae pauciseptate—the thin walls and lack of regular septation decreases the internal support of these broad hyphae and allows them to become characteristically twisted, collapsed, and folded in a ribbonlike fashion; (iii) branching that is irregularly spaced, nondichotomous, and at various angles (often at right angles) to the parent hypha. (Color Plate 24.)

The special fungus stains (GMS or PAS) generally do not color the Mucorales as deeply as they do other fungi. Moreover, some strains may be GMS negative and more evident on H&E or PAS stains.

Note: The microbiology laboratory should be notified as soon as possible if a mucormycete is suspected or seen in tissue, as special measures may be taken to improve its recovery on culture.



Courtesy of Morris Gordon.

ETIOLOGIC AGENTS: *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and other *Aspergillus* spp.

SITES OF INFECTION: Lung, paranasal sinus, ear, eye, skin, multiple systemic sites.

TISSUE REACTION: Invasive aspergillosis is characterized by acute inflammation (neutrophils) and necrotic debris. In severely neutropenic patients, the acute inflammatory response is greatly reduced. Occasionally, a granulomatous response occurs. *Aspergillus* spp. have a predilection for invading blood vessels (angioinvasion; see p. 25), causing blockage of blood vessels (thrombosis), tissue death and necrosis (infarction), and presence of red blood cells outside of the blood vessels (hemorrhage). Some other hyaline fungi, such as *Fusarium* spp. and *Scedosporium* spp., may cause similar tissue reactions.

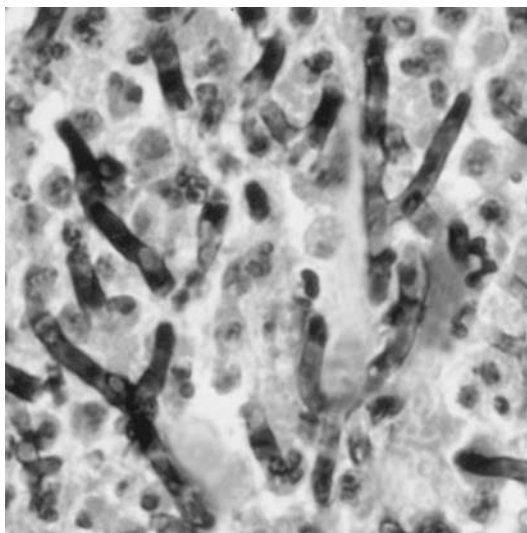
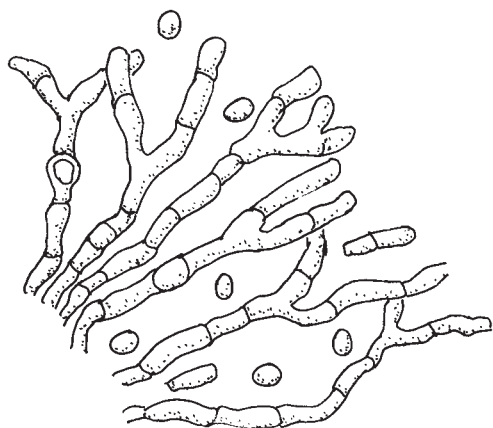
Allergic or hypersensitivity aspergillosis may occur in several forms, but most will result in tissue reactions that involve hypersecretion of mucus with neutrophils and eosinophils. Charcot-Leyden crystals are sometimes seen; they are formed from breakdown products of eosinophils and are intensely pink, long, narrow, widening at the center, and tapered to a point at each end. Granulomatous inflammation develops in some instances. Mucus plugs are sometimes expectorated and have concentric layers of eosinophils, epithelial cells, necrotic cellular debris, and possibly Charcot-Leyden crystals.

When fungus balls (aspergillomas; see p. 25) form, the wall of the cavity is often eroded and composed of inflammatory granulation tissue; calcium oxalate crystals may be seen in the ball and wall of the cavity (especially if composed of *A. niger*).

MORPHOLOGY OF ORGANISM: The key features include (i) septate hyphae, mostly 3–6 μm in diameter; (ii) dichotomous branching (i.e., each branch is approximately equal in width to the originating parent hypha) at 45° angles; and (iii) tendency to grow from hematogenous lesions in tissues in a radial fashion, like the spokes of a wheel, with the hyphae appearing nearly parallel to one another. (Color Plates 25, 26, and 27.) The parallel arrangement is absent if the tissue is not intact or if the specimen is of a more liquid nature. *Aspergillus terreus* is unique in having the ability to produce conidia (aleurioconidia) along the hyphae *in vivo* during invasive infection. In chronic lesions, short, distorted hyphae may be as wide as 12 μm . Conidial heads (vesicle, phialides, and conidia) may occasionally form in areas exposed to air, e.g., in pulmonary cavities, nasal-sinus aspergillomas, and ear or skin infections. *A. niger* is often involved in this type of infection and can be suspected if calcium oxalate crystals are present, as *A. niger* is known to produce large amounts of oxalic acid in this setting.

Aspergillosis (continued)

Many other fungi appear similar to the aspergilli in tissue or other clinical specimens; however, there are subtle differences. Nonetheless, definitive microbiological diagnosis is made by recovery of the organism in culture followed by phenotypic or genotypic methods. Mucorales have hyphae that are almost nonseptate, are commonly broader (up to 25 μm in diameter), show random branching, often appear collapsed and twisted, are irregular and nonparallel, and usually stain lighter with GMS than do the aspergilli. *Candida* spp., in addition to hyphae, form pseudohyphae (typically showing distinct constrictions at the septa) and budding yeast cells; however, when the hyphae of *Aspergillus* are cut on cross section, they may be mistaken for nonbudding yeast cells. Hyphae of other opportunistic hyaline moulds may be indistinguishable from *Aspergillus* in tissue (see “Miscellaneous Hyalohyphomycoses,” p. 46).



Miscellaneous Hyalohyphomycoses (Other than Aspergillosis)

ETIOLOGIC AGENTS: Any mould that forms colorless, septate hyphae in host tissue, e.g., *Fusarium*, *Paecilomyces*, *Acremonium*, and *Scedosporium* (and *Pseudallescheria*).

SITES OF INFECTION: Almost any body site; much more likely to be recovered in blood culture than is *Aspergillus*.

TISSUE REACTION: The most common tissue responses are acute inflammation and necrosis with occasional granulomatous inflammation. Invasion of blood vessels with subsequent thrombosis and infarction also occurs.

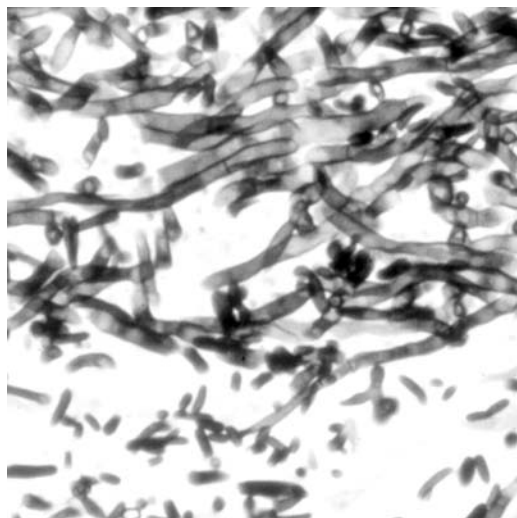
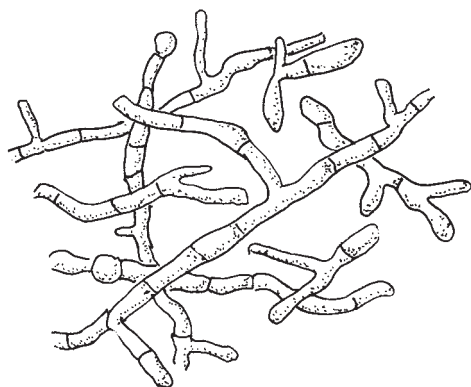
MORPHOLOGY OF ORGANISM: The hyphae are usually irregular and haphazardly arranged, with considerable variation in diameter (2–8 μm); they typically exhibit both 45 and 90° branching. Although these traits suggest an etiologic agent other than *Aspergillus* (p. 293), one cannot reliably differentiate any of these agents from *Aspergillus* in tissue unless there is production of conidia; e.g., one may occasionally observe the terminal annelloconidia of *Scedosporium* spp. The hyphomycetes differ from the Mucorales in forming hyphae that are narrower, are regularly septate, and are less prone to ribbonlike folding. These miscellaneous hyphomycetes differ from both the aspergilli and the mucormycetes in having the capacity to produce conidia and phialides within a non-aerated deep tissue lesion (*Aspergillus terreus* is the exception to this rule, as it can produce conidia along the sides of the hyphae). Phialides usually appear as tapering structures (some are slightly flask shaped or bottle necked) along the sides or at the ends of the hyphae; a broad, darkly staining band can be seen on the sides of the neck of the phialide after production of multiple conidia. The conidia commonly have a rounded apex and a flat basal scar when detached; those of *Pseudallescheria/Scedosporium* may be brown. Occasionally, hyphae that are somewhat constricted at the septa, along with the presence of phialoconidia, can resemble the pseudohyphae and budding yeasts seen in candidiasis (Liu et al., 1998). Culture or molecular diagnosis is almost always required for definitive identification of the etiologic agent (Schuetz and Walsh, 2015).

For further information, see

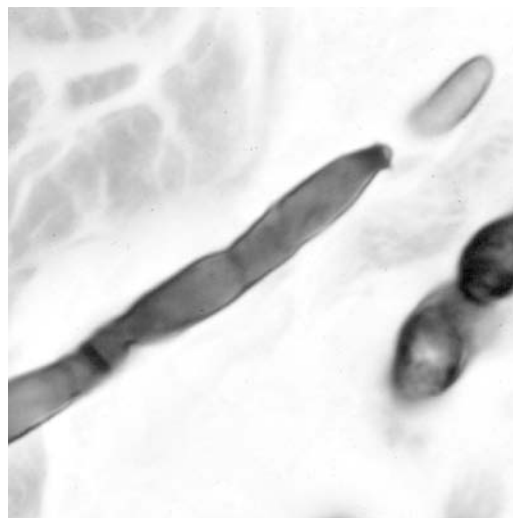
Liu et al., 1998

Schuetz and Walsh, 2015

Miscellaneous Hyalohyphomycoses (Other than Aspergillosis) (continued)



Hyphal elements; courtesy of Wiley Schell.



Conidia formation *in vivo*; courtesy of Wiley Schell.

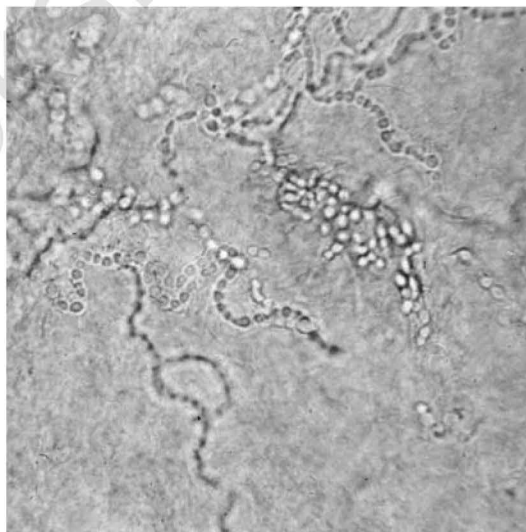
Dermatophytosis (Tinea, Ringworm)

ETIOLOGIC AGENTS: *Microsporum* spp., *Trichophyton* spp., and *Epidermophyton floccosum*.

SITES OF INFECTION: Skin, hair, nails.

TISSUE REACTION: Skin specimens usually show hyperkeratosis and various degrees of acanthosis, parakeratosis, and increased space between the cells (spongiosis). When present, the inflammatory response is usually mild and predominantly mononuclear. If the deep nonkeratinized layers of the skin are involved, a suppurative or granulomatous reaction may occur, e.g., Majocchi's granuloma. Splendore-Hoeppli material may also be present. The etiologic agent is most commonly found in the stratum corneum (the outermost layer of the epidermis, consisting of keratinized, nonnucleated cells).

MORPHOLOGY OF ORGANISM: All of the dermatophytes appear in the host as colorless, branched, septate hyphae that characteristically break up into chains of arthroconidia. Other conidia do not form in tissue. When hair is involved, the pattern of infection depends on the species of dermatophyte; i.e., arthroconidia may form on the outside of the hair shaft (ectothrix type of invasion) or inside the hair shaft (endothrix type). There is also a favic pattern of invasion in which hyphae, arthroconidia, and empty spaces form within the hair shaft.



Courtesy of Tomas Roges.

Tinea versicolor

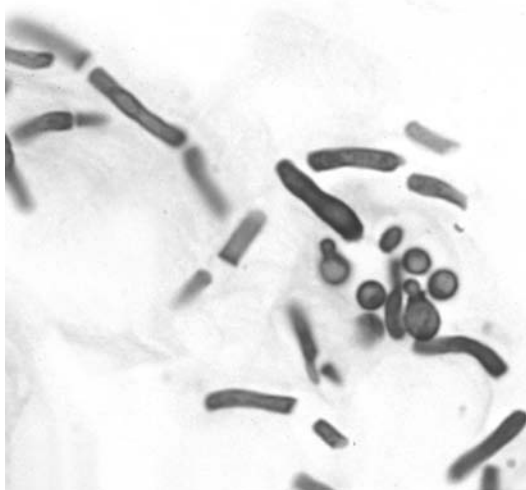
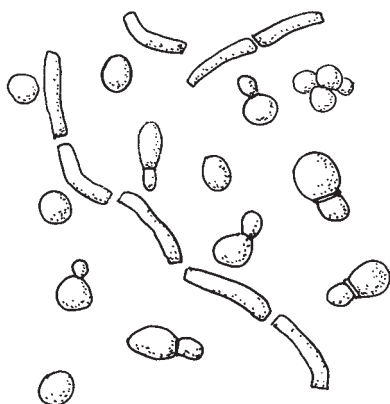
ETIOLOGIC AGENTS: *Malassezia furfur* complex (the complex contains ~12 species of *Malassezia*, but not *M. pachydermatis*).

SITES OF INFECTION: The stratum corneum (the outermost layer of the skin); the organisms can therefore be readily seen in skin scrapings.

Note: The etiologic agent can also cause fungemia and occasionally systemic infection (most frequently involving the lung) in patients receiving prolonged infusion of lipid formulation through contaminated central venous catheters (see p. 158).

TISSUE REACTION: Mild to moderate hyperkeratosis and acanthosis are usually seen. There may be a minimal mononuclear response in the dermis. The causative organism is usually seen in the stratum corneum.

MORPHOLOGY OF ORGANISM: Short, slightly curved, septate hyphal elements (2.5–4.0 μm in diameter) typically form in short chains. These are accompanied by clusters of oval to round, thick-walled cells (2.5–8 μm in diameter) that appear yeastlike but are actually phialidic. They are characteristically round at one end and have a flat collarette at the opposite end through which “buds” are produced. (Color Plate 28.) The organisms are routinely observed in skin scrapings with a KOH preparation and/or with calcofluor white; in biopsy specimens, they can be seen with H&E but are best demonstrated by special stains for fungus, preferably PAS to better see the defining lines of the organism. The etiologic agents, members of the *M. furfur* complex, will grow only on media that have been overlaid or supplemented with olive oil or another long-chain fatty acid.



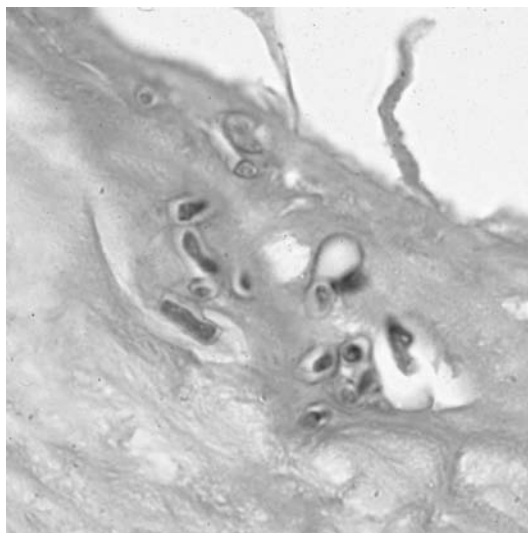
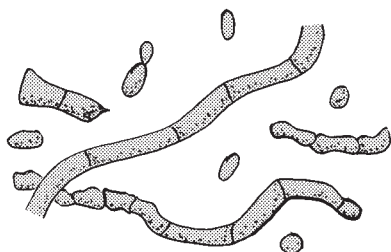
Tinea nigra

ETIOLOGIC AGENT: *Hortaea werneckii* (*Phaeoannellomyces werneckii*).

SITES OF INFECTION: Palms of hands, soles of feet; rarely on other skin surfaces. Only the stratum corneum (outermost layer of the skin) is involved.

TISSUE REACTION: Mild to moderate hyperplasia and/or hyperkeratosis is seen in the epidermis. A minimal mononuclear cell infiltrate may occur in the dermis. The fungi are easily detected in the stratum corneum.

MORPHOLOGY OF ORGANISM: Darkly pigmented, branched, septate, narrow hyphae (1.5–3.0 μm wide) usually accompanied by elongated budding cells (1.5–5.0 μm in diameter). The organism stains well with the special fungus stains, but the natural pigmentation can be best seen with H&E.

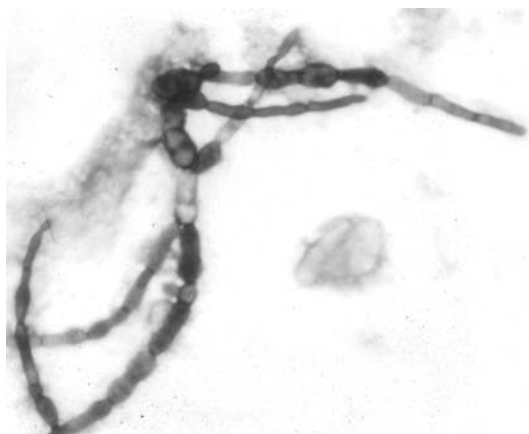
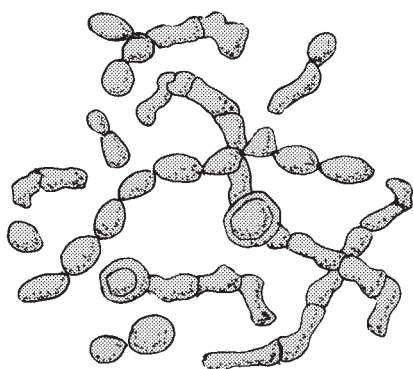


ETIOLOGIC AGENTS: *Exophiala jeanselmei* complex, *Phialophora verrucosa*, *Phaeoacremonium parasiticum*, *Pleurostomophora richardsiae*, *Exophiala dermatitidis*, *Bipolaris* spp., *Alternaria* spp., *Cladophialophora bantiana* (in the brain), and a large variety of other dematiaceous fungi.

SITES OF INFECTION: Most commonly in skin and subcutaneous tissue; also occurs in the brain and occasionally in a variety of other body sites. Severe disseminated infection is also reported.

TISSUE REACTION: The more common etiologic agents elicit encapsulated cystic granulomas in the dermis and subcutaneous tissue in immunocompetent hosts. However, the tissue reaction in immunocompromised patients may be devoid of granuloma formation and be evident as a mixed acute and chronic inflammatory response with features overlapping those of other invasive mould infections. The center of the granuloma may contain a suppurative exudate surrounded by a wide zone of granulation tissue. Multiple abscesses may occur in early infection. The less common etiologic agents may produce a dispersed granulomatous inflammation. In cerebral infections, the characteristic inflammatory response is suppurative and granulomatous with abscess formation.

MORPHOLOGY OF ORGANISM: Variably brown- or olivaceous-pigmented hyphae (2–6 μm wide) occur singly or in small aggregates. They often have closely spaced, constricted septations producing a moniliform (“string of beads”) appearance. Large, bizarre, thick-walled vesicular swellings ($\geq 25 \mu\text{m}$ in diameter) resembling chlamydoconidia may be seen along, or at the ends of, the hyphae. Yeastlike cells producing buds singly or in chains are also commonly present, depending on the species. The pigment is usually visible in unstained or H&E-stained specimens, but may be difficult to observe in individual strains. Although the Fontana-Masson stain can be used to further demonstrate melanin in the cell wall, it is not necessarily diagnostic for dematiaceous moulds. (Color Plate 29.)



Courtesy of Joan Barenfanger.

Chromoblastomycosis

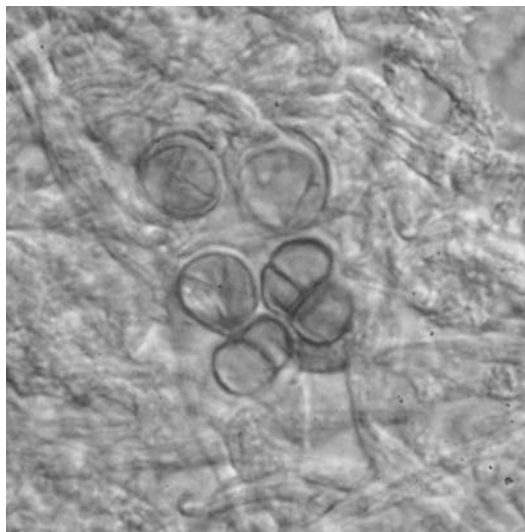
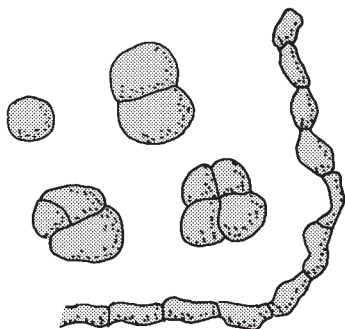
ETIOLOGIC AGENTS: *Fonsecaea pedrosoi*, *Fonsecaea compacta*, *Phialophora verrucosa*, *Cladophialophora carrionii*, *Exophiala jeanselmei* complex, and *Rhinocladiella aquaspersa*.

SITES OF INFECTION: Skin and subcutaneous tissue.

TISSUE REACTION: In skin, a characteristic pseudoepitheliomatous hyperplasia with hyperkeratosis and parakeratosis occurs. Inflammation is generally granulomatous; it is often accompanied by a suppurative reaction, possibly from a secondary infection, causing satellite microabscesses.

MORPHOLOGY OF ORGANISM: Brown-pigmented, round to polyhedral, thick-walled sclerotic bodies (5–12 μm in diameter) having horizontal and/or vertical septations are diagnostic. These structures are also referred to as Medlar bodies or “copper pennies.” (Color Plate 30.) Brown, branched, distorted, septate hyphae (3–5 μm in diameter) may also be present. The fungi typically migrate to the surface of the skin and are seen as black dots in the keratin scales; sclerotic bodies and sometimes short hyphae are seen microscopically.

The structure and pigment of the organism are usually well demonstrated with H&E stain.



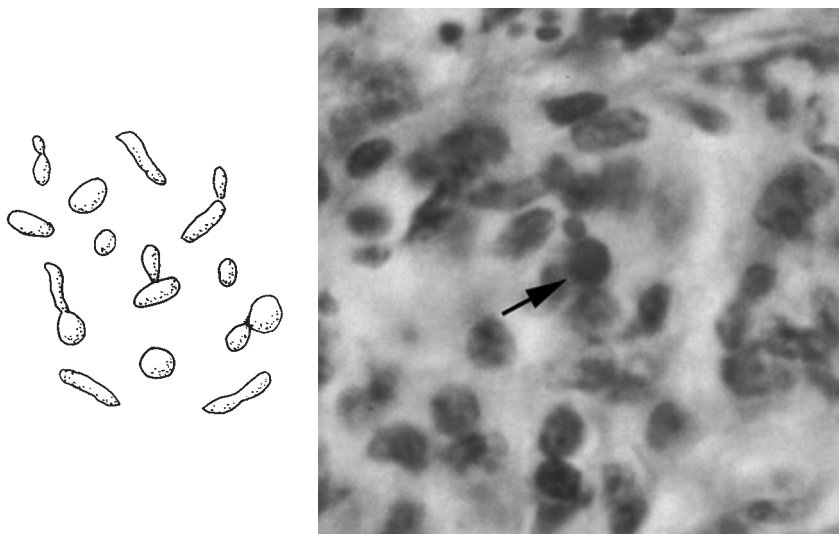
ETIOLOGIC AGENTS: *Sporothrix schenckii* complex.

SITES OF INFECTION: Skin, subcutaneous tissue, and contiguous lymphatics; rare dissemination to bones, joints, lungs, and other internal organs.

TISSUE REACTION: *S. schenckii* usually provokes a mixed suppurative and granulomatous inflammatory reaction that is often accompanied by fibrosis and microabscess formation; this mixed inflammatory reaction is seen in the dermis and subcutaneous tissue as well as in disseminated disease. Splendore-Hoepli material typically forms around the yeastlike cells. Lesions of cutaneous infections usually exhibit hyperkeratosis, parakeratosis, and pseudoepitheliomatous hyperplasia.

MORPHOLOGY OF ORGANISM: Yeastlike cells are of various sizes and shapes; they may be round to oval (usually 2–6 μm in diameter) and often have elongated “pipe stem” buds. Budding is on a narrow base. Characteristic elongated “cigar bodies” are most commonly seen in disseminated lesions. In cutaneous lesions, the organisms are usually very sparse, and may not be seen even with the preferred special stains for this fungus (GMS or PAS). Fluorescent-antibody and other immunohistologic staining techniques are extremely helpful in observing the organism, but they are not readily available in many laboratories.

S. schenckii and *Histoplasma capsulatum* var. *capsulatum* may both appear as small, round or oval cells, but the neutrophilic inflammatory reaction seen in the mixed suppurative and granulomatous reaction of sporotrichosis is not characteristic of histoplasmosis. *Candida glabrata*, which also appears as small, oval yeast cells, produces a suppurative tissue reaction but does not usually generate granulomas; this fact might assist in differentiating it from *Sporothrix*.



Courtesy of Evelyn Koestenblatt.

ETIOLOGIC AGENT: *Histoplasma capsulatum* var. *capsulatum*.

SITES OF INFECTION: Lung, blood, bone marrow, multiple systemic sites.

TISSUE REACTION: The initial reaction is histiocytic. In acute pulmonary infections, macrophages and giant cells that are packed with yeast cells fill the alveolar spaces. Parenchymal necrosis develops, along with epithelioid and giant-cell granulomas, and often eventual cavitation. Healing is typified by the granulomas becoming fibrocaseous nodules that commonly calcify. (Color Plates 14 and 15.) Although *Candida glabrata* is similar in size and shape to *H. capsulatum*, the infections differ histologically in that *Candida* almost always produces a suppurative tissue reaction; *Sporothrix schenckii*, which is also a small yeast, differs in commonly producing a mixed suppurative and granulomatous reaction.

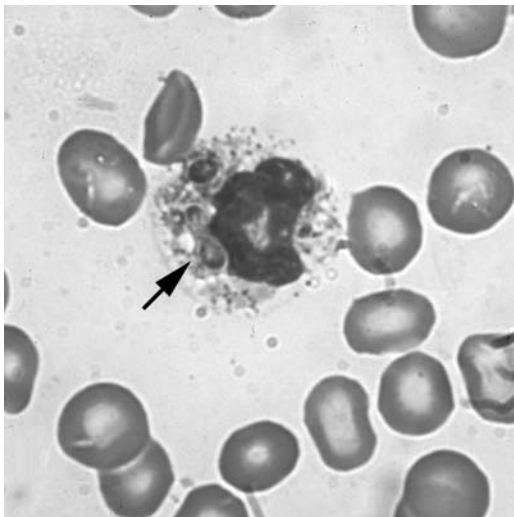
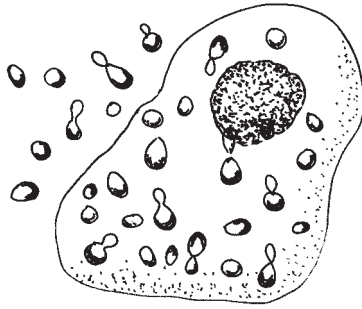
MORPHOLOGY OF ORGANISM: Small yeast cells (2–4 μm in length) are usually ovoid with budding on a narrow base at the smaller end. The yeasts reproduce within monocytes or macrophages and, when released, often remain in clusters. In Giemsa- or Wright-stained preparations, a pale blue ring (the fungus cell wall) surrounds the darker blue cytoplasm that retracts from the wall, often giving the false impression of a capsule; the chromatin stains dark violet and appears as a crescent-shaped mass within the cell. A halo or pseudocapsule also appears with H&E, but the organism stains well and evenly with GMS or PAS.

Differentiation of *Histoplasma* from other small yeasts and a few parasites can be difficult, but a few factors may be helpful.

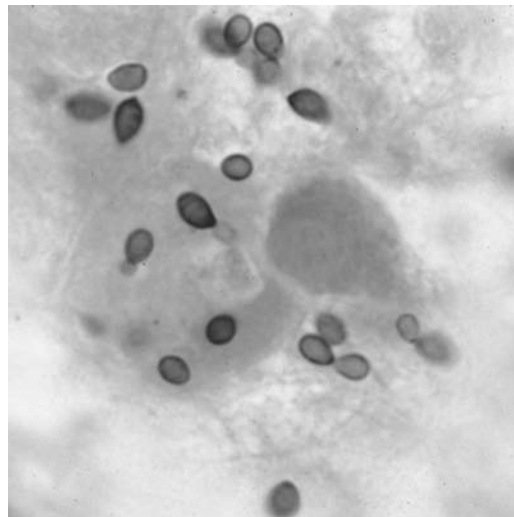
- The yeastlike cells of *Talaromyces marneffei* do not bud; they have a prominent transverse septum and reproduce by fission.
- The yeast cells of *Candida glabrata* are more variable in size, stain better with H&E, and do not demonstrate a pseudocapsule. Also, *Candida* spp. generate a pyogenic tissue reaction, not a granulomatous one.
- *Sporothrix schenckii* causes a mixed suppurative and granulomatous reaction rather than the purely granulomatous reaction seen in histoplasmosis. In sporotrichosis, the yeast cells are often fewer, and they may be more elongated and variably shaped than those of *Histoplasma*.
- *Cryptococcus* yeast cells have a dark wall (accentuated with Fontana-Masson stain), are rounder, and consistently appear in a large variety of sizes. Their capsular material stains with mucicarmine.
- The endospores of *Coccidioides* are about the same size as the yeast cells of *Histoplasma*, but the endospores are rounder, do not bud, and are typically accompanied by spherules.
- Some protozoans can mimic *Histoplasma* but are different in that they stain entirely with H&E, do not form a pseudocapsule, do not reliably stain with special histologic fungal stains, and do not bud. Additionally, amastigotes of *Leishmania* sp. and *Trypanosoma cruzi* have paranuclear bar-shaped kinetoplasts that can be seen with H&E under oil immersion.

Histoplasmosis (continued)

The yeast cells of *Histoplasma capsulatum* var. *duboisii* are 8–15 μm in diameter in tissue, i.e., much larger than those of *H. capsulatum* var. *capsulatum*, but they are small and identical to var. *capsulatum* when grown on culture. The var. *duboisii* is endemic in central and western Africa, most commonly reported in Nigeria.



Bone marrow; intracellular.



Lung; extracellular; courtesy of Joan Barenfanger.

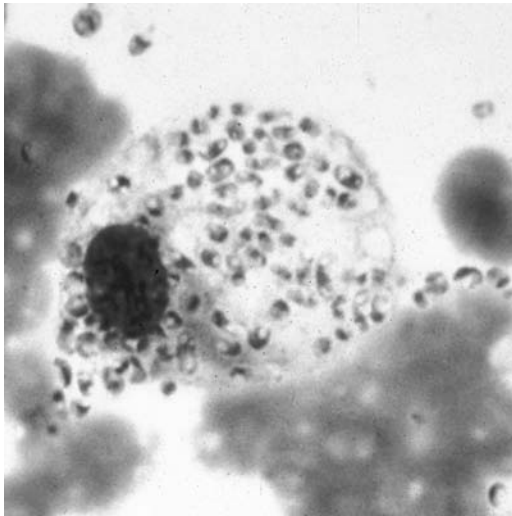
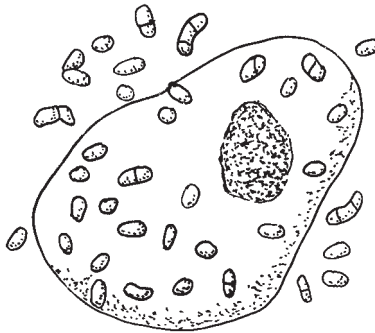
Talaromycosis (Penicilliosis)

ETIOLOGIC AGENT: *Talaromyces marneffe* (formerly *Penicillium marneffe*).

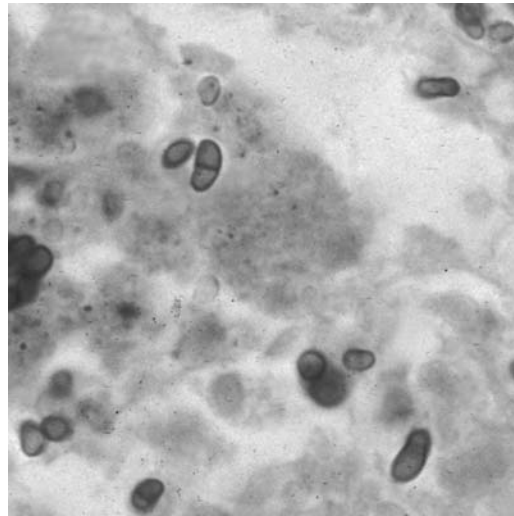
SITES OF INFECTION: Blood, bone marrow, skin, lung, liver, lymph nodes, multiple systemic sites.

TISSUE REACTION: Histiocytes predominate in early lesions. As the lesion progresses, central necrosis develops, with infiltration of neutrophils and formation of abscesses. Granulomas slowly evolve in the lung and may lead to fibrosis and cavitation. Calcification does not usually occur (in contrast to histoplasmosis). In the severely immunocompromised patient, the inflammatory response may be minimal or absent.

MORPHOLOGY OF ORGANISM: Oval, yeastlike cells are 2.5–5 μm in length and multiply within histiocytes in tissue (or within monocytes in blood or bone marrow). Budding does not occur; a prominent central septum forms, and reproduction is by fission (arthroconidium-like). Outside of histiocytes, yeast cells are up to 8 μm in length, may have several septa, and are sometimes curved. The organisms stain well with GMS or PAS; they give a false impression of having a capsule when stained with H&E, so they can very closely mimic *Histoplasma capsulatum* var. *capsulatum*, especially when intracellular (but the yeast cells of *Histoplasma* reproduce by budding).



Bone marrow; intracellular; courtesy of William Merz.



Bone marrow; extracellular; courtesy of Ron C. Neafie.

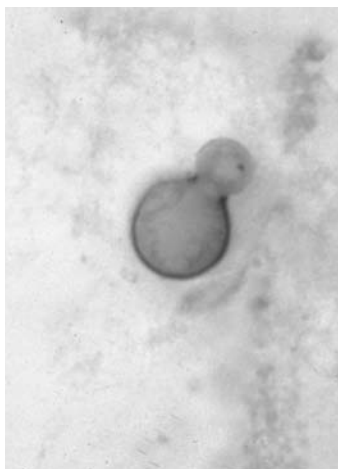
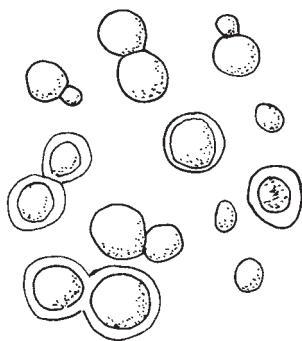
ETIOLOGIC AGENT: *Blastomyces dermatitidis*.

SITES OF INFECTION: Lung, skin, bones, multiple systemic sites.

TISSUE REACTION: *Blastomyces* evokes a suppurative and/or granulomatous response. In young lesions, neutrophils predominate (eosinophils may also be present). In older lesions, the suppurative reaction decreases and granulomas, which may contain central abscesses and/or caseation, predominate. Long-standing infections commonly show fibrosis and sometimes cavitation. In skin, hyperplasia occurs.

MORPHOLOGY OF ORGANISM: Yeastlike cells (3–30 μm in diameter; most commonly 8–15 μm ; there have been several reported cases with yeast cells as large as 40 μm) are round to oval, with sharply defined refractile cell walls that are commonly referred to as “double contoured.” The thick wall is seen with H&E and PAS, and it is sometimes lightly colored with mucicarmine stain, but it is not always conspicuous with GMS stain. Shrinkage of cytoplasmic contents and retraction from the rigid cell walls during fixation and processing create clear spaces, or “halos,” in the fungal cells. Each yeast cell produces only one bud, which is distinctively attached to the parent cell on a very **broad base** (average, 4–5 μm); the bud characteristically grows to the same size as the parent cell before detaching. (Color Plates 31 and 32.) Budding *in vivo* may be relatively infrequent.

Small spherules of *Coccidioides* may be easily mistaken for yeast cells of *Blastomyces*, especially if two spherules abut one another and give the impression of broad-based budding. Both organisms have thick, refractile walls, but close examination should reveal typical single spherules with endospores.



Young blastoconidium; courtesy of Joan Barenfanger.



Mature blastoconidium; courtesy of Joan Barenfanger.

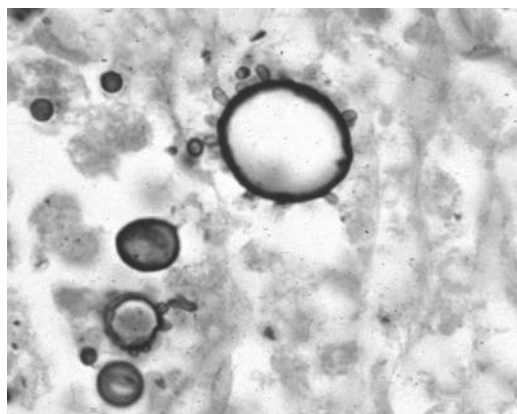
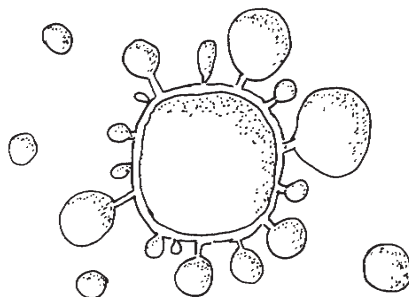
Paracoccidioidomycosis

ETIOLOGIC AGENT: *Paracoccidioides brasiliensis*.

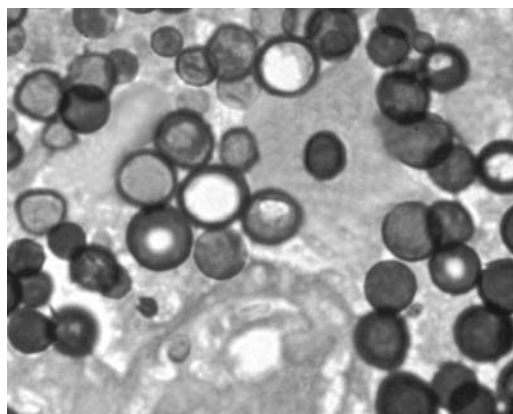
SITES OF INFECTION: Lung, skin, mucous membranes, multiple systemic sites.

TISSUE REACTION: A mixed suppurative and granulomatous inflammatory response is elicited by *P. brasiliensis* in pulmonary infections. Neutrophilic abscesses may predominate in one area of tissue while another area contains mainly granulomas. In rapidly progressing lesions, necrosis is frequently observed. In long-standing disease, fibrosis is common and calcification may occasionally occur. In mucocutaneous infections, ulceration and pseudoepitheliomatous hyperplasia may be seen.

MORPHOLOGY OF ORGANISM: Yeastlike cells are round to oval and can become quite large (3–30 μm or more in diameter). The outstanding characteristic is the presence of multiple buds that are attached to the parent cell by narrow connections. Buds may be small and all approximately the same size or fairly large and of unequal sizes and shapes. A large parent cell surrounded by small buds creates the classic ship's-wheel appearance; parent cells with fewer, but larger, buds are common, and single budding may also be seen.



Courtesy of Ron C. Neafie.



Courtesy of Douglas Flieder.

Candidiasis (Candidosis)

ETIOLOGIC AGENTS: *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and other *Candida* spp.

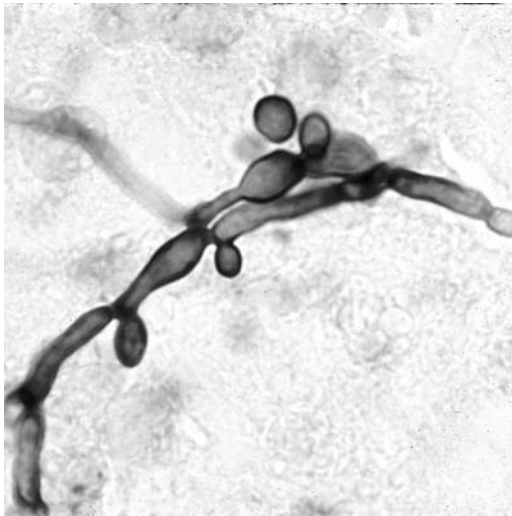
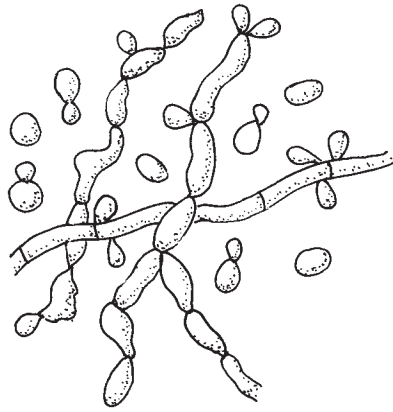
SITES OF INFECTION: Blood, mucous membranes, skin, nails, multiple systemic sites (appearance in the urine can be an early indication of candidemia).

TISSUE REACTION: In systemic infections, *Candida* spp. most commonly elicit an acute suppurative inflammation, sometimes forming abscesses composed of polymorphonuclear as well as mononuclear cells. Coagulative necrosis replaces the suppurative reaction in neutropenic patients. Granulomas only rarely occur (generally with chronic systemic candidiasis); other yeastlike fungi are more likely than *Candida* to form granulomatous lesions. *Candida* spp. are also known to invade blood vessels and produce infarcts (see p. 25).

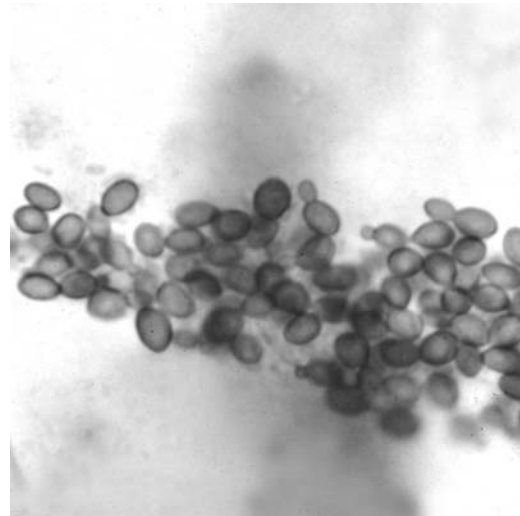
MORPHOLOGY OF ORGANISM: All species of *Candida* form round to oval budding yeast cells (blastoconidia, 3–6 μm in diameter) singly, in chains, or in small, loose clusters. Most species, when invading tissue, form both pseudohyphae and true hyphae. Pseudohyphae are actually chains of blastoconidia that have elongated and have not separated from one another. They can be recognized by the distinct constrictions at the septa; also, pseudohyphal branching will only occur at the site of a septation. True hyphae have no, or only slight, constrictions at the septa, and there is often no septation at the initiation of a branch. Blastoconidia develop along the sides of both types of hyphae.

C. glabrata is unique in that it is slightly smaller (2–5 μm in diameter) than the other species of *Candida* and it does not produce any hyphal forms. The small yeast cells often aggregate in clusters and may closely resemble *Histoplasma capsulatum*, but no halo or pseudocapsular effect is seen when the cells are stained with H&E.

Culture is required to identify any *Candida* to species level.



Candida albicans; courtesy of Joan Barenfanger.



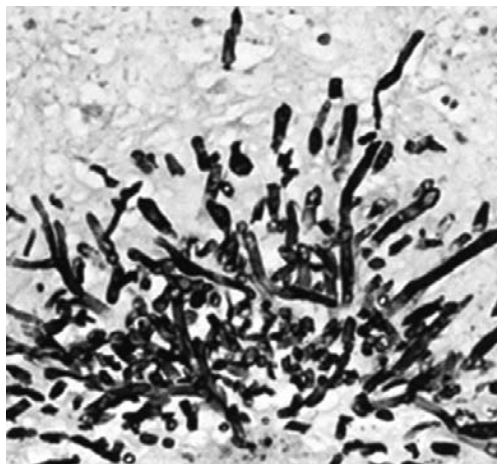
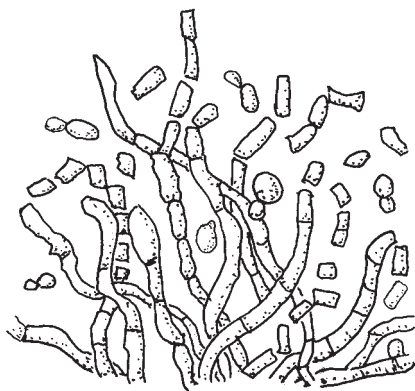
Candida glabrata; courtesy of Joan Barenfanger.

ETIOLOGIC AGENTS: *Trichosporon asahii*, *Trichosporon mucoides*, *Trichosporon inkin*, *Trichosporon ovoides*, *Trichosporon cutaneum*, *Trichosporon asteroides*, and occasionally other species of *Trichosporon*.

SITES OF INFECTION: Lung, liver, spleen, kidney, gastrointestinal tract, bone marrow, heart, brain, eye, multiple systemic sites (most frequently *T. asahii* and, much less often, *T. mucoides* and *T. inkin*); hair (*T. ovoides* in white piedra on scalp hairs, *T. cutaneum* on axilla hair, *T. inkin* on pubic hair); very occasionally skin (*T. asteroides*, *T. cutaneum*, *T. inkin*, *T. ovoides*).

TISSUE REACTION: In systemic *Trichosporon* infections, acute inflammatory cells are generally absent due to the neutropenic state of the patient. Nodular infarcts are more regularly formed as a result of fungal occlusion of blood vessels. Abscesses and granulomatous inflammation are occasionally seen in nonneutropenic patients.

MORPHOLOGY OF ORGANISM: In systemic infections, the fungal elements of *Trichosporon* spp. commonly grow from a focal point in a parallel radiating fashion, similar to that of aspergillosis. They clearly differ from *Aspergillus* by characteristically forming (i) pleomorphic yeastlike cells (blastoconidia) 3–8 μm in diameter; (ii) pseudohyphae; (iii) septate true hyphae (without predominant dichotomous branching); and (iv) arthroconidia, formed by the fragmentation of the true hyphae. Although the yeastlike cells of *Trichosporon* are usually larger than those of *Candida*, it can be very difficult to differentiate *Trichosporon* spp. from *Candida* spp. if arthroconidia are not seen (it is fairly common for arthroconidia to be sparse or seemingly absent in *Trichosporon* spp. in tissue). To assist in such instances, diluted periodic acid methenamine silver stain exhibits a substantially more intense staining of *Trichosporon* than it does of *Candida* (Obana et al., 2010). In most cases, direct immunohistochemical procedures or culture is required for definitive identification.



Courtesy of Thomas Walsh (from Walsh et al., 1992).

ETIOLOGIC AGENTS: *Cryptococcus neoformans* and *Cryptococcus gattii*.

SITES OF INFECTION: Lung, meninges and cerebrospinal fluid, blood, skin, mucous membranes, multiple systemic sites.

TISSUE REACTION: In immunocompetent patients, a mixed suppurative and granulomatous reaction or a purely granulomatous reaction with various degrees of necrosis is seen. Chronic pulmonary infection results in the formation of residual granulomas (cryptococcomas). Healing is by fibrosis, generally without calcification.

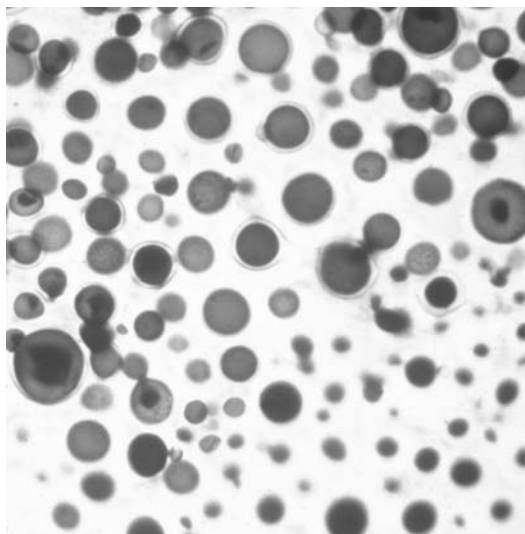
In immunocompromised patients, the reaction may be minimal or absent. In this case, the yeasts proliferate abundantly, creating mucoid “cystic” lesions packed with round, encapsulated cryptococci that resemble soap bubbles; these are seen most frequently in the brain. The number of organisms present in the tissue is inversely proportional to the number of inflammatory cells.

Candidiasis differs from cryptococcosis by most commonly producing a purely suppurative reaction.

MORPHOLOGY OF ORGANISM: The yeast cells (i) are 2–20 μm in diameter (usually 4–10 μm); (ii) vary in size within the microscopic field; (iii) may be oval, but are more typically round with thin, dark walls; (iv) bud on a narrow base; and (v) characteristically produce thick capsules, but cells relatively deficient in capsular material sometimes occur.

Capsules should be suspected when the yeast cells do not appear to touch one another (due to the surrounding mucopolysaccharide capsular material). In smeared specimens, capsules can be demonstrated with India ink. (Color Plate 33.) Tissues stained with mucicarmine show the capsule as bright carmine red, often with a spiny or scalloped appearance. (Color Plate 34.) The mucicarmine stain may also color the cell walls of *Rhinosporidium seeberi* and *Blastomyces dermatitidis*. The cryptococcal yeast cell (but not the capsule) is stained by GMS. Because the cell walls of *C. neoformans* and *C. gattii* contain melaninlike substances, they become brown to black with the Fontana-Masson stain. Drying, fixing, and staining may cause the yeast cells to collapse or become crescent shaped.

(continued on following page)



Courtesy of Joan Barenfanger.

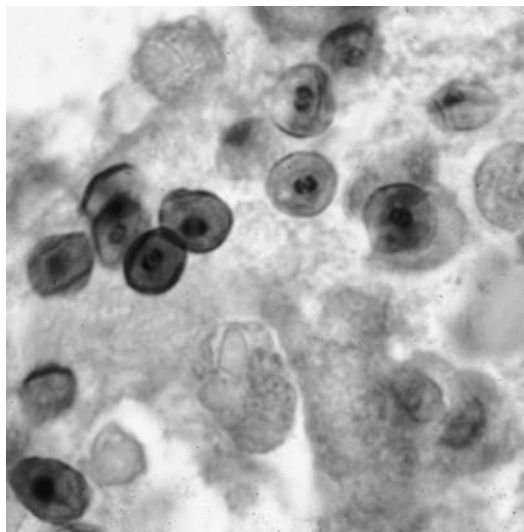
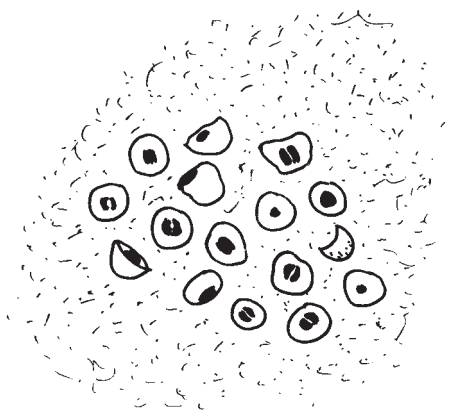
Pneumocystosis

ETIOLOGIC AGENT: *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*).*

SITE OF INFECTION: Lung.

TISSUE REACTION: The prominent characteristic in the lung is a foamy exudate in the alveolar spaces; this foam can be observed in lung biopsies as well as in specimens of bronchoalveolar lavage and, in many cases, induced sputum. This reaction is almost diagnostic of infection with *P. jirovecii*. The organisms are seen in the foamy exudate. Granulomatous reaction has been reported on rare occasions.

MORPHOLOGY OF ORGANISM: The most commonly used stains for *Pneumocystis* (GMS, calcofluor white, and toluidine blue O) stain the cyst form, not the trophozoite. The cysts (4–7 μm in diameter) are nonbudding and are round, ovoid, or collapsed crescent forms. They characteristically have focal thickenings in the wall that, when stained with GMS, appear as dark dots in the shape of single or double commas or a set of parentheses. The organisms occur in small clusters on a thick, foamy background. (Color Plate 35.) Immunospecific stain is commercially available. *P. jirovecii* does not grow on routine culture.



Courtesy of Joan Barenfanger.

* *P. jirovecii* is the species found in humans, and *P. carinii* is the species found in rats. The acronym PCP, originally for *Pneumocystis carinii* pneumonia, is still used, but meaning *Pneumocystis* pneumonia.

ETIOLOGIC AGENTS: *Prototheca wickerhamii* (the species more commonly encountered in human infections) and *Prototheca zopfii*. They are actually achlorophyllous algae.

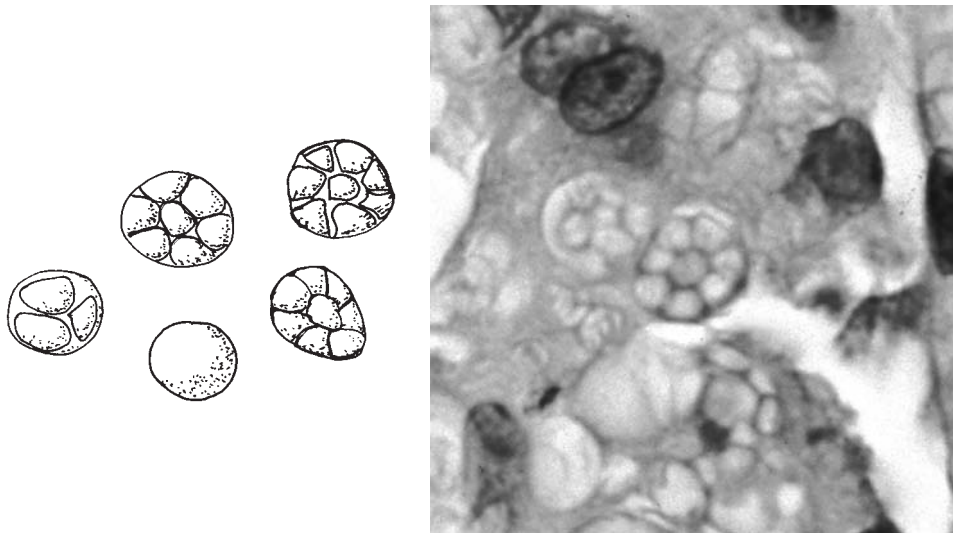
SITES OF INFECTION: Skin and subcutaneous tissue, bursa of the elbow, very rarely systemic sites.

TISSUE REACTION: Skin biopsies show varying degrees of nonspecific changes, including hyperkeratosis, parakeratosis, and acanthosis. The inflammatory response may be minor or absent, or it may consist of mononuclear and/or granulomatous infiltrates, with or without necrosis. Bursitis of the elbow typically exhibits necrotizing granulomas.

MORPHOLOGY OF ORGANISM: Round or oval sporangia of *P. wickerhamii* vary from 3–15 μm in diameter, while those of *P. zopfii* are 7–30 μm in diameter. Each sporangium of *P. wickerhamii* contains 2–20 endospores; only 4–8 are usually visible in one plane, and they appear round, polyhedral, or wedge shaped in a radial arrangement. The spores are densely basophilic, staining deep purplish blue with H&E. The organisms are best seen with the special stains for fungi. Hyaline, nonviable, ghostlike forms may also be present.

P. zopfii can be similar to *P. wickerhamii*, but it frequently forms oval, nonendosporulating cells, each having vacuolated cytoplasm and a single discrete, basophilic nucleus.

The size of the mother cells and differences in the number and morphology of endospores help differentiate *Prototheca* from *Coccidioides*.



Courtesy of Evelyn Koestenblatt.

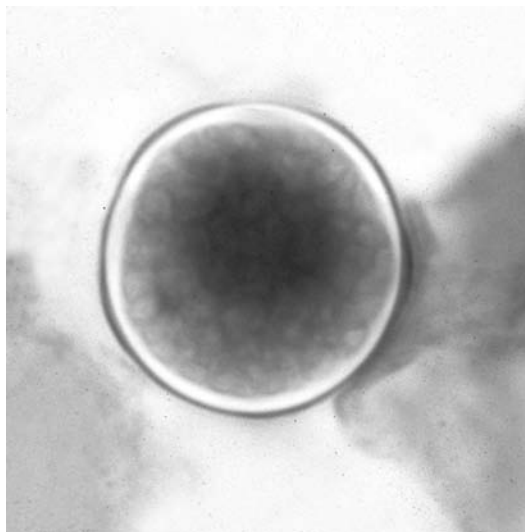
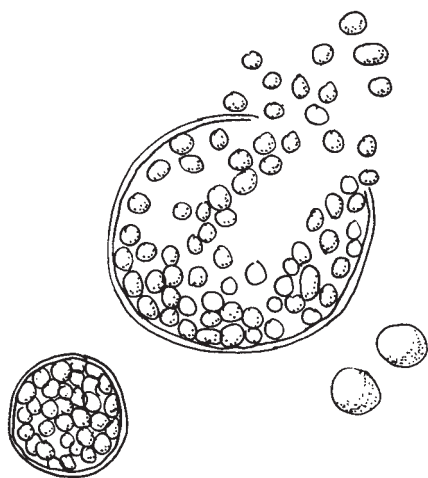
ETIOLOGIC AGENTS: *Coccidioides immitis* and *Coccidioides posadasii*.

SITES OF INFECTION: Lung, skin, bone, meninges; dissemination to multiple systemic sites.

TISSUE REACTION: A granulomatous reaction develops in the presence of spherules, and a predominantly suppurative reaction occurs in response to released endospores; both of the forms and reactions commonly occur simultaneously. Splendore-Hoeppli material sometimes surrounds spherules and aggregates of endospores.

MORPHOLOGY OF ORGANISM: Round spherules can be as small as 5 μm in diameter when immature (nonendosporeulating) and grow to 30–100 μm or more in diameter upon maturity (endosporeulating). Immature spherules stain well with PAS and GMS, but mature spherules are GMS variable and PAS negative due to the high phospholipid content of the mature cell wall (1–2 μm in width). Endospores are round (2–5 μm in diameter) and uninucleate and have walls and cytoplasmic inclusions that are GMS and PAS positive. (Color Plate 36.) The spherules and endospores stain with H&E and are readily visible in sufficient numbers. Fragmented or empty ruptured spherules are common. Septate hyphae, barrel-shaped arthroconidia, and rarely small conidial forms singly (some budding) or in chains (Schuetz et al., 2011) have been observed in cavitory and necrotic lesions.

When only endospores are seen, they may initially be confused with yeasts, but endospores do not bud. When two immature spherules abut one another, they can give the impression of broad-based budding cells of *Blastomyces dermatitidis*.



Courtesy of Joan Barenfanger.

ETIOLOGIC AGENT: *Rhinosporidium seeberi* (does not grow on synthetic media). It is now actually classified as a parasite rather than a fungus, but due to its similarities to fungi, it is commonly and traditionally regarded as one.

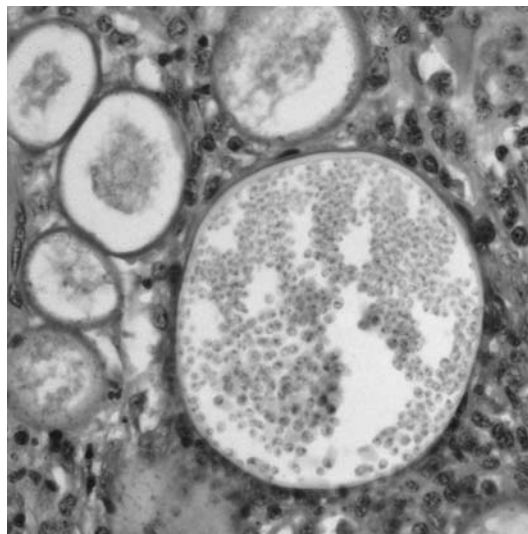
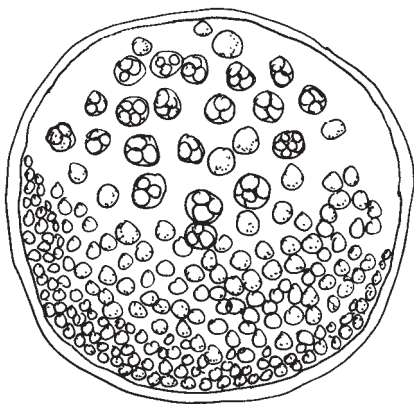
SITES OF INFECTION: Mucocutaneous tissue, primarily involving the nasal cavity, nasopharynx, and oral cavity; secondary skin infections and rarely limited systemic dissemination have been reported.

TISSUE REACTION: In the submucosa or dermis, a chronic inflammatory response (mostly lymphocytes, plasma cells, and various numbers of epithelioid cells and neutrophils) with granulation tissue is typically seen. If the sporangia rupture and endospores are released, a granulomatous reaction is likely, but in some instances a suppurative response may occur.

MORPHOLOGY OF ORGANISM: Mature sporangia are thick walled ($\sim 5\ \mu\text{m}$ wide), round, large (100–350 μm in diameter; most commonly 100–200 μm), and contain numerous sporangiospores (endospores) that range from 1–10 μm in diameter. A zonal pattern of sporangiospore development is uniquely characteristic of this pathogen: small, young spores are seen peripherally along the inner wall or form a crescentlike mass at one pole of the sporangium; medium-size, enlarging spores are between the periphery and the center; and the larger, mature spores are centrally located. Mature sporangiospores appear lobulated due to globular eosinophilic inclusions and, when released into the tissue, can be suggestive of *Prototheca* (p. 162). The walls of the sporangiospores and the mature sporangia are GMS and PAS positive. Mucicarmine also will stain the walls of the spores and the inner surface of the sporangial wall. (Color Plate 37.) Special fungal stains are seldom necessary, as the organism is readily seen with H&E.

Trophocytes (immature sporangia) are 10–100 μm in diameter with refractile eosinophilic walls $\sim 2\text{--}3\ \mu\text{m}$ thick. Trophocytes contain granular or flocculent cytoplasm and a round, pale nucleus with a prominent nucleolus or karyosome. They are readily seen with H&E but do not stain well with GMS.

The etiologic agent, *R. seeberi*, cannot be cultured; diagnosis depends on direct examination of infected tissue.



Courtesy of Douglas Flieder.

Adiaspiromycosis

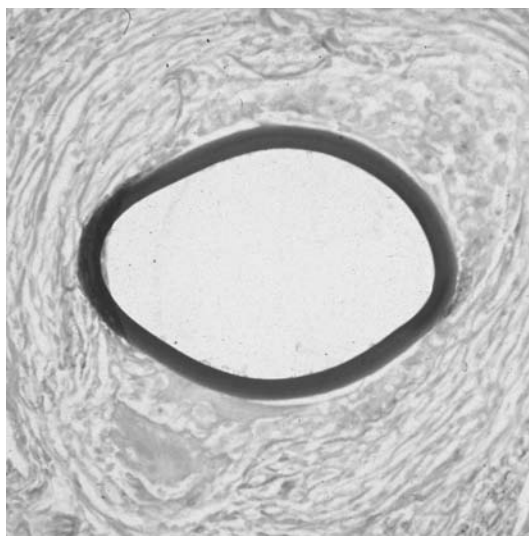
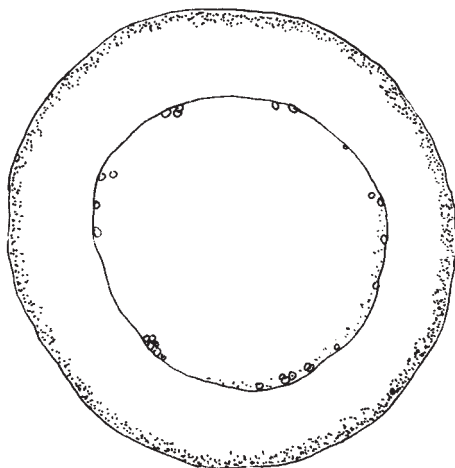
ETIOLOGIC AGENT: *Emmonsia crescens*.

SITE OF INFECTION: Lung.

TISSUE REACTION: The typical inflammatory response is granulomatous and fibrotic. A granuloma forms around each adiaconidium (formerly adiaspore), which in turn is surrounded by dense fibrous connective tissue. Giant cells are often in contact with the outer spore wall. Polymorphonuclear leukocytes, especially eosinophils, may also be seen in the presence of small, immature adiaconidia. Necrosis or caseation is almost never seen. In some individuals, there is little, or no, host response (regardless of the level of immunocompetence).

MORPHOLOGY OF ORGANISM: Mature adiaconidia are round, large (200–400 μm in diameter), and thick walled (20–70 μm in width). The narrow outer layer of the adiaconidial wall is eosinophilic; a thin middle layer of the wall has irregular perforations that may appear as unstained spots; the inner layer of the wall is broad, hyaline, and composed predominantly of chitin. The walls can be readily seen with H&E and stain extremely well with GMS and PAS stains. The interior of the conidium usually appears empty, but small (1–3 μm in diameter), refractile, eosinophilic hyaline globules may be seen along the inner surface of the wall. There is no evidence of replication, i.e., no budding or endosporulation.

If starch granules of lentils and other legumes are aspirated into the lungs, a pneumonitis may develop that can somewhat resemble adiaspiromycosis, but the starch granules of the legumes will have thinner walls and contain loculated material that can be seen with PAS and other stains.



Courtesy of Ron C. Neafie.

Special References

For further information and additional photomicrographs, see:

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PART II

Identification of Fungi in Culture

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Guide to Identification of Fungi in Culture

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(very thin, branching filaments)

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(yeastlike at 25–30°C and also at 35–37°C if growth occurs)

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having only hyphae with chlamydoconidia 84

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Pink to Violet Surface 100

Green Surface

Light reverse 102

Dark Gray or Black Surface

Light reverse 103

Greenish, Dark Gray, or Black Surface

Dark reverse

having small conidia 104

having large conidia 106

having arthroconidia 107

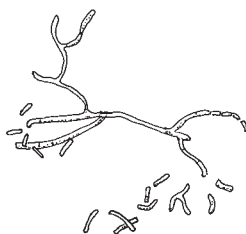
having only hyphae (with or without chlamydoconidia) 107

having large fruiting bodies 107

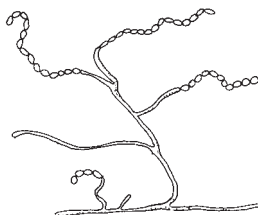
FILAMENTOUS BACTERIA

Very thin (1 μm or less in diameter), branching filaments*

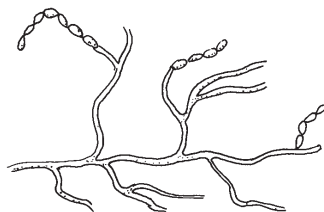
Aerobic Actinomycetes



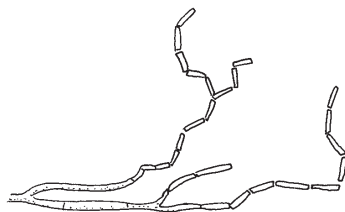
Nocardia (p. 116)



Streptomyces (p. 119)



Actinomadura (p. 120)



Nocardioopsis (p. 121)

* Growth characteristics and biochemical tests must be utilized for differentiation of genera; these are summarized in [Table 2](#) (p. 115).

MONOMORPHIC YEASTS AND YEASTLIKE ORGANISMS

Yeastlike at 25–30°C and also at 35–37°C if growth occurs

All rapid growers except *Ustilago* spp.

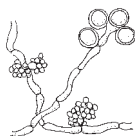
All WHITE, CREAM, or TAN except *Rhodotorula* and *Sporobolomyces* spp.

Microscopic morphology on cornmeal-Tween 80 agar (Dalmat plate)*

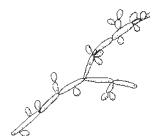
Pseudohyphae with blastoconidia



Candida albicans (p. 127)



Candida dubliniensis (p. 129)



Candida tropicalis (p. 133)



Candida parapsilosis (p. 134)



Candida lusitanae (p. 135)



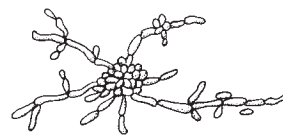
Candida krusei (p. 136)



Candida kefyr
(*pseudotropicalis*) (p. 138)



Candida rugosa (p. 139)



Candida guilliermondii (p. 141)



Candida lipolytica (p. 143)



Candida zeylanoides (p. 144)



Saccharomyces (p. 155)



Wickerhamomyces anomalus
(p. 157)

* Morphology alone cannot be relied upon for identification. Use procedure on p. 374 and Tables 4 through 11 (pp. 128, 130, 132, 137, 142, 151, and 152) for identification of genus and species.

MONOMORPHIC YEASTS AND YEASTLIKE ORGANISMS

Yeastlike at 25–30°C and also at 35–37°C if growth occurs (*continued*)

All rapid growers except *Ustilago* spp.

All WHITE, CREAM, or TAN except *Rhodotorula* and *Sporobolomyces* spp.

Microscopic morphology on cornmeal-Tween 80 agar (Dalmay plate)*

Yeastlike cells only; usually no hyphae or pseudohyphae



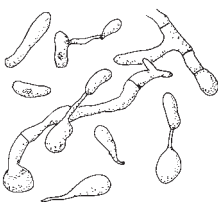
Cryptococcus (p. 148)



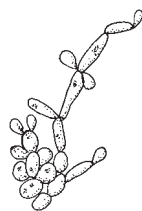
Candida glabrata (p. 145)



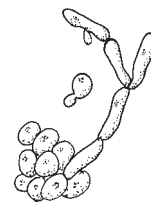
Rhodotorula (p. 153)
(pink or coral pigment)



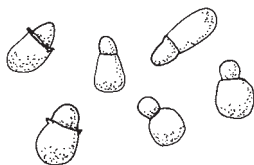
Sporobolomyces (p. 154)
(pink or coral pigment)



Saccharomyces (p. 155)



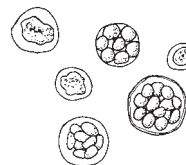
Wickerhamomyces anomalus
(p. 157)



Malassezia (p. 158)



Ustilago (p. 161)



Prototheca (p. 162)



Candida auris (p. 146)



Candida haemulonii (p. 147)

* Morphology alone cannot be relied upon for identification. Use procedure on p. 374 and Tables 4 through 11 (pp. 128, 130, 132, 137, 142, 151, and 152) for identification of genus and species.

MONOMORPHIC YEASTS AND YEASTLIKE ORGANISMS

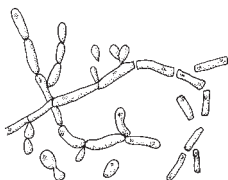
Yeastlike at 25–30°C and also at 35–37°C if growth occurs (*continued*)

All rapid growers except *Ustilago* spp.

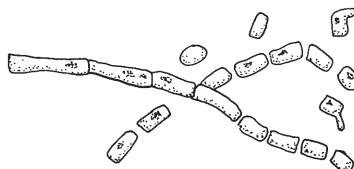
All WHITE, CREAM, or TAN except *Rhodotorula* and *Sporobolomyces* spp.

Microscopic morphology on cornmeal-Tween 80 agar (Dalmau plate)*

Hyphae and arthroconidia or annelloconidia



Trichosporon (p. 163)



Geotrichum (p. 166)

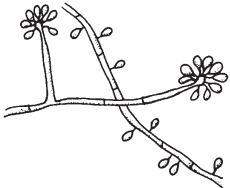
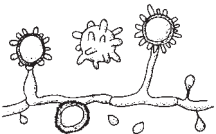
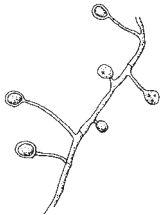
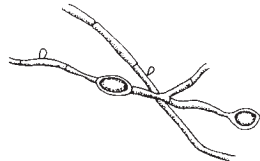
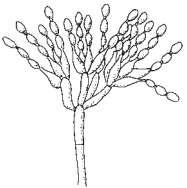
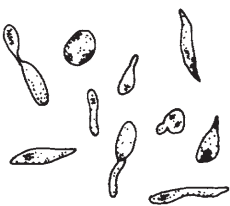
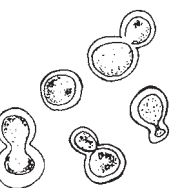
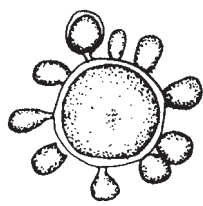


Magnusiomyces
(*Blastoschizomyces*) (p. 165)

* Morphology alone cannot be relied upon for identification. Use procedure on p. 374 and Tables 4 through 11 (pp. 128, 130, 132, 137, 142, 151, and 152) for identification of genus and species.

THERMALLY DIMORPHIC FUNGI

Filamentous when cultured at 25–30°C; yeastlike when cultured at 35–37°C*

<i>Sporothrix schenckii</i> (p. 178)	<i>Histoplasma capsulatum</i> (p. 170)	<i>Blastomyces dermatitidis</i> (p. 172)	<i>Paracoccidioides brasiliensis</i> (p. 174)	<i>Talaromyces (Penicillium) marneffei</i> (p. 176)
25°C mould phase on Sabouraud dextrose agar				
MACROSCOPICALLY:				
Wrinkled, leathery, some short aerial mycelium when old	Loose, cottony	Smooth, then prickly, then cottony	Heaped; short mycelium	Flat, velvety
White, then tan or black	White or brownish	White, then brownish	White, then brownish	Tan, then reddish yellow and bluish green; red, diffusing pigment
MICROSCOPICALLY:				
Fine, branched, septate hyphae; "florette" conidial form	Branched, septate hyphae; microconidia; knobby macroconidia in 3–4 weeks	Branched, septate hyphae; single small conidia	Septate hyphae, chlamydoconidia, few microconidia	Septate hyphae, metulae, phialides, chains of conidia
				
37°C yeast phase on brain heart infusion agar				
MACROSCOPICALLY: All cream or tan				
MICROSCOPICALLY:				
Round, oval, or cigar shaped	Small budding cells	Large, double-contoured cells budding on a broad base	Large, multiple budding cells; "ship's wheel"	Oval cells with central septum; no budding
				

* See p. 381 for method of converting filamentous forms to yeast phase.

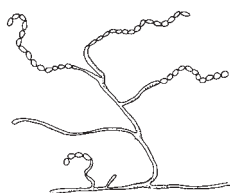
THERMALLY MONOMORPHIC MOULDS

Filamentous when cultured at 25–30°C and also at 35–37°C if growth occurs

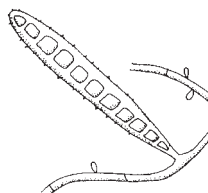
SURFACE: WHITE, CREAM, OR LIGHT GRAY*

REVERSE: NONPIGMENTED

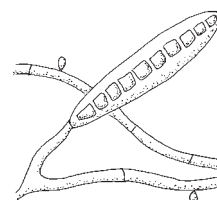
Having microconidia or macroconidia



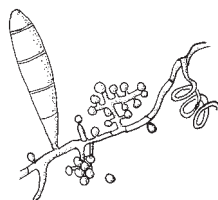
*Streptomyces*** (p. 119)



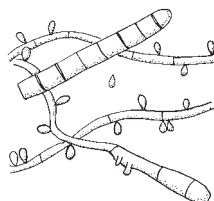
Microsporum vanbreuseghemii
(p. 268)



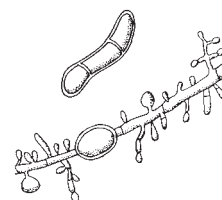
Trichophyton ajelloi (p. 281)



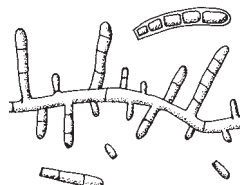
Trichophyton mentagrophytes
(p. 270)



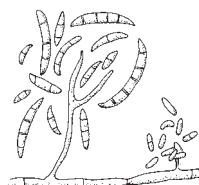
Trichophyton rubrum (p. 272)



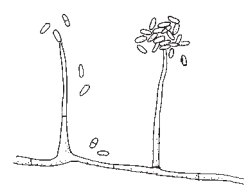
Trichophyton tonsurans
(p. 273)



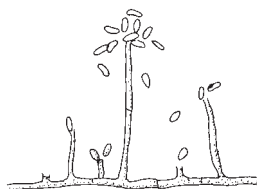
Trichophyton terrestre (p. 274)



Fusarium (p. 322)



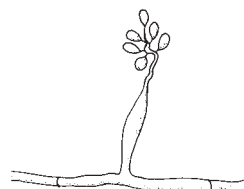
Acremonium (p. 215)



Phialemonium (p. 215)



Verticillium (p. 320)



Beauveria (p. 319)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

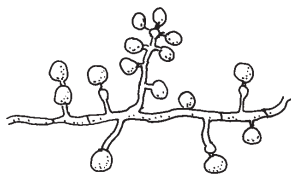
** *Streptomyces* is a filamentous bacterium.

THERMALLY MONOMORPHIC MOULDS

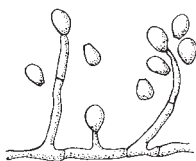
SURFACE: WHITE, CREAM, OR LIGHT GRAY*

REVERSE: NONPIGMENTED (*continued*)

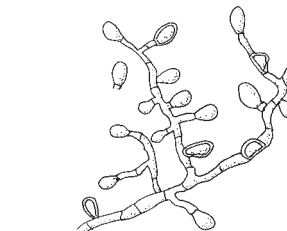
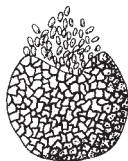
Having microconidia or macroconidia (continued)



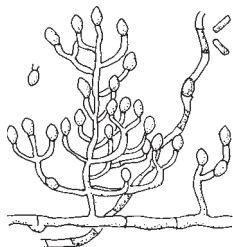
*Emmonsia**** (p. 317)



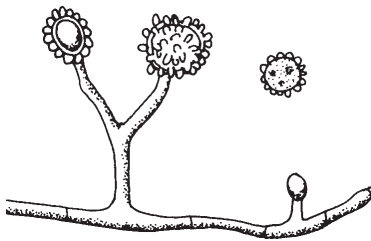
Pseudallescheria/Scedosporium
(p. 222)



Chrysosporium (p. 326)



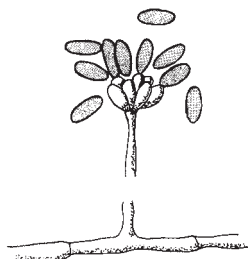
Sporotrichum (p. 329)



Scedosporium (p. 330)



Graphium (p. 242)



Stachybotrys (p. 241)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

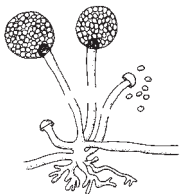
*** Large adlaconidia form at 37°C.

THERMALLY MONOMORPHIC MOULDS

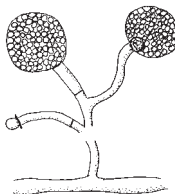
SURFACE: WHITE, CREAM, OR LIGHT GRAY*

REVERSE: NONPIGMENTED (*continued*)

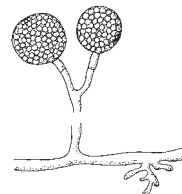
Having sporangia or sporangiola



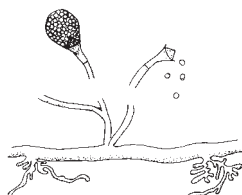
Rhizopus (p. 188)



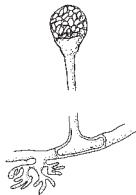
Mucor (p. 189)



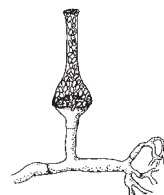
Rhizomucor (p. 190)



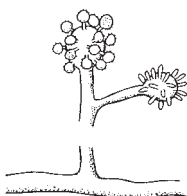
Lichtheimia (p. 191)



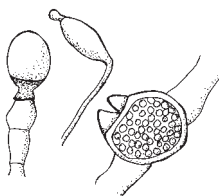
Apophysomyces (p. 193)



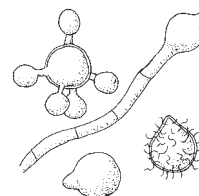
Saksenaea (p. 195)



Cunninghamella (p. 198)

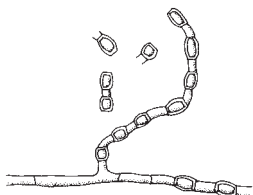


Basidiobolus (p. 200)

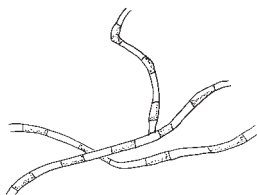


Conidiobolus (p. 201)

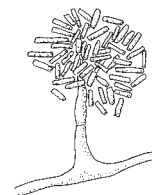
Having arthroconidia



Coccidioides (p. 287)



Malbranchea (p. 289)



Hormographiella (p. 292)

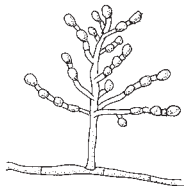
* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

THERMALLY MONOMORPHIC MOULDS

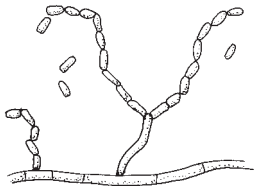
SURFACE: WHITE, CREAM, OR LIGHT GRAY*

REVERSE: NONPIGMENTED (*continued*)

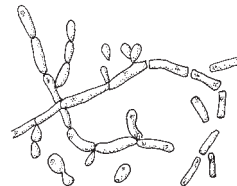
Having arthroconidia (continued)



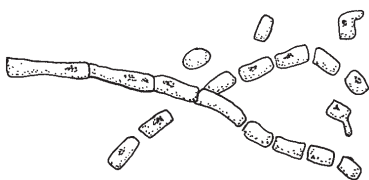
Geomyces (p. 290)



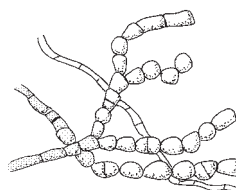
Arthrographis (p. 291)



Trichosporon (p. 163)

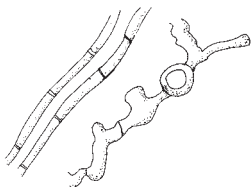


Geotrichum (p. 166)



Neoscytalidium (p. 238)

Having only hyphae with chlamydoconidia



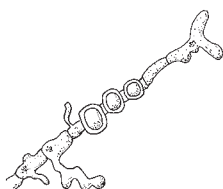
Microsporium ferrugineum
(p. 269)



Trichophyton schoenleinii
(p. 278)



Trichophyton verrucosum
(p. 279)



Trichophyton violaceum
(p. 280)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

THERMALLY MONOMORPHIC MOULDS

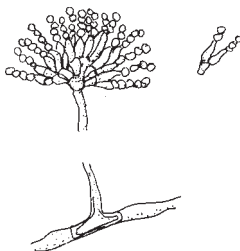
SURFACE: WHITE, CREAM, OR LIGHT GRAY*

REVERSE: NONPIGMENTED (*continued*)

*Having only septate hyphae on routine primary media***



Septate hyphae



On malt extract agar



Penicillate

***Aspergillus tanneri* (p. 303)**

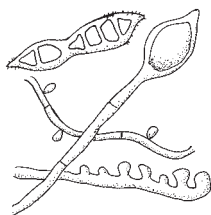
* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

** On routine media, *A. tanneri* produces only hyphae. Chlamydial heads are seen only on Czapek or malt extract agar. See p. 303.

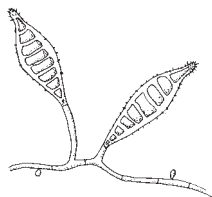
THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

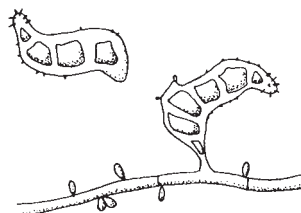
REVERSE: YELLOW, ORANGE, OR REDDISH



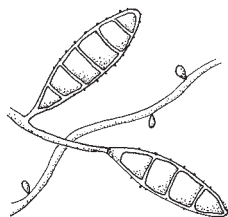
Microsporium audouinii
(p. 261)



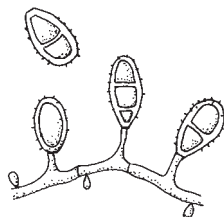
Microsporium canis var. *canis*
(p. 262)



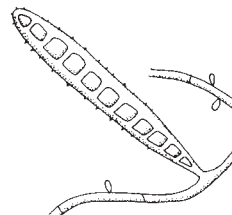
Microsporium canis var. *distortum* (p. 263)



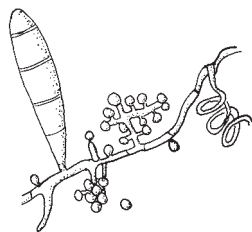
Microsporium gypseum
(p. 265)



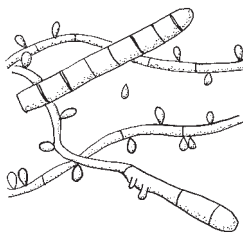
Microsporium nanum (p. 267)



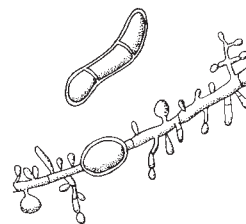
Microsporium vanbreuseghemii
(p. 268)



Trichophyton mentagrophytes
(p. 270)



Trichophyton rubrum (p. 272)

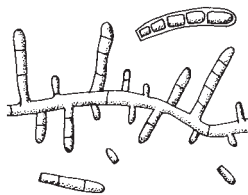


Trichophyton tonsurans
(p. 273)

THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

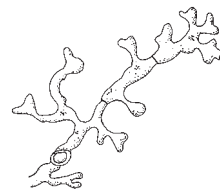
REVERSE: YELLOW, ORANGE, OR REDDISH (*continued*)



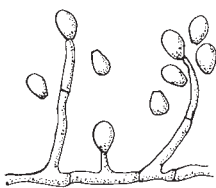
Trichophyton terrestre (p. 274)



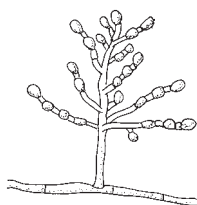
Trichophyton verrucosum
(p. 279)



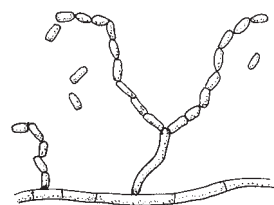
Trichophyton schoenleinii
(p. 278)



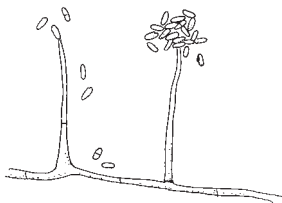
*Pseudallescheria/
Scedosporium* (p. 222)



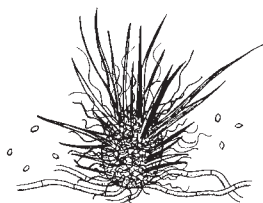
Geomyces (p. 290)



Arthrographis (p. 291)



Acremonium (p. 321)

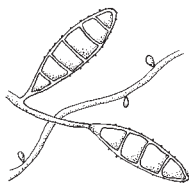


Chaetomium (p. 255)

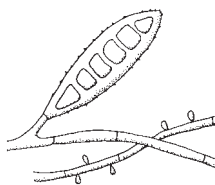
THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

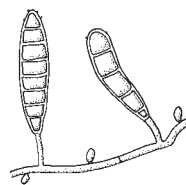
REVERSE: DEEP RED TO PURPLE



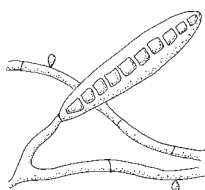
Microsporum gypsum
(p. 265)



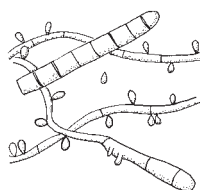
Microsporum cookei (p. 264)



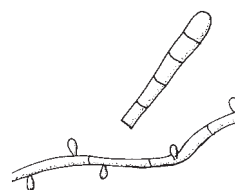
Microsporum gallinae (p. 266)



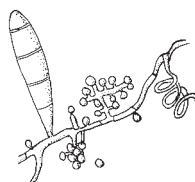
Trichophyton ajelloi (p. 281)



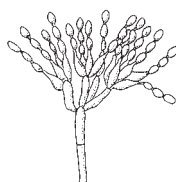
Trichophyton rubrum (p. 272)



Trichophyton megninii (p. 275)



Trichophyton mentagrophytes
(p. 270)



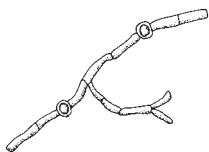
Talaromyces (Penicillium)
*marneffeii** (p. 176)

* *T. marneffeii* is thermally dimorphic.

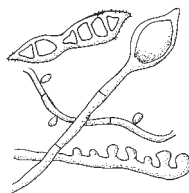
THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

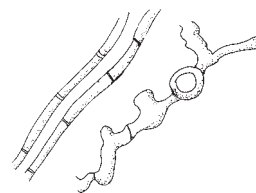
REVERSE: BROWNISH



Madurella mycetomatis
(p. 231)



Microsporum audouinii
(p. 261)



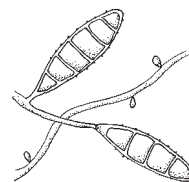
Microsporum ferrugineum
(p. 269)



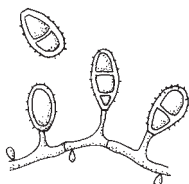
Trichophyton schoenleinii
(p. 278)



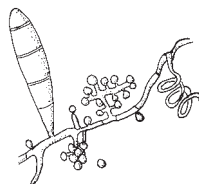
Microsporum canis (p. 262)



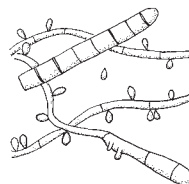
Microsporum gypsum
(p. 265)



Microsporum nanum (p. 267)



Trichophyton mentagrophytes
(p. 270)

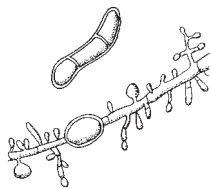


Trichophyton rubrum (p. 272)

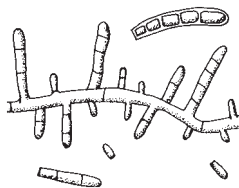
THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

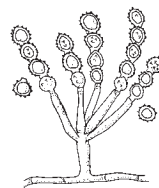
REVERSE: BROWNISH (*continued*)



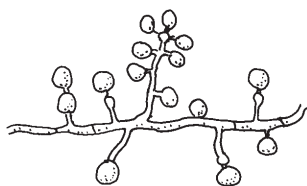
Trichophyton tonsurans
(p. 273)



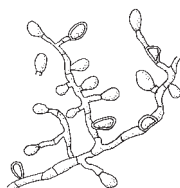
Trichophyton terrestre (p. 274)



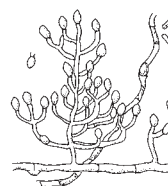
Scopulariopsis (p. 312)



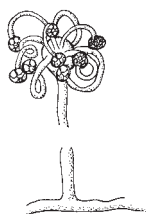
*Emmonsia** (p. 317)



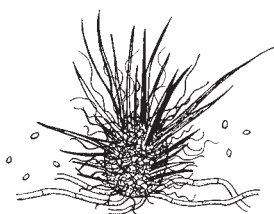
Chrysosporium (p. 326)



Sporotrichum (p. 329)



Cokeromyces (p. 196)



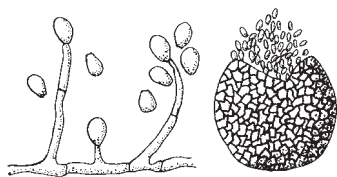
Chaetomium (p. 255)

* Large adiaconidia form at $\geq 37^{\circ}\text{C}$.

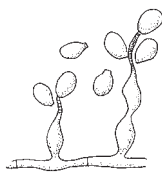
THERMALLY MONOMORPHIC MOULDS

SURFACE: WHITE, CREAM, BEIGE, OR LIGHT GRAY

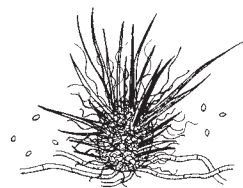
REVERSE: BLACKISH



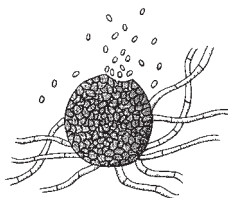
*Pseudallescheria/
Scedosporium* (p. 222)



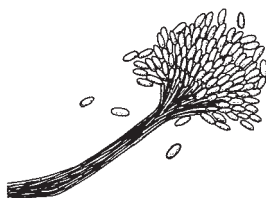
Lomentaspora prolificans
(formerly *Scedosporium
prolificans*) (p. 225)



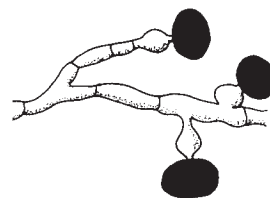
Chaetomium (p. 255)



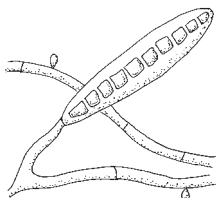
Phoma (p. 256)



Graphium (p. 242)



Nigrospora (p. 254)



Trichophyton ajelloi (p. 281)

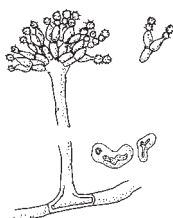
THERMALLY MONOMORPHIC MOULDS

SURFACE: TAN TO BROWN*

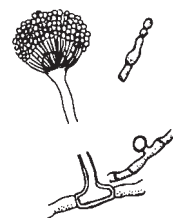
Having small conidia



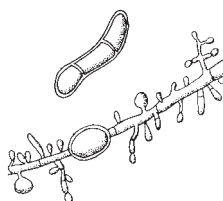
Aspergillus versicolor (p. 301)



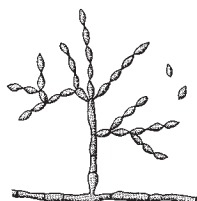
Aspergillus calidoustus (p. 302)



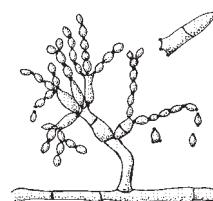
Aspergillus terreus (p. 307)



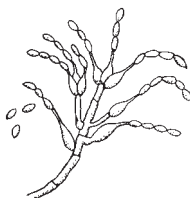
Trichophyton tonsurans
(p. 273)



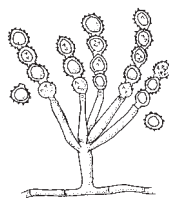
Cladophialophora carrionii
(p. 219)



Cladosporium spp. (p. 217)



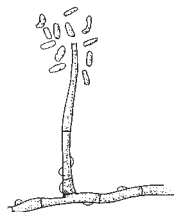
Paecilomyces variotii (p. 310)



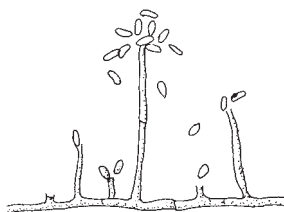
Scopulariopsis brevicaulis
(p. 312)



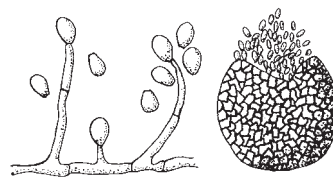
Verticillium (p. 320)



Phaeoacremonium (p. 214)



Phialemonium (p. 215)



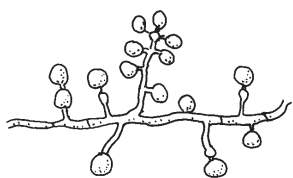
Pseudallescheria/
Scedosporium (p. 222)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

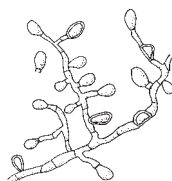
THERMALLY MONOMORPHIC MOULDS

SURFACE: TAN TO BROWN* (*continued*)

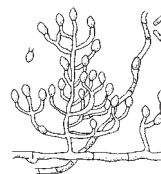
Having small conidia (continued)



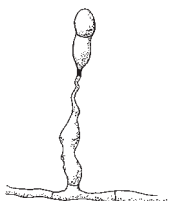
*Emmonsia*** (p. 317)



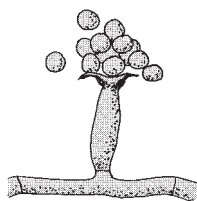
Chrysosporium (p. 326)



Sporotrichum (p. 329)



Ochroconis (p. 226)

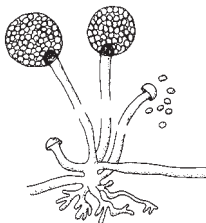


Pleurostomophora richardsiae
(p. 213)

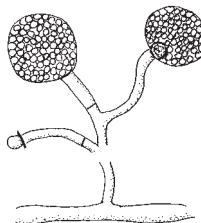


Botrytis (p. 240)

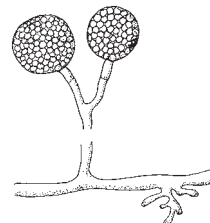
Having large conidia or sporangia



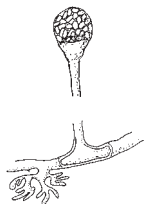
Rhizopus (p. 188)



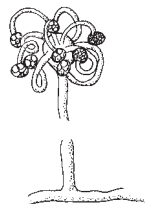
Mucor (p. 189)



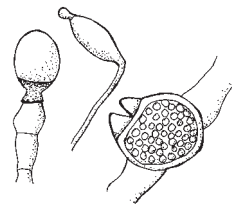
Rhizomucor (p. 190)



Apophysomyces (p. 193)



Cokeromyces (p. 196)



Basidiobolus (p. 200)

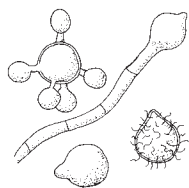
* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

** Large adiaconidia form at $\geq 37^\circ\text{C}$.

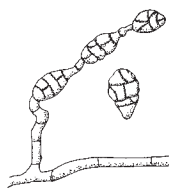
THERMALLY MONOMORPHIC MOULDS

SURFACE: TAN TO BROWN* (*continued*)

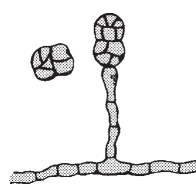
Having large conidia or sporangia (continued)



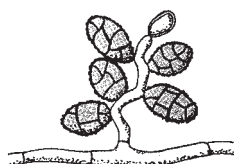
Conidiobolus (p. 201)



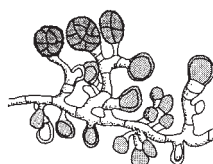
Alternaria (p. 249)



Stemphylium (p. 251)



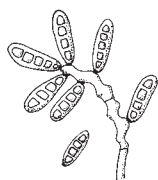
Ulocladium (p. 250)



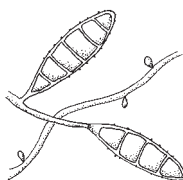
Epicoccum (p. 253)



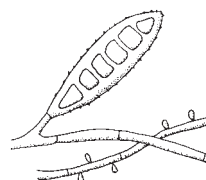
Curvularia (p. 243)



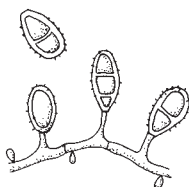
Bipolaris (p. 244)



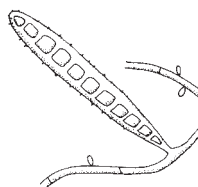
Microsporium gypseum
(p. 265)



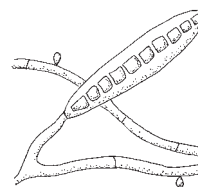
Microsporium cookei (p. 264)



Microsporium nanum (p. 267)



Microsporium vanbreuseghemii
(p. 268)



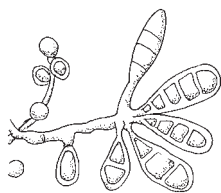
Trichophyton ajelloi (p. 281)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

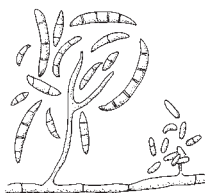
THERMALLY MONOMORPHIC MOULDS

SURFACE: TAN TO BROWN* (*continued*)

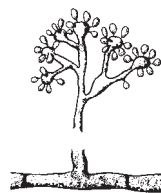
Having large conidia or sporangia (*continued*)



Epidermophyton floccosum
(p. 282)

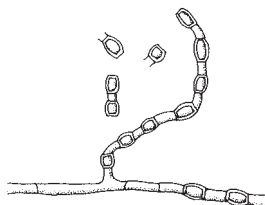


Fusarium (p. 322)

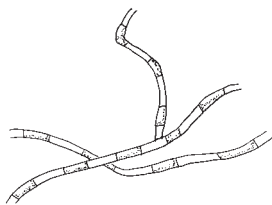


Botrytis (p. 240)

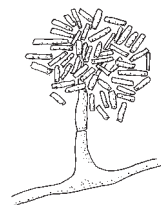
Having arthroconidia



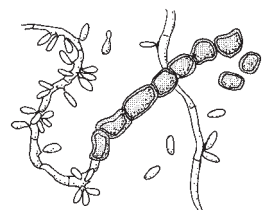
Coccidioides (p. 287)



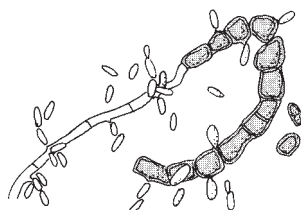
Malbranchea (p. 289)



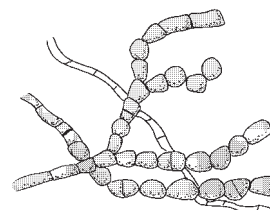
Hormographiella (p. 292)



Aureobasidium pullulans
(p. 234)



Hormonema dematioides
(p. 237)



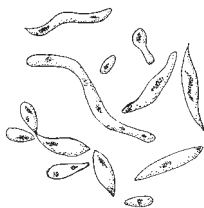
Neoscytalidium (p. 238)

* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

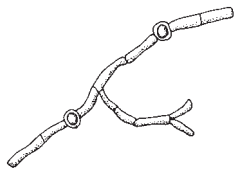
THERMALLY MONOMORPHIC MOULDS

SURFACE: TAN TO BROWN* (*continued*)

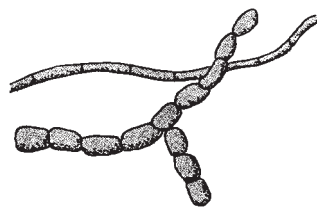
Having miscellaneous microscopic morphology



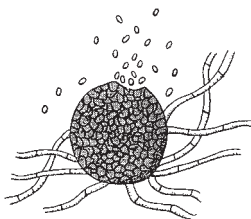
Ustilago (p. 161)



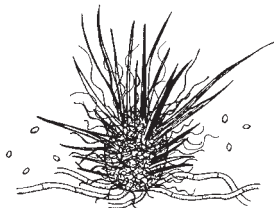
Madurella mycetomatis
(p. 231)



Madurella grisea (p. 232)



Phoma (p. 256)

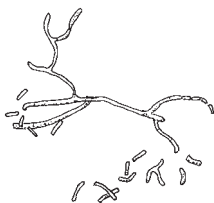


Chaetomium (p. 255)

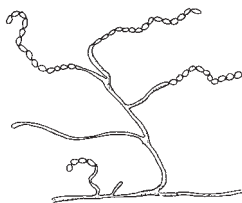
* Also see p. 169, as several thermally dimorphic fungi may fit this description at 25–30°C.

THERMALLY MONOMORPHIC MOULDS

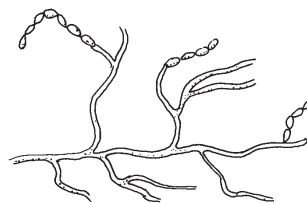
SURFACE: YELLOW TO ORANGE



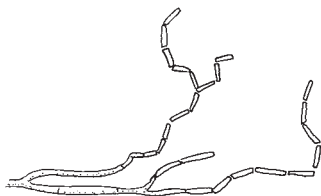
*Nocardia** (p. 116)



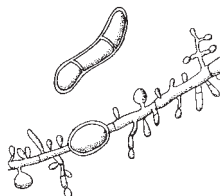
*Streptomyces** (p. 119)



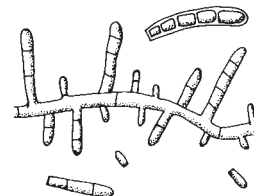
*Actinomadura** (p. 120)



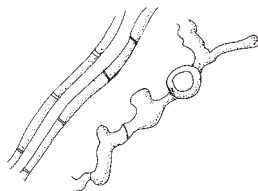
*Nocardiosis** (p. 121)



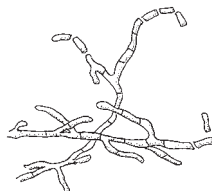
Trichophyton tonsurans
(p. 273)



Trichophyton terrestre (p. 274)



Microsporium ferrugineum
(p. 269)



Trichophyton soudanense
(p. 276)

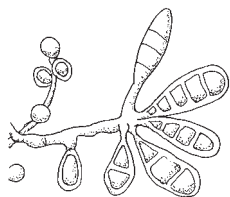


Trichophyton verrucosum
(p. 279)

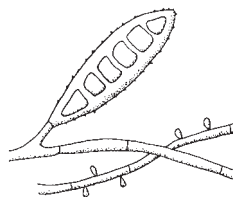
* These organisms are filamentous bacteria.

THERMALLY MONOMORPHIC MOULDS

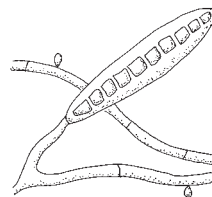
SURFACE: YELLOW TO ORANGE (*continued*)



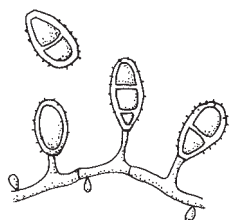
Epidermophyton floccosum
(p. 282)



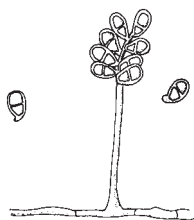
Microsporum cookei (p. 264)



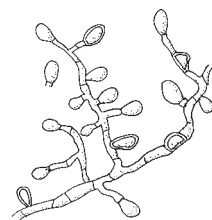
Trichophyton ajelloi (p. 281)



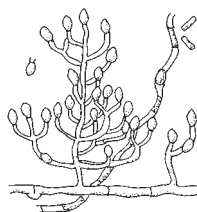
Microsporum nanum (p. 267)



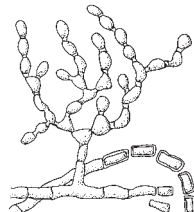
Trichothecium (p. 325)



Chrysosporium (p. 326)



Sporotrichum (p. 329)



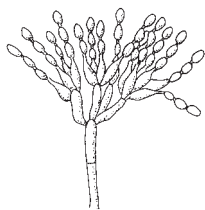
Chrysonilia sitophila (p. 331)



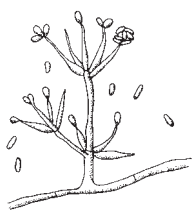
Aspergillus flavus (p. 298)

THERMALLY MONOMORPHIC MOULDS

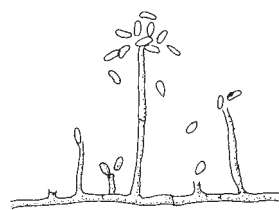
SURFACE: YELLOW TO ORANGE (*continued*)



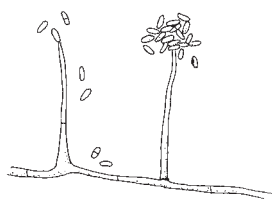
*Talaromyces marneffeii***
(p. 176)



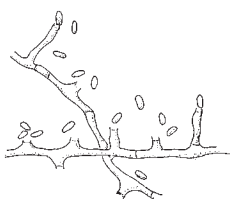
Verticillium (p. 320)



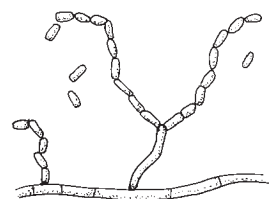
Phialemonium (p. 215)



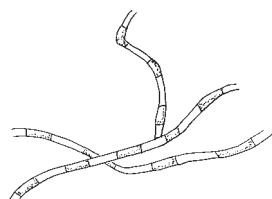
Acremonium (p. 321)



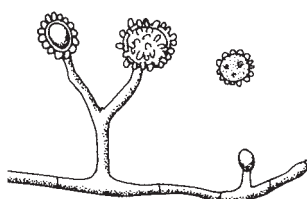
Lecythophora (p. 324)



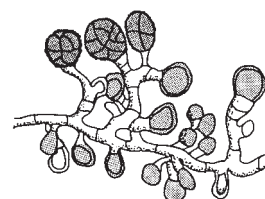
Arthrographis (p. 291)



Malbranchea (p. 289)



Sepedonium (p. 330)

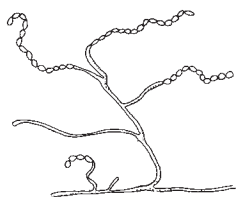


Epicoccum (p. 253)

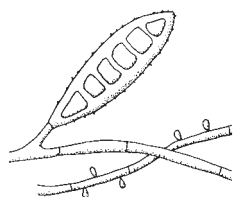
** *T. marneffeii* is thermally dimorphic.

THERMALLY MONOMORPHIC MOULDS

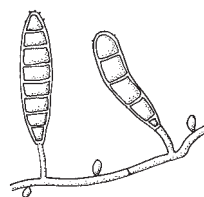
SURFACE: PINK TO VIOLET



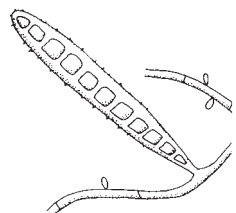
*Streptomyces** (p. 119)
(or *Actinomadura* (p. 120))



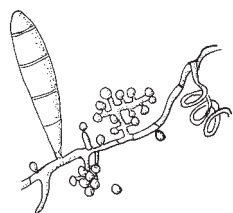
Microsporium cookei (p. 264)
(grape-red)



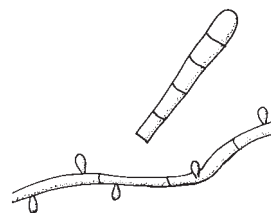
Microsporium gallinae (p. 266)



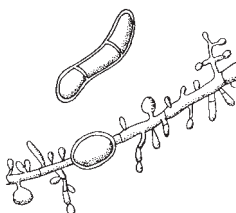
Microsporium vanbreuseghemii
(p. 268)



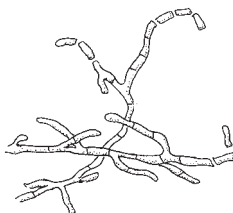
Trichophyton mentagrophytes
(p. 270)



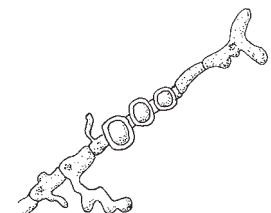
Trichophyton megninii (p. 275)



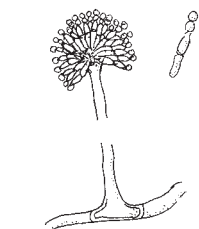
Trichophyton tonsurans
(p. 273)



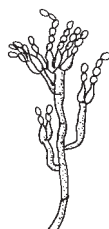
Trichophyton soudanense
(p. 276)



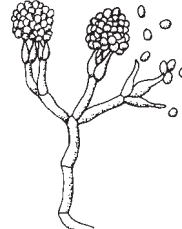
Trichophyton violaceum
(p. 280)



Aspergillus versicolor (p. 301)



Purpureocillium lilacinum
(p. 311)

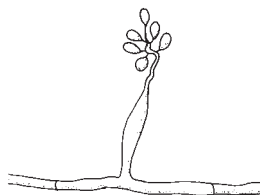


Gliocladium (p. 315)

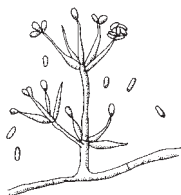
* *Streptomyces* and *Actinomadura* are filamentous bacteria.

THERMALLY MONOMORPHIC MOULDS

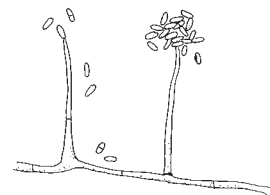
SURFACE: PINK TO VIOLET (*continued*)



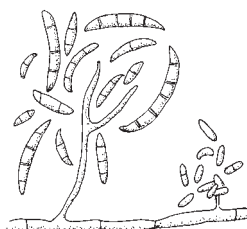
Beauveria (p. 319)



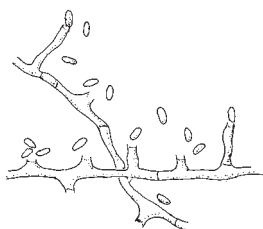
Verticillium (p. 320)



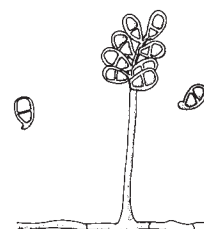
Acremonium (p. 321)



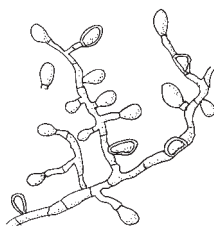
Fusarium (p. 322)



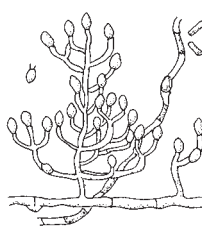
Lecythophora (p. 324)



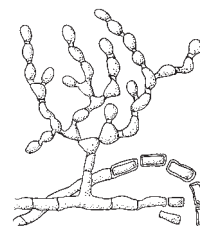
Trichothecium roseum (p. 325)



Chrysosporium (p. 326)



Sporotrichum (p. 329)

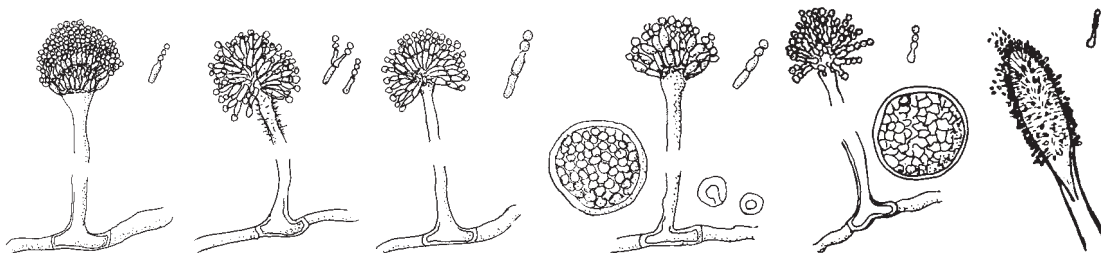


Chrysonilia sitophila (p. 331)

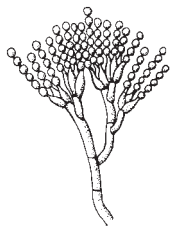
THERMALLY MONOMORPHIC MOULDS

SURFACE: GREEN

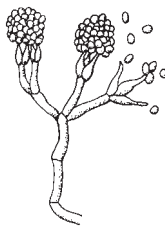
REVERSE: LIGHT



Aspergillus fumigatus, *A. flavus*, *A. versicolor*, *A. nidulans*, *A. glaucus*, or *A. clavatus* (pp. 295–308)



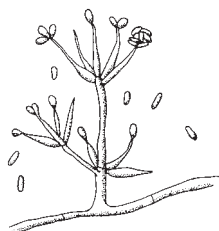
Penicillium (p. 309)



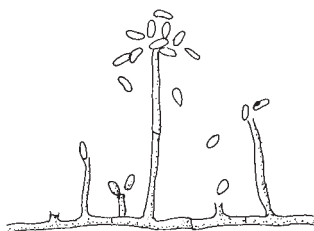
Gliocladium (p. 315)



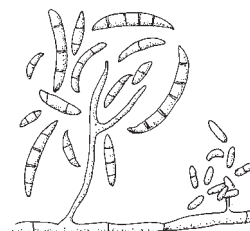
Trichoderma (p. 316)



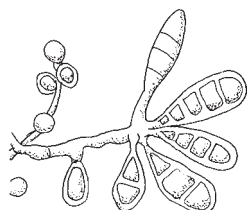
Verticillium (p. 320)



Phialemonium (p. 215)



Fusarium (p. 322)

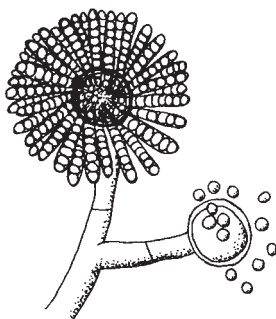


Epidermophyton floccosum
(p. 282)

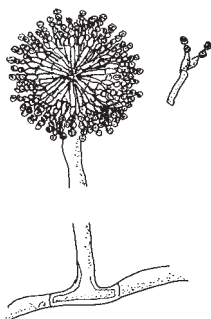
THERMALLY MONOMORPHIC MOULDS

SURFACE: DARK GRAY OR BLACK

REVERSE: LIGHT



Syncephalastrum (p. 199)



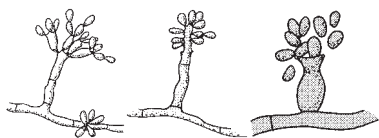
Aspergillus niger (p. 297)

THERMALLY MONOMORPHIC MOULDS

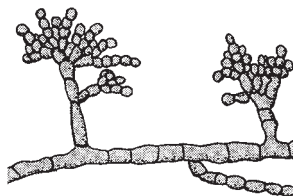
SURFACE: GREENISH, DARK GRAY, OR BLACK

REVERSE: DARK

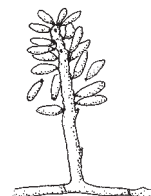
*Having small conidia**



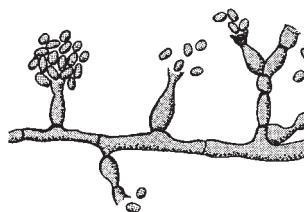
Fonsecaea pedrosoi (p. 207)



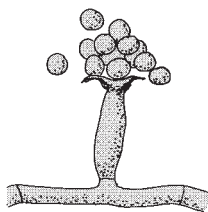
Fonsecaea compacta (p. 209)



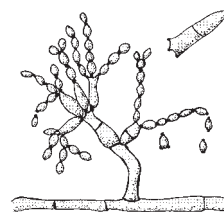
Rhinocladiella (p. 210)



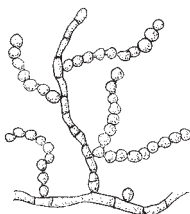
Phialophora verrucosa (p. 211)



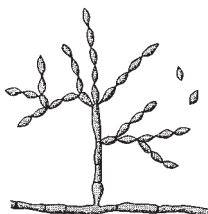
Pleurostomophora richardsiae
(p. 213)



Cladosporium sp. (p. 217)



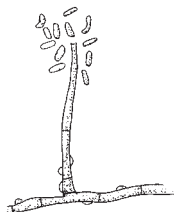
Cladophialophora boppii
(p. 221)



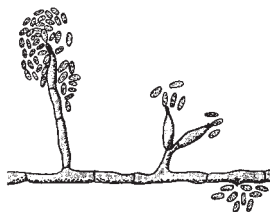
Cladophialophora carrionii
(p. 219)



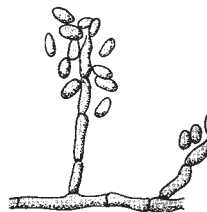
Cladophialophora bantiana
(p. 220)



Phaeoacremonium
parasiticum (p. 214)



Exophiala jeanselmei complex
(p. 228)



Exophiala dermatitidis (p. 229)

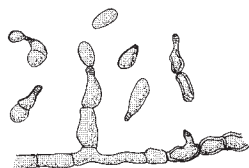
* Also see p. 178, as *Sporothrix schenckii* may fit this description at 25–30°C.

THERMALLY MONOMORPHIC MOULDS

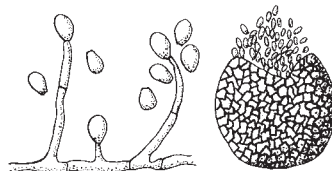
SURFACE: GREENISH, DARK GRAY, OR BLACK

REVERSE: DARK (*continued*)

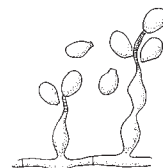
*Having small conidia** (*continued*)



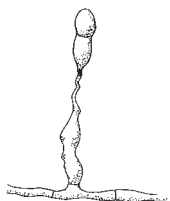
Hortaea werneckii (p. 230)



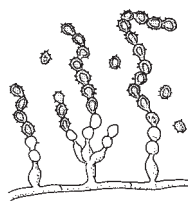
Pseudallescheria/
Scedosporium (p. 222)



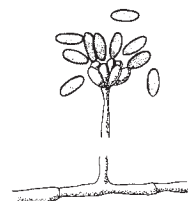
Lomentaspora prolificans
(formerly *Scedosporium*
prolificans) (p. 225)



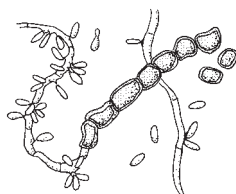
Ochroconis (p. 226)



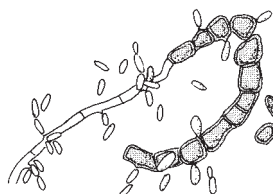
Scopulariopsis brumptii
(p. 312)



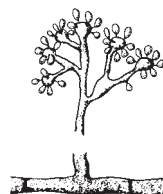
Stachybotrys (p. 241)



Aureobasidium pullulans
(p. 234)



Hormonema dematioides
(p. 237)



Botrytis (p. 240)

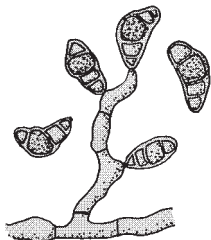
* Also see p. 178, as *Sporothrix schenckii* may fit this description at 25–30°C.

THERMALLY MONOMORPHIC MOULDS

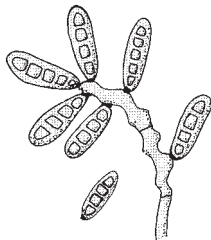
SURFACE: GREENISH, DARK GRAY, OR BLACK

REVERSE: DARK (*continued*)

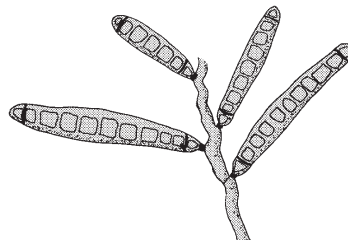
Having large conidia



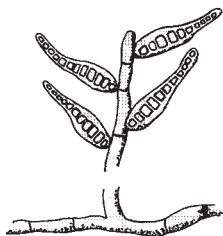
Curvularia (p. 243)



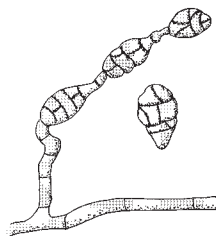
Bipolaris (p. 244)



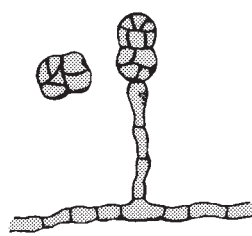
Exserohilum spp. (p. 247)



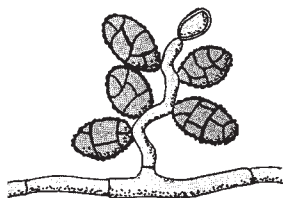
Helminthosporium (p. 248)



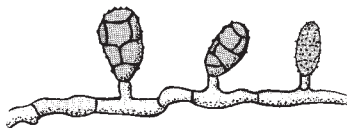
Alternaria (p. 249)



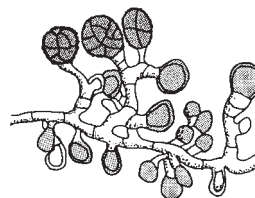
Stemphylium (p. 251)



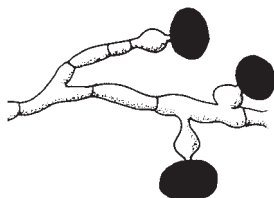
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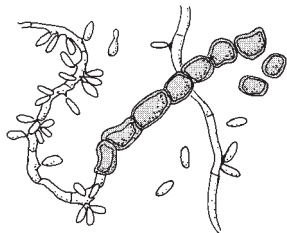
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THERMALLY MONOMORPHIC MOULDS

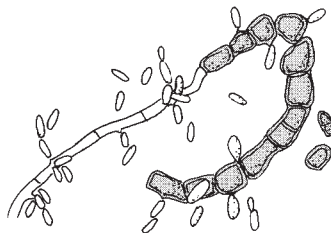
SURFACE: GREENISH, DARK GRAY, OR BLACK

REVERSE: DARK (*continued*)

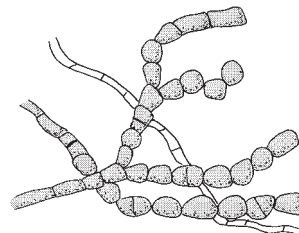
Having arthroconidia



Aureobasidium pullulans
(p. 234)

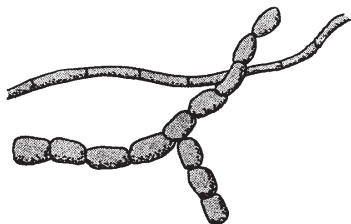


Hormonema dematioides
(p. 237)

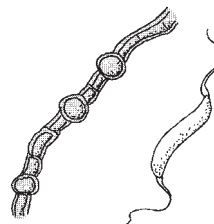


Neoscytalidium (p. 238)

Having only hyphae (with or without chlamydoconidia)

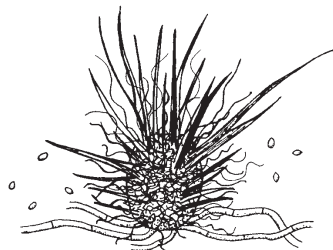


Madurella grisea (p. 232)

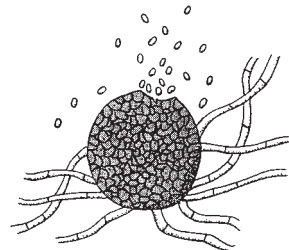


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Introduction to Filamentous Bacteria

Some of the aerobic actinomycetes resemble fungi in that they form filaments that are well developed and branched (commonly referred to as hyphae). However, the results of cell wall analysis, lack of a membrane-bound nucleus, lack of mitochondria, small size, and susceptibility to antibacterial agents have long defined these organisms as bacteria rather than fungi.

The aerobic actinomycetes are Gram positive and have filaments that are 1 μm or less in diameter. Some may be partially acid-fast when stained by a modified Kinyoun method (p. 387). These organisms grow on routine bacteriology and mycology media without some particular antibacterial additives and on routine mycobacteriology media. In order to isolate these organisms from contaminated specimens, it is often imperative to utilize selective media (see p. 395), along with nonselective media, to enhance their recovery. The colonies are usually glabrous and often become covered with aerial hyphae, forming a chalky or powdery coat. Visualization of aerial hyphae can be enhanced by examining the colony with a dissecting microscope. The microscopic morphology is best observed by slide culture on minimal medium, such as cornmeal-Tween 80 agar or 2% plain agar prepared with tap water.

In addition to morphology and staining characteristics, a battery of traditional observations and biochemical tests can be useful for preliminary differentiation of the organisms in this group (see Tables 2 and 3, pp. 115 and 118). However, nucleic acid-based molecular methods are strongly recommended, and very often essential, for accurate species identification. These procedures are performed in many reference laboratories.

The most commonly encountered pathogenic aerobic actinomycetes belong to the genus *Nocardia*; they cause pulmonary, systemic, and cutaneous diseases, including mycetomas (swollen tumorlike subcutaneous lesions that yield granular pus through draining sinuses). Members of the other genera are etiologic agents of mycetoma and occasionally other infections. Many *Streptomyces* spp. are considered nonpathogenic saprobes.

*For a complete review of the aerobic actinomycetes, see
Conville and Witebsky, 2015*

TABLE 2 Differentiation of filamentous aerobic actinomycetes encountered in clinical specimens^{a,b}

Organism	Colony on Sabouraud dextrose agar	Aerial hyphae	Fragmentation of hyphae ^c	Acid-fast (partially) ^d	Growth with lysozyme	Hydrolysis of starch
<i>Nocardia</i> spp.	White to pink or orange; glabrous or powdery; wrinkled	+	+	+	+	0 ^V
<i>Nocardiosis dassonvillei</i>	Yellowish; heaped; wrinkled; chalky or velvety	+	+	0	0	+
<i>Actinomadura madurae</i>	White, tan, pink, red, or orange; glabrous; wrinkled; hard; adherent; slow growing	0 ^V	0	0	0	+
<i>Actinomadura pelletieri</i>	Bright red; heaped; glabrous	0 ^V	0	0	0	0
<i>Streptomyces somaliensis</i>	Cream to brown or black; slow growing; leathery; folded	+	0	0	0	V
<i>Streptomyces griseus</i>	White or grayish; glabrous, chalky, or velvety	+	0	0 (spores often +)	0	+
<i>Streptomyces</i> spp.	Variety of colors; glabrous, chalky, or velvety	+	0	0 (spores often +)	V	+ ^V

^a Molecular methods are highly recommended, and often essential, for definitive identification. Abbreviations: +, positive; 0, negative; V, variable.

^b Other clinically encountered aerobic actinomycetes are not filamentous and are more likely handled in the bacteriology laboratory.

^c Smears must be made with care to detect spontaneous fragmentation rather than that caused by trauma; slide culture may be best for evaluation of hyphal fragmentation.

^d Stained by modified acid-fast method (p. 387).

***Nocardia* spp.**

PATHOGENICITY: Cause nocardiosis, which symptomatically may be similar to tuberculosis or actinomycosis. The disease, especially in immunocompromised hosts, most frequently begins as a pulmonary infection and disseminates to the brain, kidneys, and other organs. Traumatic inoculation is usually the source of infection in immunocompetent hosts, and skin lesions or subcutaneous abscesses occur. Mycetomas, which typically involve the subcutaneous tissues, muscle, tendon, and bone, usually develop in the extremities. *Nocardia* species are the most common cause of actinomycotic mycetoma. Approximately 88 species have been identified, and only about half have been isolated from humans. The organisms are ubiquitous in nature and may therefore be encountered as colonizers or specimen contaminants.

RATE OF GROWTH: Moderately rapid; usually mature in 7–9 days, but should be incubated for at least 2–3 weeks in case growth is delayed. Optimal growth is at 35–37°C. Grow on Sabouraud dextrose agar (SDA) without antibiotics and on Lowenstein-Jensen and Middlebrook 7H11 media (frequently survive decontamination procedures used for isolation of acid-fast bacilli when exposure to the reagents does not exceed 15 minutes). Excellent recovery occurs on nonselective and selective buffered charcoal-yeast extract agar (the formulation containing vancomycin rather than cefamandole is preferred) and on other selective media such as colistin-nalidixic acid agar and modified Thayer-Martin agar (containing vancomycin, colistin, and nystatin). Inclusion of a selective medium is usually essential for inhibition of faster-growing organisms and recovery of *Nocardia* spp. from nonsterile sites. Media containing chloramphenicol or gentamicin are not recommended, as they may prevent growth of *Nocardia* spp.

COLONY MORPHOLOGY: Grow aerobically on SDA without antibiotics, forming raised, irregular, folded colonies varying from white to orange, depending on the species. May be glabrous, velvety, or develop a white chalky coating of short aerial mycelia (Color Plates 38 to 41.) If aerial hyphae are sparse, they may be best observed with a dissecting microscope.

MICROSCOPIC MORPHOLOGY: Delicate, branching, often beaded, intertwining filaments that fragment into bacillary and coccoid forms; best exhibited on slide culture using a minimal medium, such as cornmeal-Tween 80 agar or tap water agar. They are Gram positive and often, but not always, partially acid-fast (use a modified Kinyoun method [p. 387]). Young primary cultures may be the most acid-fast; or acid-fastness may be enhanced on Middlebrook 7H11 medium or by growing cultures for 3–4 weeks in a proteinaceous medium, such as litmus milk or bromocresol purple milk.

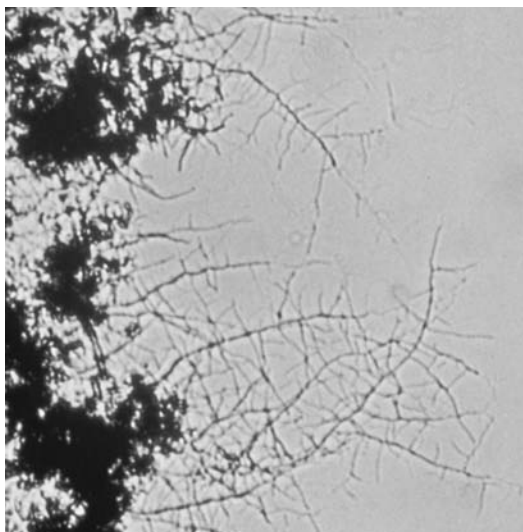
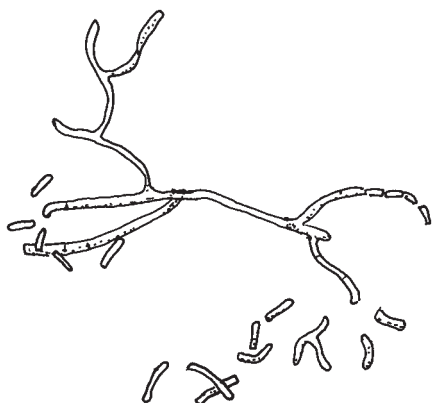
See Table 2 (p. 115) for differentiation of filamentous aerobic actinomycetes and Table 3 (p. 118) for presumptive phenotypic identification of the most commonly encountered *Nocardia* spp.

(continued on following page)

***Nocardia* spp. (continued)**

Note: Clinical isolates formerly identified as *Nocardia asteroides* complex are now separated (based on molecular studies) into *N. abscessus*, *N. cyriacigeorgica*, *N. farcinica*, *N. nova*, and *N. transvalensis* complex. *N. asteroides* (*sensu stricto*) is seldom isolated from humans and is considered nonpathogenic. Use of molecular methods for definitive identification of all etiologically significant *Nocardia* isolates is highly recommended and often essential.

In vitro antimicrobial susceptibility testing is recommended in cases of disseminated or refractory infection or if the patient cannot tolerate sulfonamides (the usual drug of choice for treating nocardiosis). Susceptibility profiles may assist in the phenotypic identification of *Nocardia* species (Brown-Elliott et al., 2006); conversely, accurate identification may predict the isolate's probable susceptibility profile. Only laboratories having extensive experience and expertise in performing susceptibility tests on *Nocardia* ought to do so; all others are advised to send their isolates to a qualified reference laboratory.



From Joseph Staneck, Aerobic actinomycetes: *Nocardia* and related organisms, ASM Teleconference of 9 August 1994.

For further information, see
 Brown-Elliott et al., 2006
 Conville and Witebsky, 2015
 Girard et al., 2017
 Saubolle, 2009
 Wilson, 2012
 Wolfsohn et al., 2009

TABLE 3 Phenotypic characteristics of most common clinically encountered *Nocardia* spp.^{a,b}

Species	Urease	Hydrolysis of: ^c					Utilization of:		Nitrate reduction	Arylsulfatase 14 days	Opacity on MB 7H11 ^d	Growth at 45°C
		Casein	Tyrosine	Xanthine	Hypoxanthine	Esculin	Acetamide	Citrate				
<i>N. abscessus</i>	+	0	0	0	0	0	0	+	+	0	0	0
<i>N. nova</i> ^e	+	0	0	0	0	0	0	0	+	+	0	0
<i>N. cyriacigeorgica</i>	0	0	0	0	0	0	+	0	+	NA	0	+
<i>N. farcinica</i>	+	0	0	0	0	+	+	0	0	0	+	+
<i>N. transvalensis</i> ^f	+	0	0	0	+	+	0	+	+	0	0	V
<i>N. otitidiscaviarum</i>	+	0	0	+	+	+	0	0	+	NA	0	V
<i>N. brasiliensis</i>	+	+	+	0	+	+	0	+	+	0	0	0
<i>N. pseudobrasiliensis</i>	+	+	+	0	+	+	NA	+	0	0	0	0

^a References: Brown-Elliott et al., 2006; Wolfsohn et al., 2009. Abbreviations: +, positive; 0, negative; V, variable; NA, not available; MB 7H11, Middlebrook 7H11 agar.

^b Use of molecular methods for definitive identification of all etiologically significant *Nocardia* isolates is highly recommended and often imperative. *In vitro* antimicrobial susceptibility profiles may also contribute to identification, but the testing should only be performed by laboratories with extensive experience and expertise in susceptibility testing of *Nocardia* spp.

^c Within 2 weeks at 25–30°C.

^d Within 10 days (see p. 415).

^e *N. nova* complex includes *N. africana*, *N. kruczakiae*, and *N. veterana*.

^f In the United States, *N. wallacei* is the most commonly isolated member of the *N. transvalensis* complex (see Conville et al., 2008); *N. blacklockiae* is also included in this complex.

Streptomyces spp.

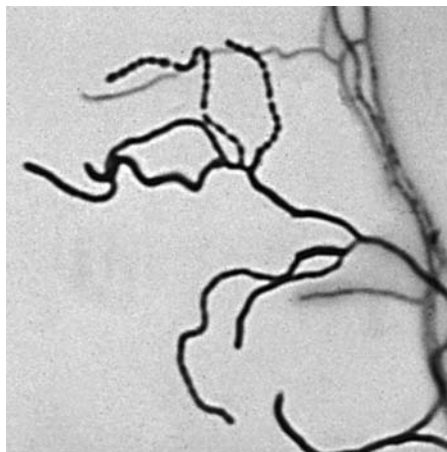
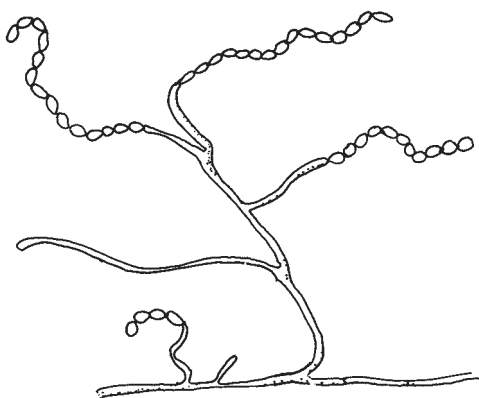
PATHOGENICITY: Most of the many (>500) *Streptomyces* spp. are considered non-pathogenic contaminants or colonizers. The major exception is *S. somaliensis*, which causes mycetomas and occasionally invasive infections. Other species only rarely cause disease. *S. griseus* (also known as *S. anulatus*) is the most commonly recovered species from human specimens, but only occasionally appears to be an etiologic agent of infection; it is better known as the original source of streptomycin. Isolates are usually identified only to genus level (molecular testing is required for even a presumptive species identification); more studies are needed to improve identification.

RATE OF GROWTH: Rapid or moderate; mature in 4–10 days. Optimum growth usually occurs at 25–30°C.

COLONY MORPHOLOGY: Surface is slightly folded, hard, leathery; may develop a fine chalky or powdery aerial mycelium. Many strains have various pigments of gray, black, brown, tan, orange, rose, red, or occasionally green. Culture often produces the characteristic odor of freshly tilled soil. (Color Plates 42 and 43.)

MICROSCOPIC MORPHOLOGY: Hyphae are long, thin (1 μm or less in diameter), and abundantly branching, with filaments that may be straight, wavy, or spiral. Small, oblong conidia (also referred to as spores) are produced at distinct points on the filament, often in chains; this is best observed on slide culture. Conidia may stain partially acid-fast; some species do not form conidia readily.

See Table 2 (p. 115) for differentiation of filamentous aerobic actinomycetes.



Courtesy of Morris Gordon.

For further information, see
Kapadia et al., 2007
Saubolle, 2009
van de Sande, 2013

***Actinomadura* spp.**

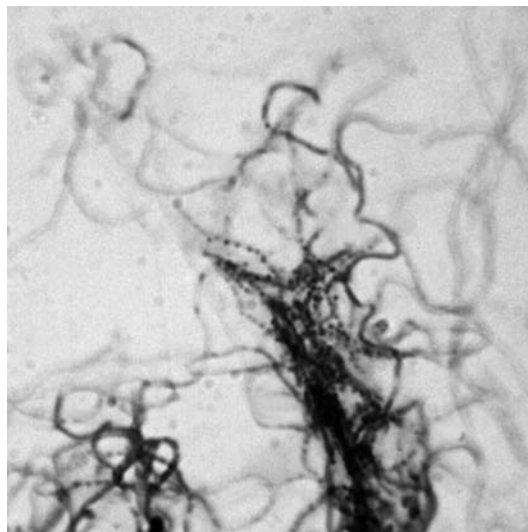
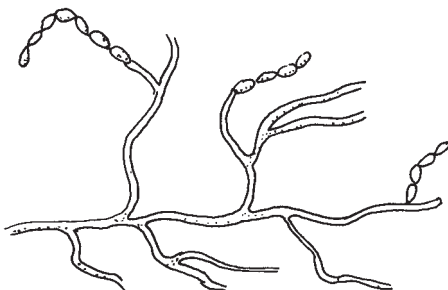
PATHOGENICITY: A frequent cause of mycetoma. Although there are many species, *A. madurae* and *A. pelletieri* are the only two species of clinical importance. They are closely related, but *A. madurae* is the more commonly encountered and has on rare occasion caused severe, deeply invasive infections (e.g., pneumonia, peritonitis, and bacteremia) in immunocompromised patients. It has also been isolated as a saprobic contaminant from a variety of body sites.

RATE OF GROWTH: Usually rapid on Lowenstein-Jensen (LJ) medium; slower on Sabouraud dextrose agar. Optimum growth is at 35–37°C.

COLONY MORPHOLOGY: Waxy, folded, membranous, or mucoid. May be white, tan, pink, orange, or red. White aerial hyphae may develop after 2 weeks of incubation; best seen on LJ medium.

MICROSCOPIC MORPHOLOGY: Narrow, abundantly branched filaments (0.5–1 μm in diameter) that are Gram positive, non-acid-fast, and nonfragmenting. Short chains of round conidia may be produced from limited portions of the aerial hyphae; this is best observed on slide culture. It is very similar to *Streptomyces*.

See Table 2 (p. 115) for differentiation of filamentous aerobic actinomycetes.



For further information, see
McNeil and Brown, 1994, pp. 380–381
Saubolle, 2009
van de Sande, 2013

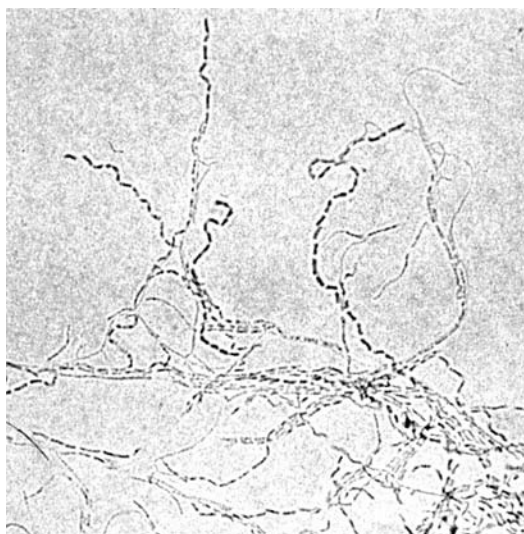
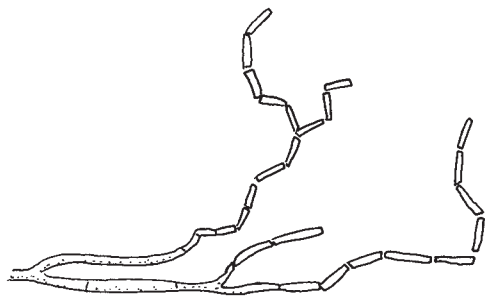
PATHOGENICITY: Occasional etiologic agent of mycetoma; also considered a rare potential cause of cutaneous, pulmonary, conjunctival, and blood infections. There have been relatively few reports of clinical disease.

RATE OF GROWTH: Moderately rapid; mature in 4–10 days.

COLONY MORPHOLOGY: Yellowish, heaped, irregularly wrinkled. Aerial hyphae may develop to form a velvety coating. A recently described species, *N. synnemataformans*, forms a deep pimento-red colony.

MICROSCOPIC MORPHOLOGY: Narrow filaments (1 μm or less in diameter) that are long, extensive, and sometimes branched; they are Gram positive and non-acid-fast. The filaments fragment into chains of arthroconidia, giving a characteristic zigzag appearance. The total length of the aerial hyphae turns into conidial chains, in contrast to *Actinomadura* spp. and *Streptomyces* spp., which produce conidial chains only at distinct parts of the hyphae.

See Table 2 (p. 115) for differentiation of filamentous aerobic actinomycetes.



From McNeil and Brown, 1994.

For further information, see
Kwon-Chung and Bennett, 1992, pp. 587–588
McNeil and Brown, 1994, p. 394
Saubolle, 2009

Yeasts and Yeastlike Organisms

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Introduction to Yeasts and Yeastlike Organisms

In this guide, the terms “yeast” and “yeastlike” refer to unicellular organisms that generally reproduce by budding. If the buds (blastoconidia) elongate and remain attached to the parent cell, they form chains known as pseudohyphae. Some of the organisms included here produce true septate hyphae, while others form no hyphal elements of any sort. A few are capable of producing ascospores (the sexual state). Organisms that are actually algae but that grow in a yeastlike manner are traditional members of this group.

Colonies are smooth and glabrous and may be moist or dry; they are usually white to cream colored, but some are tan, pinkish, or orangey.

The ability to produce pseudohyphae, true hyphae, and/or terminal chlamydospores and the shape and arrangement of blastoconidia are used along with other morphologic characteristics and biochemical tests to identify the yeasts to the genus and species levels. A number of commercial systems are available for biochemical testing (see pp. 375–376). Microscopic morphology is studied on agar, such as cornmeal-Tween 80 agar, by using the Dalmau method (p. 409), which ensures the decreased oxygen environment required for the production of structures upon which identification depends. Isolates of *Candida* spp. from patients being treated with antimicrobial agents occasionally exhibit atypical microscopic morphologies, most often exceptionally large yeast cells. Identification of *Candida* species and other yeasts is achieved biochemically on a wide range of systems characterizing differential assimilation properties. Identification of *Candida* spp. and other yeasts is being increasingly performed by proteomics using MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) technology (see p. 353).

Yeasts are the most common fungi isolated in the clinical laboratory. They are ubiquitous in our environment and also live as normal inhabitants in our bodies. Thus, it is often difficult to determine the clinical significance of an isolate. Implication of the yeast as the etiologic agent of infection often requires repeated recovery from the site and direct microscopic demonstration of the yeast in infected tissue. The yeasts and yeastlike organisms are considered

opportunistic pathogens, causing disease in patients (i) with an impairment of the body's immune system; (ii) receiving prolonged treatment with antibiotics, corticosteroids, or cytotoxic drugs; (iii) with intravascular catheters; (iv) with diabetes mellitus; or (v) who are intravenous illicit drug users.

Candidiasis (also called candidosis) is by far the most common fungal infection (other than ringworm) seen in humans. Mucocutaneous infections are seen in individuals with defects in cell-mediated immunity, while systemic infections are primarily seen in neutropenic patients. Although *Candida* spp. are often isolated from lower respiratory specimens, they are seldom of clinical significance; definitive diagnosis of lower respiratory infection due to *Candida* requires histologic confirmation.

For further information, see

Anaissie et al. (ed.), 2009, chapters 8, 9, 10, and index

Kullberg and Arendrup, 2015

Kurtzman et al. (ed.), 2010

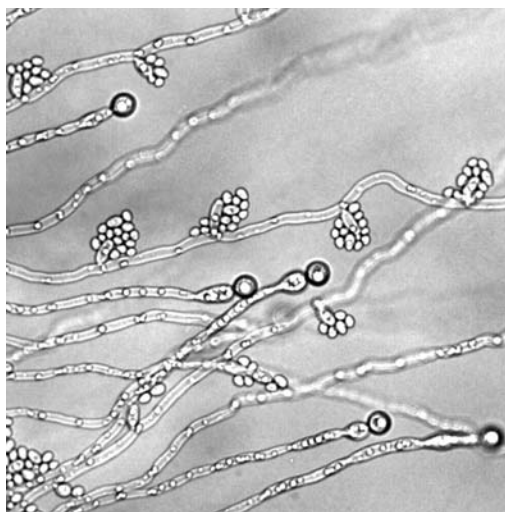
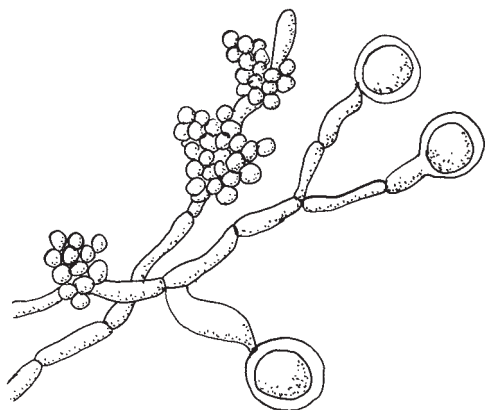
PATHOGENICITY: Most common cause of candidiasis (candidosis) (see p. 60), which is an acute, subacute, or chronic infection involving any part of the body. This organism is found as part of the normal flora in the skin, mouth, vaginal tract, and gastrointestinal tract (therefore, it is often present in stools without significance).

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Cream colored, pasty, smooth. On enriched media (e.g., blood agar or chocolate agar), extensions commonly called “feet” develop at the border of the colony. (Color Plate 44.) Colonies are green on CHROMagar Candida. (Color Plate 45.)

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval ($3.5\text{--}7 \times 4\text{--}8 \mu\text{m}$). On cornmeal-Tween 80 agar (Dalmau plate [p. 409]) at 25°C for 72 h, pseudohyphae (and some true hyphae) form with clusters of round blastoconidia at the septa. Large, thick-walled, usually single terminal chlamydospores are characteristically formed; they are most likely to be seen near the edge of the coverslip. Chlamydospore formation is inhibited at 30–37°C. *C. albicans* yields a positive reaction on the germ tube test (p. 378), as does *Candida dubliniensis*; to differentiate the two species, see Table 6 (p. 132).

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 37
Kwon-Chung and Bennett, 1992, pp. 310–316
Rippon, 1988, pp. 536–570

TABLE 4 Characteristics of the genera of clinically encountered yeasts and yeastlike organisms^a

Organism	On cornmeal-Tween 80 agar at 25°C					Asco-spores	Sporangia	Capsule	Urease	Growth:		
	Pseudo-hyphae	True hyphae	Blasto-conidia along hyphae	Arthro-conidia	Annello-conidia					With cycloheximide at 25°C	On SDA at 37°C	In Sabouraud broth
<i>Candida</i>	+	Few	+	0	0	0	0	0	0 ^V	V	+ ^V	Some species show surface growth
<i>Rhodotorula</i>	0 ^R	0		0	0	0	0	V	+	0 ^V	+ ^V	NSG
<i>Cryptococcus</i>	0 ^R	0		0	0	0	0	+	+	0	V	NSG
<i>Saccharomyces</i>	V	0		0	0	+	0	0	0	0	+	NSG
<i>Wickerhamomyces</i> (formerly <i>Pichia</i> , <i>Hansenula</i>)	0 ^V	0		0	0	+	0	0	0	0	V	NSG
<i>Malassezia</i>	0 ^R	0 ^R		0	0	0	0	0	+	+ ^{W,V}	+ ^V	NSG
<i>Prototheca</i>	0	0		0	0	0	+	0 ^V	0	0	+ ^V	Surface growth
<i>Geotrichum</i>	0	+	0	+	0	0	0	0	0	0	0 ^W	Pellicle forms
<i>Trichosporon</i>	+	+	+	+	0	0	0	0	+	+ ^V	+ ^V	Pellicle forms
<i>Blastoschizomyces</i>	+	+	+	0 ^V	+	0	0	0	0	+	+	Pellicle forms

^a Abbreviations: SDA, Sabouraud dextrose agar; +, positive; 0, negative; V, species or strain variation; W, weak; R, rarely few rudimentary forms; NSG, no surface growth.

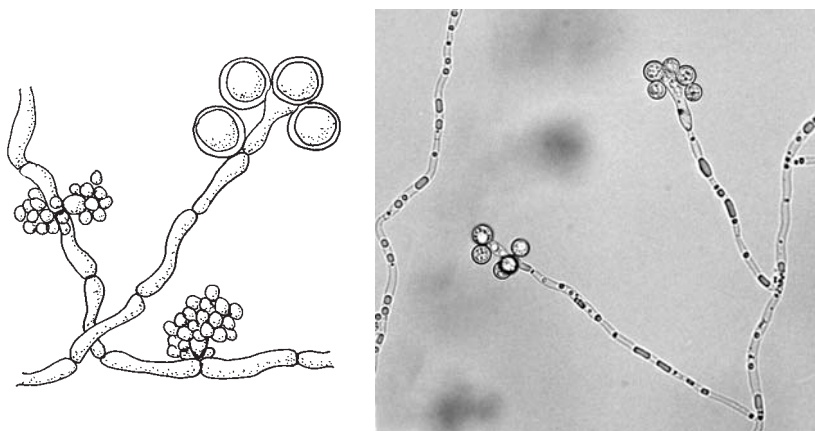
PATHOGENICITY: Primarily associated with recurrent erythematous oral candidiasis, and only occasionally with infections at other body sites, in HIV-infected patients. It also occasionally appears in bloodstream and other infections in immunocompromised patients who are HIV negative. The organism is widespread throughout the world and has been recovered from a variety of clinical specimens without evidence of infection. *In vitro* studies have shown that, upon repeated exposure to fluconazole, susceptible isolates are able to rapidly develop decreased susceptibility or frank resistance. It is seldom encountered in the clinical laboratory, especially since the advent of successful antiretroviral treatment for HIV infection.

RATE OF GROWTH: Rapid; mature within 3 days.

COLONY MORPHOLOGY: Cream colored, pasty, smooth. On enriched media (e.g., blood agar or chocolate agar), extensions commonly called “feet” develop at the border of the colony, as occurs with *Candida albicans* (Color Plate 44), but not as well when incubated in 5% CO₂. On CHROMagar Candida, colonies are green, but a lighter green than *C. albicans*; the shade differences are only discernible on initial isolation.

MICROSCOPIC MORPHOLOGY: Very similar to that of *C. albicans*. On cornmeal-Tween 80 agar (Dalmau plate [p. 409]) at 25°C for 72 h, forms pseudohyphae, and some true hyphae, with clusters of round blastoconidia at the septa. Large, thick-walled terminal chlamydospores characteristically form in pairs or small clusters (as opposed to *C. albicans*, which usually produces terminal chlamydospores singly). *C. dubliniensis* (like *C. albicans*) yields a positive reaction with the germ tube test (p. 378); to differentiate the two species, see Table 6 (p. 132).

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4; Pinjon et al., 2005; Sullivan and Coleman, 1998; Sullivan et al., 1999

TABLE 5 Characteristics of *Candida* spp. most commonly encountered in the clinical laboratory^a

Organism	Microscopic morphology on cornmeal-Tween 80 agar at 25°C	Growth:			Germ tubes	Urease (25°C)
		In Sabouraud broth	With cycloheximide at 25°C	On SDA at 37°C		
<i>C. albicans</i> <i>C. dubliniensis</i>	Pseudohyphae with terminal chlamydospores; clusters of blastoconidia at septa	NSG	+	+	+	0
<i>C. tropicalis</i>	Blastoconidia anywhere along pseudohyphae	Narrow surface film with bubbles	0 ^V	+	0	0
<i>C. parapsilosis</i>	Blastoconidia along curved pseudohyphae; giant mycelial cells	NSG	0	+	0	0
<i>C. lusitanae</i>	Short chains of elongate blastoconidia along curved pseudohyphae	NSG	0	+	0	0
<i>C. guilliermondii</i>	Fairly short, fine pseudohyphae; clusters of blastoconidia at septa	NSG	+	+	0	0
<i>C. kefyr</i> (<i>C. pseudotropicalis</i>)	Elongated blastoconidia resembling logs in a stream along pseudohyphae	NSG	+	+	0	0
<i>C. rugosa</i>	Pseudohyphae with elongated blastoconidia, some in chains	NSG	0	+	0	0
<i>C. zeylanoides</i>	Pseudohyphae give featherlike appearance at low power	Pellicle (delayed)	+	0 ^V	0	0
<i>C. glabrata</i>	No pseudohyphae; cells small; terminal budding	NSG	0	+	0	0
<i>C. krusei</i>	Pseudohyphae with cross-matchsticks or treelike blastoconidia	Wide surface film up sides of tube	0	+	0	+ ^V
<i>C. lipolytica</i>	Elongated blastoconidia in short chains along pseudohyphae	Pellicle (delayed)	+	+	0	+

^a Abbreviations: SDA, Sabouraud dextrose agar; +, positive; 0, negative; W, reaction may be weak; V, strain variation; NSG, no surface growth.

^b Fermentation is demonstrated by the production of gas (acid does not indicate fermentation).

Assimilation of:													Fermentation of: ^b						
Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	KNO ₃	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose	Cellobiose
+	+	V	0	+	0	0	0	⁺ ₀ ^V	0	⁺ 0 ^V	0	0	+	+	0	0	V	V	0
+	+	⁺ ₀ ^V	0	+	0	⁺ ₀ ^V	0	+	0	+	0	0	+	+	⁺ ₀ ^V	0	⁺ ₀ ^V	⁺ ₀ ^V	0
+	+	+	0	+	0	0	0	+	0	+	0	0	+	0	0	0	V	0	0
+	+	+	0	+	0	+	0	+	0	+	0	0	+	0 ^V	V	0	⁺ ₀ ^V	V	+
+	+	+	0	+	+	+	0	+	+	+	+	0	+	0	⁺ ₀ ^W	0	⁺ ₀ ^W	⁺ ₀ ^W	0
+	0	+	⁺ ₀ ^V	+	0	⁺ ₀ ^V	0	⁺ ₀ ^V	+	0	0	0	+	0	+	⁺ ₀ ^V	+	0	0
+	0	0	0	+	0	0	0	V	0	0	0	0	0	0	0	0	0	0	0
+	0	0	0	0 ^V	0	0 ^V	0	0	0	+	0	0	0 ^W	0	0	0	0	0 ^V	0
+	0	0	0	0	0	0	0	0	0	+	0	0	+	0	0	0	0	⁺ ₀ ^V	0
+	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0
+	0	0	0	V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 6 Characteristics that assist in differentiating *Candida dubliniensis* from *Candida albicans*^a

Organism	Colonies on CHROMagar, 37°C ^b	Growth at 42–45°C at 48 h ^c	Chlamydospores on appropriate agar ^d	Colonies on Staib agar ^e	Assimilation at ≤48 h ^f		
					XYL	MDG	TRE
<i>C. dubliniensis</i>	Usually dark green; most distinct at 72 h	0 or poor	Usually abundant and in pairs or small clusters	Rough; may have hyphal fringe	0	0	0 ^V
<i>C. albicans</i>	Usually light green or light bluish green	+ ^V	Usually single, very occasionally in pairs or small clusters	Smooth, shiny	+ ^V	+ ^V	+

^a Variability has been reported in all of the above tests; there is no single phenotypic test that can reliably discriminate *C. dubliniensis* from *C. albicans*. It is advisable to perform several of the tests simultaneously. Abbreviations: XYL, xylose; MDG, α-methyl-D-glucoside; TRE, trehalose; +, positive; 0, negative; V, variable.

^b CHROMagar Candida (p. 406) must be incubated at 37°C (not 25–30°C) for clearest results. The ability of *C. dubliniensis* to produce dark green colonies on CHROMagar is best observed upon primary isolation of the organism; the property has been found to be unstable following subculture or storage of the isolate.

^c Inoculate isolate onto two Sabouraud dextrose agar slants or plates; incubate one at 42–45°C and the other at 37°C (as a comparative growth control). *C. albicans* grows well at the higher temperatures, but often at a slower rate. It is essential that the temperature of the incubator be carefully controlled to ensure accuracy.

^d Media used for the production of chlamydospores (room temperature): cornmeal-Tween 80 agar, Tween 80-oxgall-cafeic acid agar, rice agar Tween. Clusters of chlamydospores are best seen on primary isolates; storage of isolate may diminish chlamydospore production.

^e Inoculate a Staib agar (p. 403) plate for isolated colonies and incubate at 30°C for 48–72 h.

^f The assimilation results refer only to commercial rapid miniature systems, not to the conventional Wickerham tube method. Commercial databases may not be updated to include findings of recent studies; therefore, the individual significant substrate reactions must be examined. For further information on the various commercial systems and their ability to identify *C. dubliniensis*, see Pincus et al. (1999).

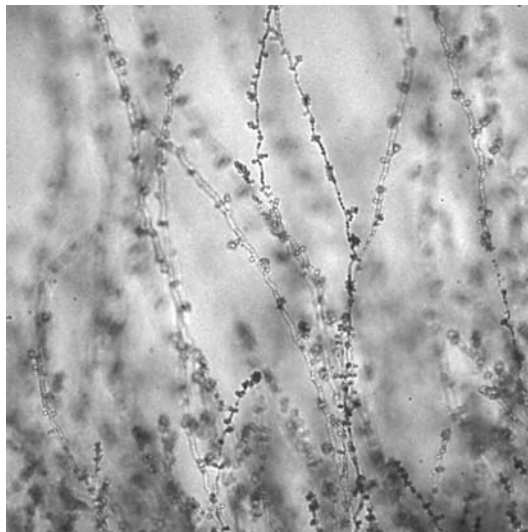
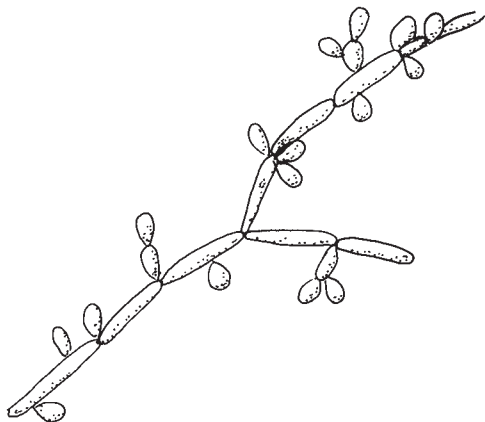
PATHOGENICITY: As is true of many species of *Candida* and other yeasts, *C. tropicalis* is known to cause infection, especially in immunocompromised, predisposed patients, as discussed on pp. 125–126. It is especially virulent in neutropenic patients with leukemia or other hematological malignancies. It is also recovered from mucosal sites without evidence of disease.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Creamy; near the edge it may be wrinkled or have a slight mycelial fringe. (Color Plate 46.) Colonies are metallic blue on CHROMagar *Candida*. (Color Plate 45.)

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval ($3.5\text{--}7 \times 5.5\text{--}10\text{ }\mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, *C. tropicalis* forms blastoconidia singly or in very small groups all along graceful, long pseudohyphae. True hyphae may also be present. A few teardrop-shaped chlamydospores may rarely be produced.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Candida parapsilosis complex*

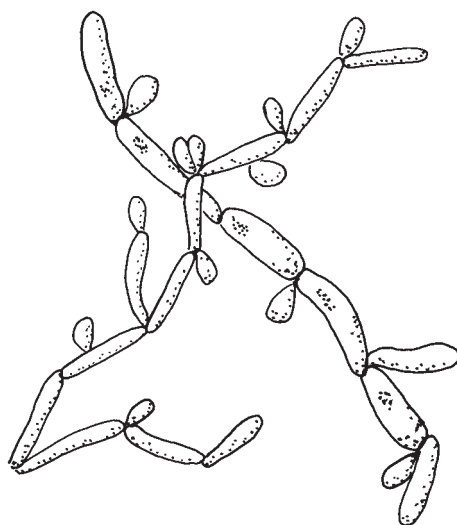
PATHOGENICITY: Known to cause infections in particularly susceptible individuals, as discussed on pp. 125–126. It is the second most common agent of fungal endocarditis (*Candida albicans* being the most common). It is also frequently responsible for invasive fungal infections in neonates. The MICs of echinocandins are elevated in *C. parapsilosis* compared to other *Candida* species.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Creamy, sometimes developing a lacy appearance.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are ovoid ($2.5\text{--}4 \times 3\text{--}8 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, blastoconidia, singly or in small clusters, are seen along the pseudohyphae. Outstanding characteristics are the crooked or curved appearance of relatively short pseudohyphae (compared to those of *Candida tropicalis*) and the occasional presence of large hyphal elements called giant cells.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Tavanti et al., 2005 (designation of the new species)

* The complex includes two former subgroups of *C. parapsilosis* that are now considered separate species, i.e., *Candida orthopsilosis* and *Candida metapsilosis*. The three species cannot be differentiated phenotypically; molecular methods are required.

*Candida lusitanae**

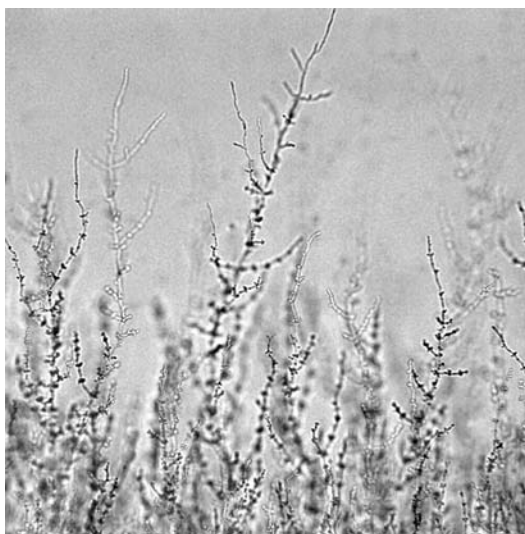
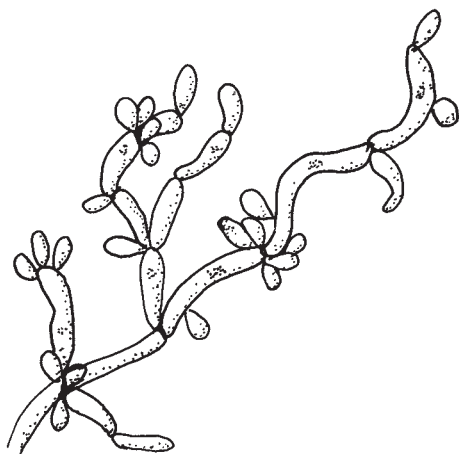
PATHOGENICITY: Encountered as an opportunistic pathogen in immunocompromised patients. Although resistance to amphotericin B has frequently been reported, studies have found that it does not exhibit significantly more primary resistance than do other species of *Candida*, but it is more likely to develop resistance upon treatment with the drug.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Cream colored, smooth, glistening.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval ($2-6 \times 3-10 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, pseudohyphae are slender, branched, and curved with short chains of elongate blastoconidia. Morphologically *C. lusitanae* resembles *Candida tropicalis* and *Candida parapsilosis* but differs in its ability to ferment cellobiose and usually to assimilate rhamnose.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kwon-Chung and Bennett, 1992, pp. 318-319

* Some sources may refer to *Candida lusitanae* as the teleomorph *Clavispora lusitanae*.

Candida krusei*

PATHOGENICITY: Known to cause infections in particularly susceptible individuals, as discussed on pp. 125–126. It is considered to be innately resistant to fluconazole.

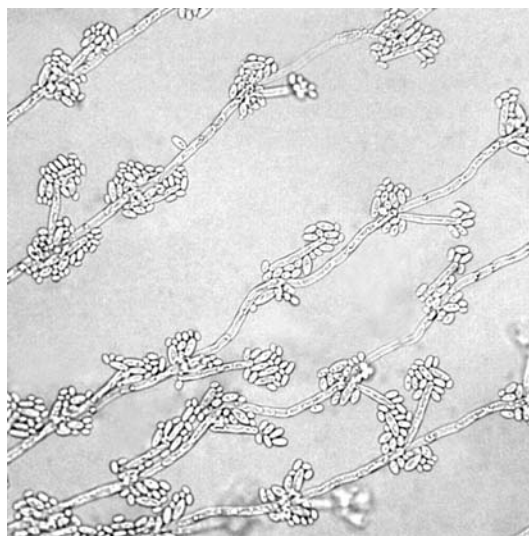
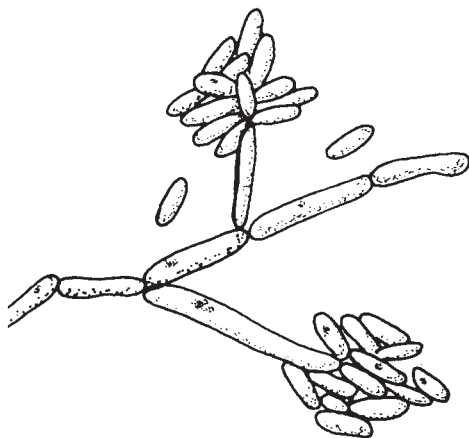
RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Flat, dry, dull, developing a mycelial fringe. Cream colored. (Color Plates 47 and 48) On CHROMagar Candida agar, colonies are rough with a pink center and white border. (Color Plate 45.)

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval or elongate ($2\text{--}6 \times 4\text{--}10 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, *C. krusei* forms pseudohyphae with elongate blastoconidia forming a cross-matchsticks or treelike appearance. The formations may be confused with the annellides of *Magnusiomyces capitatus* (formerly *Blastoschizomyces capitatus* [p. 165]); see Table 7 (p. 137) for the differentiation of these two yeasts.

Although the biochemical profiles of *Candida inconspicua* and *Candida norvegensis* are extremely similar to that of *C. krusei*, they can be distinguished by the characteristics in Table 8 (p. 137).

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 156

* Some sources may refer to *Candida krusei* as the teleomorph *Pichia kudriavzevii*.

TABLE 7 Differentiating characteristics of *Blastoschizomyces capitatus* versus *Candida krusei*^a

Organism	Growth with cycloheximide	Galactose assimilation	Glucose fermentation	Urease	Conidia
<i>B. capitatus</i>	+	+	0	0	Anelloconidia
<i>C. krusei</i>	0	0	+	+ ^V	Blastoconidia

^a Abbreviations: +, positive; 0, negative; V, variable.

TABLE 8 Differentiating characteristics of *Candida krusei*, *Candida inconspicua*, and *Candida norvegensis*^a

Organism	Colonies on CHROMagar Candida	Pseudohyphae on cornmeal-Tween 80 agar	Growth on Trichophyton agar #1 ^b	Fermentation of glucose	Assimilation of cellobiose	Esculin hydrolysis
<i>C. krusei</i>	Rough; pink center with white border	+	+	+	0	0
<i>C. inconspicua</i>	May resemble <i>C. krusei</i>	0 Short chains of oval cells may form	0 ^c	0	0	0
<i>C. norvegensis</i>	May resemble <i>C. krusei</i>	0 ^V Pseudohyphae may form	0	+ ^D	+	+

^a All three of these species show resistance to fluconazole. If definitive identification of *C. inconspicua* versus *C. norvegensis* is required, molecular analysis is recommended. Abbreviations: +, positive; 0, negative; V, variable; D, delayed/slow.

^b Casein agar, vitamin free (p. 406).

^c May grow after an initial lag period.

Candida kefyr* (formerly *Candida pseudotropicalis*)

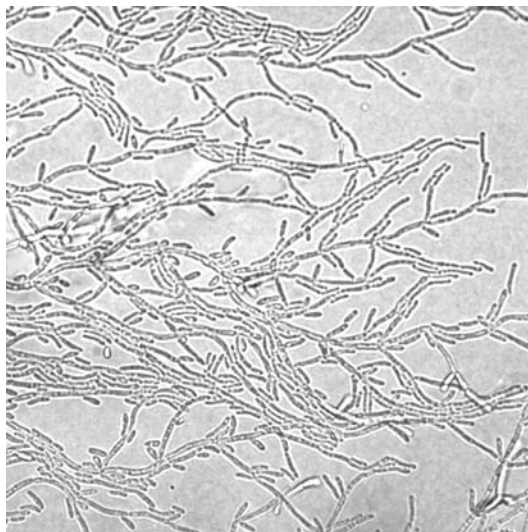
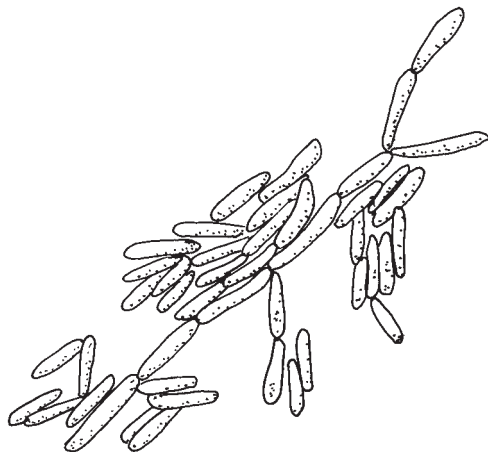
PATHOGENICITY: Usually considered nonpathogenic but occasionally causes infection in particularly susceptible individuals, as discussed on pp. 125–126.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Smooth, white to cream colored.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval ($3\text{--}8 \times 5\text{--}12 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, *C. kefyr* forms pseudohyphae with elongate blastoconidia that characteristically line up in parallel, giving the appearance of logs in a stream.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 109

* Some sources may refer to *Candida kefyr* as the teleomorph *Kluyveromyces marxianus*.

Candida rugosa species complex*

PATHOGENICITY: Initially known as a major cause of mastitis in cattle. Now considered an emerging fungal pathogen in humans; occasionally reported in individual cases as well as in outbreaks of central venous catheter-related candidemia and urinary tract infections, especially in burn and trauma centers. The organism has proved difficult to treat, some isolates exhibiting reduced susceptibility (*in vitro* or *in vivo*) to fluconazole, voriconazole, amphotericin B, and caspofungin. The susceptibility patterns vary by geographical region; isolates from Asia-Pacific and Latin American regions appear to be more resistant than those from Europe and North America. Antifungal susceptibility testing of clinically significant isolates is usually necessary.

RATE OF GROWTH: Rapid; mature within 3 days.

COLONY MORPHOLOGY: White to cream colored, dry, often wrinkled, may have mycelial fringe. Unique in forming dry, fairly rough, blue-green colonies with a white border when grown on CHROMagar *Candida* agar. (Color Plate 49.)

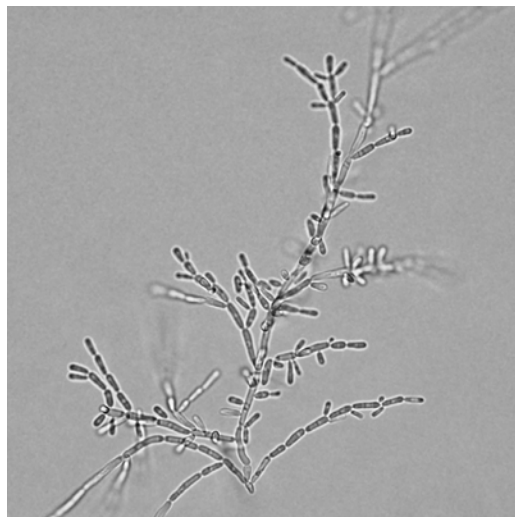
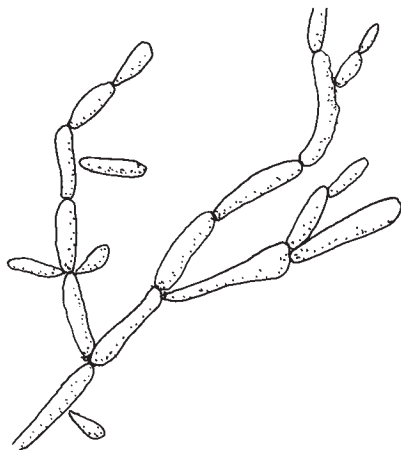
MICROSCOPIC MORPHOLOGY: On routine primary media, budding yeast cells are oval to almost cylindrical ($2.0\text{--}3.5 \times 6\text{--}12 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, *C. rugosa* forms pseudohyphae (sometimes short) with elongate blastoconidia, some in chains.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.

(continued on following page)

* *Candida rugosa* has been found to consist of several species, including *C. rugosa*, *Candida pararugosa*, *Candida pseudorugosa*, *Candida mesorugosa*, and *Candida neorugosa*. Thus, *C. rugosa* is now considered to be best termed as *Candida rugosa* species complex. While *C. rugosa* and *C. pseudorugosa* may be distinguished biochemically, the other species, which are genetically distinct, are not distinguishable by conventional culture-based biochemical methods. Instead, MALDI-TOF mass spectrometry may be used to distinguish the different species. Although a proposal has been recently made to transfer members of the *Candida rugosa* species complex into the new genus *Diutina* gen. nov., the data supporting this change in nomenclature have not been independently validated and thus the term *Candida rugosa* species complex will be used in this text.

Candida rugosa* species complex (continued)



For further information, see

Colombo et al., 2007

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Hospenthal et al., 2006

Khunnamwong et al., 2015

Padovan et al., 2013

Pfaller et al., 2006

Candida guilliermondii complex*

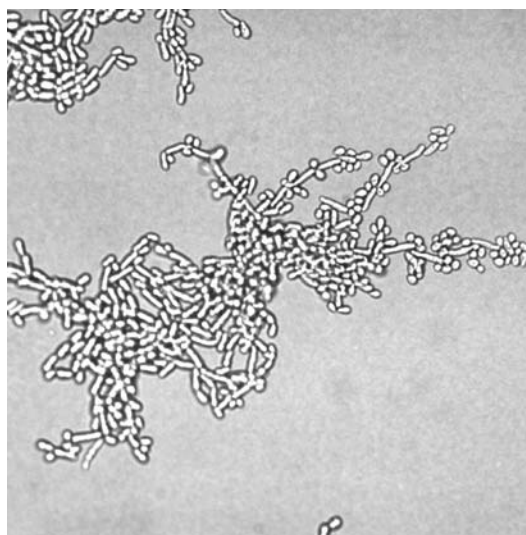
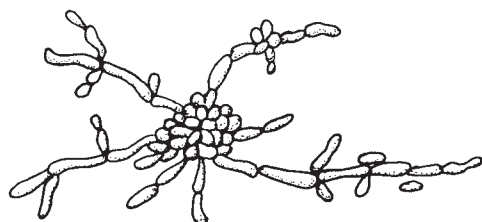
PATHOGENICITY: Has occasionally been reported to cause endocarditis, osteomyelitis, cutaneous and urinary tract infections, and disseminated disease. Infections can be fatal in immunocompromised patients. Infects particularly susceptible individuals, as described on pp. 125–126. *C. guilliermondii* also has been reported to become resistant to amphotericin B during therapy.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Flat, glossy, smooth edged, and usually cream colored but may become tan or pinkish with age.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are ovoid to elongate ($2\text{--}5 \times 3\text{--}7 \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, *C. guilliermondii* forms clusters of yeast cells with relatively few, short pseudohyphae often having small groups of blastoconidia at the septa. True hyphae are not produced.

See Table 4 (p. 128) for differentiation of yeastlike genera, Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*, and Table 9 (p. 142) for distinguishing *C. guilliermondii* from *Candida famata*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Girmenia et al., 2006
Kidd et al., 2016, p. 122
Savini et al., 2011

* The complex consists of *Candida guilliermondii*, *Candida fermentati*, and *Candida carpophila*; they are phenotypically indistinguishable. Some sources may refer to *Candida guilliermondii* as *Meyerozyma guilliermondii*.

TABLE 9 Differentiating characteristics of *Candida guilliermondii* versus *Candida famata*^a

Organism	Shape of cell	Growth at 37°C	Growth at 42°C	Pseudohyphae on cornmeal-Tween 80 agar	Glucose fermentation
<i>C. guilliermondii</i>	Ovoid or elongate	+	V	V (often short, primitive)	+
<i>C. famata</i>	Round	V	0	0	0 ^W

^a Commercial identification systems often show results with low discrimination between these species. Abbreviations: +, positive; 0, negative; V, variable; W, weak.

*Candida lipolytica** (*Yarrowia lipolytica*)

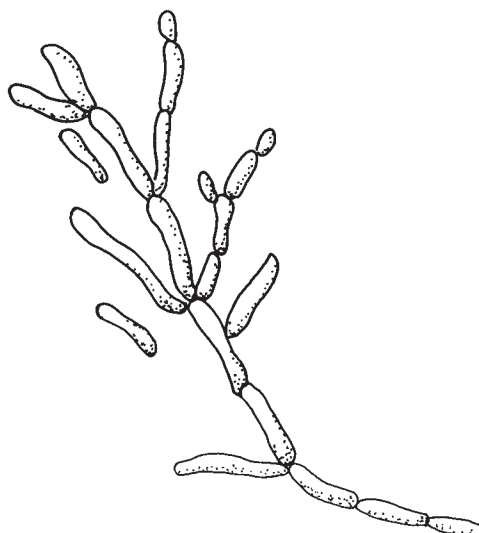
PATHOGENICITY: A rarely encountered emerging opportunistic pathogen; may cause candidemia and other disease in immunocompromised patients, as discussed on pp. 125–126.

RATE OF GROWTH: Rapid; mature in 6 days.

COLONY MORPHOLOGY: Smooth, may be delicately wrinkled, white to cream colored.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are round to oval (3–6 × 4–16 μm). On cornmeal-Tween 80 agar at 25°C for 72 h, pseudohyphae and septate true hyphae bearing elongate blastoconidia in short chains form and produce a stark, branching appearance. Arthroconidia may be present. *C. lipolytica* physiologically resembles *Candida krusei* but clearly differs by growing in the presence of cycloheximide and not fermenting glucose (in addition to the morphologic differences).

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*.



For further information, see

D'Antonio et al., 2002

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4 (as *Yarrowia lipolytica*, sexual state)

Kidd et al., 2016, p. 230

Liu et al., 2013

* The sexual state, *Yarrowia lipolytica*, may appear on culture. It produces round to oval asci (5–8 × 6–15 μm) near the septa of the hyphae; the asci usually contain two ascospores, 3–4 × 3.5–6 μm.

Candida zeylanoides

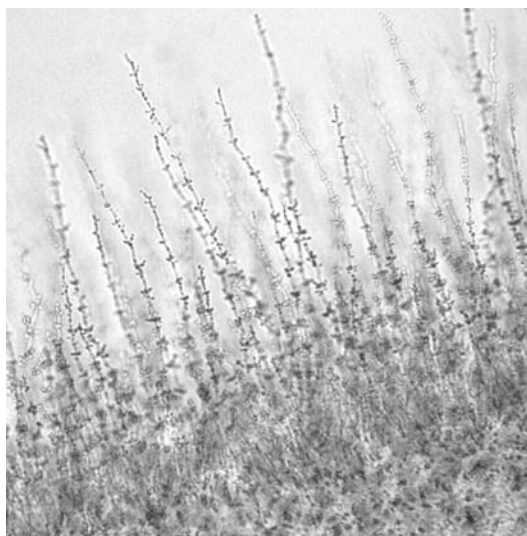
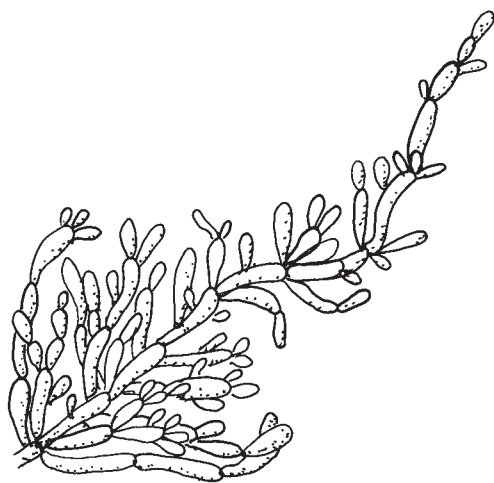
PATHOGENICITY: Rarely reported as cause of fungemia, *Candida* arthritis, and skin and nail infections.

RATE OF GROWTH: Rapid; mature in 3 days at 25–30°C; variable growth at 35–37°C.

COLONY MORPHOLOGY: Smooth, dull, cream colored to yellowish.

MICROSCOPIC MORPHOLOGY: On routine primary media, yeast cells are oval to elongate ($3\text{--}5 \times 6\text{--}11\ \mu\text{m}$). On cornmeal-Tween 80 agar at 25°C for 72 h, pseudohyphae consist of cells that are frequently curved and bear oval or elongate blastoconidia singly and in small clusters and short chains. More blastoconidia are formed at the beginnings of the pseudohyphae than at the distal ends, creating a featherlike appearance at low power.

See [Table 4 \(p. 128\)](#) for differentiation of yeastlike genera and [Table 5 \(p. 130\)](#) for characteristics of the most commonly encountered species of *Candida*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kurtzman and Fell (ed.), 2000, pp. 571–572
Levenson et al., 1991

Candida glabrata complex* (formerly *Torulopsis glabrata*)

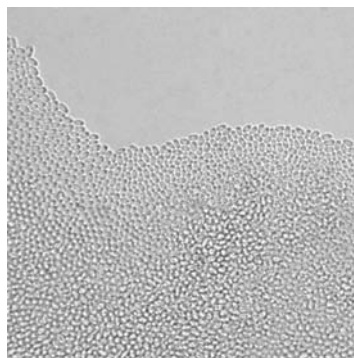
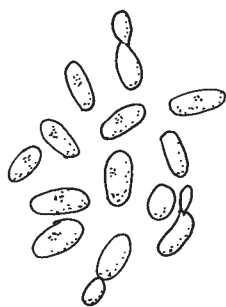
PATHOGENICITY: Causes infections usually occurring in the bloodstream or urogenital tract and occasionally disseminated disease. The organism may colonize healthy individuals but appears to cause infection only in particularly susceptible patients, as discussed on pp. 125–126. A significant number of clinical isolates have shown reduced susceptibility to fluconazole (and other azoles) and, to some extent, to amphotericin B. There are also reports of increasing frequency of resistance to echinocandins.

RATE OF GROWTH: Rapid; mature in 3–5 days; grows a bit more slowly than the other species of *Candida*. Isolates have been detected that are enhanced by, or dependent upon, cholesterol for growth. Without specialized media, these may appear as tiny colonies on MacConkey agar or grow only around a bile disk (cholesterol is the vital ingredient; disks are commercially available) on routine mycology media. These organisms are likely to show increased resistance to antifungals that target ergosterol and its synthetic pathway; adding fluconazole to culture media may therefore increase the ability to detect these organisms.

COLONY MORPHOLOGY: Small, yeastlike colonies; pasty, smooth, white to cream. (Color Plate 50.)

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, only small ($2\text{--}3 \times 3\text{--}4 \mu\text{m}$), oval yeast cells with single terminal budding are seen. No pseudohyphae are formed; on rare occasion a few short chains of ovoid cells may be seen.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 5 (p. 130) for characteristics of the most commonly encountered species of *Candida*. *C. glabrata* can be quickly identified by the rapid assimilation of trehalose (RAT) test (p. 418). (Color Plate 51.)



For further information, see

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Hazen et al., 2005 (cholesterol-dependent isolates)
 Healey et al., 2015
 Kidd et al., 2016, p. 40
 Rodrigues et al., 2013

* The complex consists of *Candida bracarensis*, *Candida glabrata*, and *Candida nivariensis*.

Candida auris

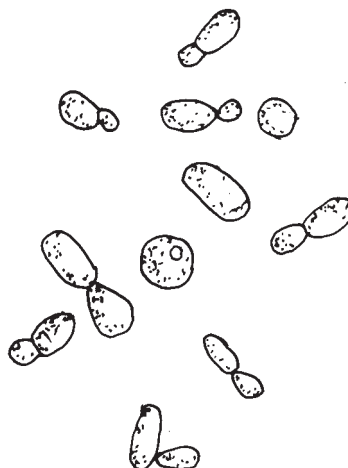
PATHOGENICITY: Initially recovered from the external ear canal of a patient in a Japanese hospital in 2008, *Candida auris* has emerged globally as a multiply drug-resistant pathogen causing candidemia and other forms of deeply invasive candidiasis. *C. auris* also has the propensity to form biofilms and to persist in the hospital environment. This pathogen is typically resistant to fluconazole and may have elevated MICs to amphotericin B; some isolates also may be resistant to echinocandins. Infection control measures are usually implemented in infected and colonized cases for prevention of nosocomial transmission.

RATE OF GROWTH: Rapid; mature within 3 days. Grows at 25–37°C, and most strains can grow at 42°C. Growth is inhibited by cycloheximide; therefore, it does not grow on Mycosel agar.

COLONY MORPHOLOGY: Cream colored, smooth on Sabouraud dextrose agar and on enriched media. The colonies of most isolates on CHROMagar have a pink to purple color and may become red with extended incubation.

MICROSCOPIC MORPHOLOGY: In nutrient broth, small, budding, ovoid, elongated, or ellipsoid yeasts measuring ~2–3 by 2.5–5 µm. The budding yeasts may grow as singlets, doublets, or in small clusters. Pseudohyphae and hyphae are not usually formed; however, rudimentary pseudohyphae occasionally may be observed. On cornmeal-Tween 80 agar at 30°C for 72 h, usually yeast cells only are seen, but isolates may very occasionally form short, undeveloped pseudohyphae.

There are no morphological or biochemical features that are sufficiently specific to identify *C. auris*. Instead, the organism may be initially misidentified as *Candida baemulonii*, *Candida duobushaemulonii*, *Candida famata*, *Saccharomyces cerevisiae*, or *Rhodotorula glutinis*. Isolates of *C. baemulonii*, *C. duobushaemulonii*, and suspicious isolates that are not obviously *S. cerevisiae* or *R. glutinis* should be further evaluated by MALDI-TOF, PCR, or direct sequencing for definitive identification.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*,
 Version 4.1.4
 Lockhart et al., 2017
 McCarthy and Walsh, 2017
 Navalkele et al., 2017
 Piedrahita et al., 2017
 Schelenz et al., 2016

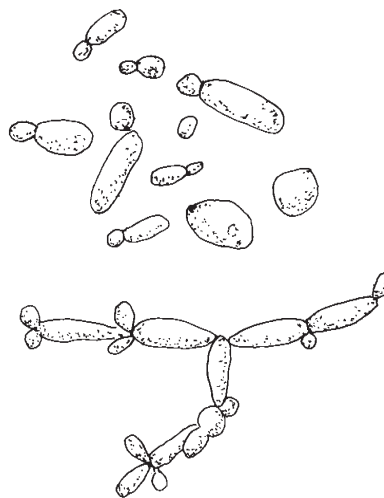
Candida haemulonii (*haemulonis*) complex*

PATHOGENICITY: *Candida haemulonii* is an increasingly recognized cause of fungemia and deeply invasive candidiasis, including peritonitis associated with peritoneal dialysis. *C. haemulonii* is phenotypically similar to *Candida auris*. As some biochemical systems include *C. haemulonii* but not *C. auris*, any clinically significant isolate of *C. haemulonii* should be confirmed by MALDI-TOF mass spectrometry or DNA sequencing.

RATE OF GROWTH: Rapid; mature within 3 days. Isolates of *C. haemulonii* complex can grow at 35°C but not at 42°C. *C. haemulonii* is not inhibited by cycloheximide and therefore grows on Mycosel agar.

COLONY MORPHOLOGY: On routine primary media, colonies are white to cream colored, smooth, and glabrous. Colonies on CHROMagar Candida are pink at 24 h and develop a darker violet center at 72 h. This is not unique to *C. haemulonii* complex; *Candida glabrata* may also become violet after 3–4 days' incubation, and *C. auris* can be somewhat similar. A novel medium employing CHROMagar Candida supplemented with Pal's agar (made mainly of powdered sunflower seeds) has been described to distinguish *C. auris* from *C. haemulonii* complex (see Kumar et al. reference below).

MICROSCOPIC MORPHOLOGY: On routine primary media, budding yeast cells are round to oval (3–5 × 3–6.5 μm). On cornmeal-Tween 80 agar at 25°C for 72 h, yeast cells are seen; true hyphae are absent; pseudohyphae are usually absent but may appear in fragmentary forms.



Yeast cells; occasional fragmentary pseudohyphae-like structures composed of clavate cells may be seen.

For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*,
 Version 4.1.4
 Kathuria et al., 2015
 Kidd et al., 2016, p. 43
 Kumar et al., 2017
 Muro et al., 2012

* The complex includes *Candida haemulonii*, *Candida duobushaemulonii*, and *Candida haemulonii* var. *vulnera*. These three species cannot be differentiated phenotypically; molecular methods are required.

Cryptococcus neoformans

PATHOGENICITY: Causes cryptococcosis, a subacute or chronic infection most frequently involving the tissue of the central nervous system but occasionally producing lesions in the skin, bones, lungs, or other internal organs. Meningitis due to *C. neoformans* is extremely common in HIV/AIDS patients. With the exception of *C. gattii* (p. 150), the other species of this genus are commonly considered nonpathogenic and only occasionally cause disease in severely immunosuppressed patients. The most common environmental source of *C. neoformans* is pigeon droppings. *C. neoformans* is intrinsically resistant to echinocandins but susceptible to amphotericin B, triazoles, and flucytosine.

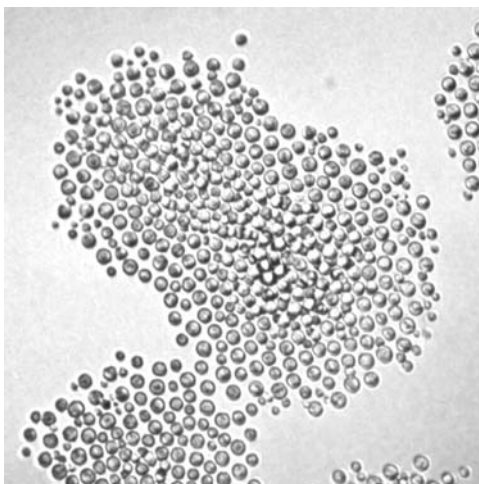
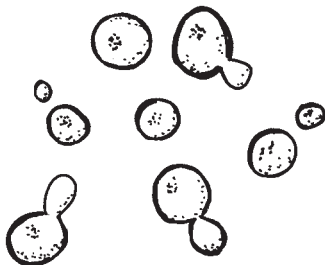
RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Colonies are flat or slightly heaped, shiny, moist, and usually mucoid, with smooth edges. Color is cream at first, later becoming tannish. Usually grows equally well at 25 and 37°C, whereas some of the other species of the genus will not grow well, if at all, at 37°C. (Color Plate 52.) Produces brown colonies on birdseed (or similar) agar (p. 403). (Color Plate 53.)

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, cells (4–8 μm in diameter) are round, dark walled, and budding. Usually no hyphae are seen. Capsules are often discernible on cornmeal-Tween 80 by the spaces (due to capsular material) between the yeast cells. The capsules are best demonstrated with an India ink preparation (p. 367). Production of capsular material may be increased by growth in 1% peptone solution. Some strains of *C. neoformans*, as well as other cryptococci, may not produce apparent capsules *in vitro*.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 10 (p. 151) for characteristics of the most commonly encountered species of *Cryptococcus*. *C. neoformans* and *C. gattii* can be rapidly differentiated from other species of *Cryptococcus* by the caffeic acid test (p. 379). (Color Plate 54.) *C. neoformans* can be differentiated from *C. gattii* with the use of canavanine glycine bromothymol blue agar (p. 405).

(continued on following page)



For further information, see

Cogliati, 2013

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Kidd et al., 2016, pp. 53–54

Kwon-Chung and Bennett, 1992, pp. 397–446

Kwon-Chung et al., 2017

May et al., 2016

Cryptococcus gattii (formerly *C. neoformans* var. *gattii*)

PATHOGENICITY: Similar to that of *Cryptococcus neoformans* (p. 148), but only recently recognized as a distinct pathogen. Infection in immunocompetent persons and development of cryptococcomas (mass lesions) in lung and brain are far more commonly associated with *C. gattii* than with *C. neoformans*. Infections occur most commonly in tropical, subtropical, and temperate regions; have recently been most numerous in Australia, Papua New Guinea, British Columbia, and the Pacific Northwest region of the United States. However, a wider geographic range of infections caused by *C. gattii* is increasingly recognized. Its preferred ecologic niche is decayed hollows and surfaces of a variety of trees throughout the world; it has been recovered from wood products, soil, water, air, various mammals, and other sources. *C. gattii* is intrinsically resistant to echinocandins.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Same as that of *C. neoformans* (p. 148).

MICROSCOPIC MORPHOLOGY: Similar to *C. neoformans* (p. 148), but in addition to round yeast cells, some more oval ones commonly form.

The major phenotypic method for differentiation of *C. gattii* from *C. neoformans* is the use of canavanine glycine bromothymol blue (CGB) agar (p. 405). *C. gattii* grows on CGB medium and turns the agar blue; *C. neoformans* does not grow on the medium, and the color of the agar remains unchanged (yellow). As treatment for both species is essentially the same, differentiation is often not required.

See Table 4 (p. 128) for differentiation of yeastlike genera and Table 10 (p. 151) for characteristics of the most commonly encountered species of *Cryptococcus*. *C. neoformans* and *C. gattii* can be rapidly differentiated from other species of *Cryptococcus* by the caffeic acid test (p. 379). (Color Plate 54.)

As *C. gattii* is considered morphologically indistinguishable from *C. neoformans*, see images on p. 149.

For further information, see

Cogliati, 2013

Datta et al., 2009

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Hoang et al., 2011

Kidd et al., 2016, pp. 52–53

Klein et al., 2009

Sorrell, 2001

Springer and Chaturvedi, 2010

TABLE 10 Characteristics of *Cryptococcus* spp.^a

Organism	Phenol oxidase production ^b	Growth at 37°C on SDA	Growth with cyclo-heximide at 25°C	Pseudo-hyphae (short)	Urease (25°C; 4 days)	Assimilation of:													Fermentation
						Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	KNO ₃	
<i>C. neoformans</i> and <i>C. gattii</i>	+	+	0	0 ^R	+	+	+	+	0	+	0	+ ^V	+	+	+ ^V	+	+	0	All species of <i>Cryptococcus</i> lack fermentative ability
<i>C. uniguttulatus</i>	0	0	0	0	+	+	+	+	0	0 ^V	0	0 ^V	+	+	+ ^V	+ ^V	0	0	
<i>C. albidus</i> var. <i>albidus</i>	0	0 ^V	0	+ ^V	+	+	+	+	+ ^V	0 ^V	0 ^V	+	+	+	+ ^W	+ ^V	+ ^V	+	
<i>C. albidus</i> var. <i>diffluens</i>	0	0 ^V	0	0 ^V	+	+	+	+	0	0 ^V	+ ^V	+	+	+	+ ^W	+	+ ^V	+	
<i>C. laurentii</i>	0	0	0 ^V	0	+	+	+	+	+	+	+ ^V	+	+	+	+ ^V	+ ^V	+	0	
<i>C. luteolus</i>	0	0	0	0	+	+	+	+	0 ^V	+	+	+	+	+	+	+	+ ^V	0	
<i>C. terreus</i>	0	V	0	0 ^V	+	+	+ ^V	0 ^V	V	+ ^V	+ ^W	+	+	+	0	+ ^V	V	+	
<i>C. gastricus</i>	0	0	0	0	+	+	+	0 ^V	0 ^V	+	0	+	+	+	0	+	0	0	

^a Abbreviations: SDA, Sabouraud dextrose agar; +, positive; 0, negative; V, strain variation; R, occur rarely; W, reaction may be weak.

^b The caffeic acid disk test (p. 379) is the preferred method for rapid and sensitive detection of phenoloxidase production. Birdseed agar (p. 403) is an alternative method, but results are significantly delayed.

TABLE 11 Characteristics of yeasts and yeastlike organisms other than *Candida* spp. and *Cryptococcus* spp.^a

Organism	Microscopic morphology on cornmeal-Tween 80 agar at 25°C	Growth at 37°C	Asco-spores	Urease (25°C; 4 days)	Assimilation of:														Fermentation of:						
					Glucose	Maltose	Sucrose	Lactose	Galactose	Melibiose	Cellobiose	Inositol	Xylose	Raffinose	Trehalose	Dulcitol	KNO ₃	Glucose	Maltose	Sucrose	Lactose	Galactose	Trehalose	Cellobiose	
<i>Saccharomyces cerevisiae</i>	Occasional short pseudohyphae	V	+	0	+	+ ^V	+	0	+ ^V	V	0	0	0	+	+ ^V	0	0	+	V	+	0	V	+ ^V	0	
<i>Wickerhamomyces anomalus</i> (<i>Pichia anomala</i>)	May form pseudohyphae	V	+	0	+	+	+	0	+ ^V	0	+	0	+ ^V	+ ^V	+	0	+	+	+ ^V	+	0	+ ^V	0 ^V	V	
<i>Geotrichum candidum</i>	True hyphae, arthroconidia; no blastoconidia	0 ^V	0	0	+	0	0	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	
<i>Blastoschizomyces capitatus</i> ^b	Pseudohyphae and true hyphae; annelloconidia; few arthroconidia	+	0	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Trichosporon</i> spp. (see Table 12 [p. 164])	Pseudohyphae and true hyphae; arthroconidia; blastoconidia	+ ^V	0	+	+	+ ^V	+ ^V	+	+ ^V	+ ^V	+ ^V	+ ^V	+	+ ^V	+ ^V	+ ^V	0	0	0	0	0	0	0	0	
<i>Rhodotorula mucilaginosa</i>	Usually no pseudohyphae	+	0	+	+	V	+	0	V	0	V	0	+	+	+	0	0	0	0	0	0	0	0	0	
<i>Rhodotorula glutinis</i>	Usually no pseudohyphae	V	0	+	+	+	+	0	V	0	V	0	V	+ ^V	+	0	+	0	0	0	0	0	0	0	
<i>Rhodotorula minuta</i>	No pseudohyphae	V	0	+	+	0	+	V	V	0	V	0	+	0	+		0	0	0	0	0	0	0	0	
<i>Sporobolomyces salmonicolor</i>	Ballistoconidia; various amounts of true and pseudohyphae	V	0	+	+	V	+	0	V	0	V	0	+ ^W	V	+		+	0	0	0	0	0	0	0	
<i>Malassezia pachydermatis</i>	Usually no hyphae	+	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Prototheca wickerhamii</i>	Sporangia; no hyphae	+ ^V	0	0	+	0	0	0	+ ^V	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	
<i>Prototheca zopfii</i>		+ ^V	0	0	+	0	0	0	0 ^V	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Prototheca stagnora</i>		0	0	0	+	0	V	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

^a Abbreviations: SDA, Sabouraud dextrose agar; +, positive; 0, negative; V, strain variation; W, reaction may be weak.^b Annelloconidia forming clusters at the ends of hyphae of *B. capitatus* may resemble the elongated blastoconidia of *Candida krusei*.

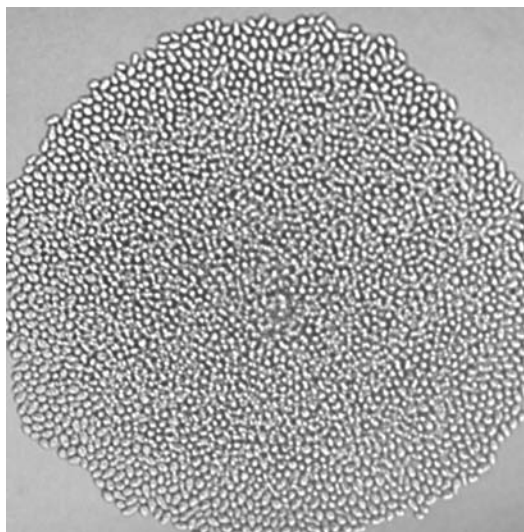
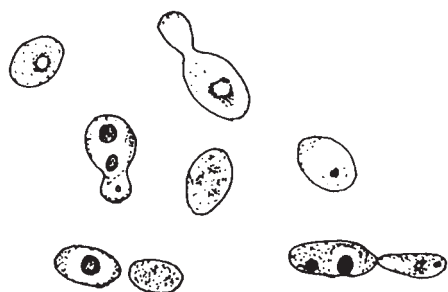
PATHOGENICITY: Commonly known as contaminants, but in recent years they have increasingly been agents of severe infections, mostly fungemia in immunosuppressed patients with central venous catheters. Endocarditis, peritonitis, meningitis, endophthalmitis, and other infections have been reported to a lesser extent. *Rhodotorula* species are intrinsically resistant to echinocandins and fluconazole.

RATE OF GROWTH: Rapid; mature in 4 days.

COLONY MORPHOLOGY: Usually pink to coral, but can also be more orange to red. Colony is yeastlike, soft, smooth, moist, and sometimes mucoid. (Color Plate 55.)

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, budding cells are round to oval or elongate ($2.5\text{--}5 \times 3\text{--}10 \mu\text{m}$); occasionally a few rudimentary pseudohyphae are seen. A capsule is sometimes formed.

See Tables 4 (p. 128) and 11 (p. 152) for differentiation of yeastlike fungi.



For further information, see
Anaissie et al. (ed.), 2009, pp. 258–259
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 125–127
Simon et al., 2014
Wirth and Goldani, 2012
Yazdani et al., 2015

* *Rhodotorula mucilaginosa* is the accepted name for the species previously known as *Rhodotorula rubra*, which is the most commonly encountered cause of infection within the genus. *Rhodotorula glutinis* is less commonly encountered, and *Rhodotorula minuta* is rare.

Sporobolomyces salmonicolor

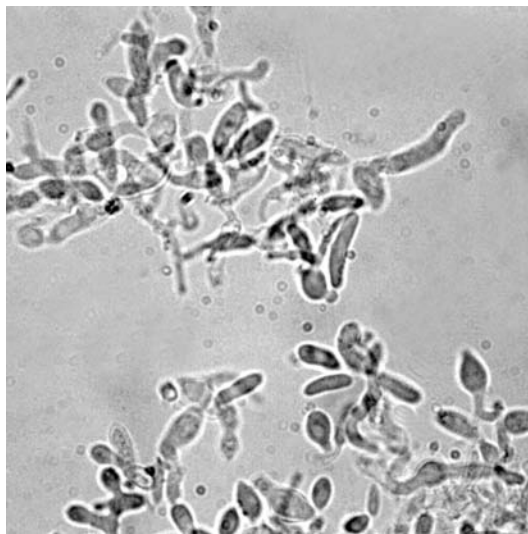
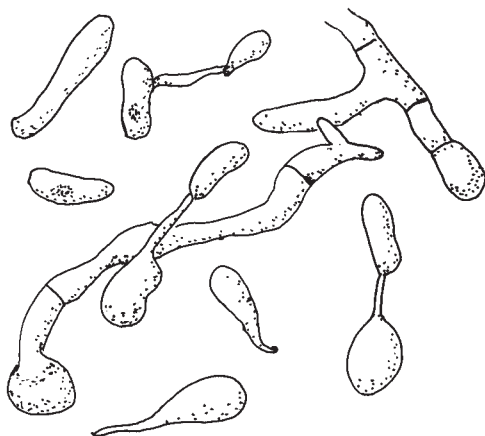
PATHOGENICITY: Most commonly isolated from environmental sources. Seldom reported as the cause of fungemia in immunocompromised patients.

RATE OF GROWTH: Rapid; mature in 5 days. Best growth is at 25–30°C; may not grow well at 35–37°C.

COLONY MORPHOLOGY: Smooth to slightly rough; characteristic salmon pink/coral color resembles that of *Rhodotorula* spp. (p. 153). Satellite colonies eventually form around the original colonies due to production of ballistoconidia. Ballistocoonidia can be best demonstrated by taping an inoculated cornmeal agar plate face to face to an uninoculated cornmeal plate. After extended incubation at 25°C, with the inoculated plate on top, the ballistoconidia that are shot off the inoculated plate will form a mirror image of the colonies on the other plate. (Color Plates 56, 57, and 58.)

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C, oval to elongate yeastlike cells (2–12 × 3–35 μm) are seen; pseudohyphae and true hyphae may be absent or abundant. Kidney-shaped ballistoconidia (3–5 × 5–12 μm) are produced on denticles; they are forcibly discharged, forming the satellite colonies.

See Tables 4 (p. 128) and 11 (p. 152) for differentiation of yeasts and yeastlike fungi.



For further information, see
 Anaissie et al. (ed.), 2009, pp. 258–259
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Tang et al., 2015

PATHOGENICITY: Previously seldom known to cause disease, but it is increasingly reported as an agent of invasive infection in a wide range of body sites; most significant is fungemia in immunocompromised or critically ill patients, as well as some relatively healthy patients. Many patients with infection caused by *Saccharomyces cerevisiae* have a history of receiving the probiotic *Saccharomyces cerevisiae* subtype *boulardii* for prophylaxis or treatment of antibiotic-associated diarrhea. Nosocomial spread of this organism to patients not being administered the probiotic also occurs; this appears to be due to the organism entering the environment when capsules or packets of the yeast are opened for deposit into the nasogastric tube of a patient being treated. The organism has been shown to persist in the environment; subsequent central vascular catheter hub or insertion-site contamination is the likely route of entry into the bloodstream of patients in the affected locale.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Smooth colonies, moist, white to cream colored.

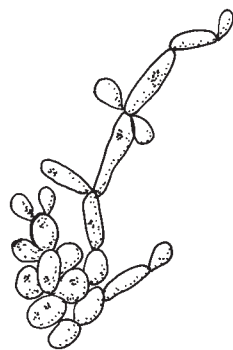
MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, round to oval yeast cells ($3-8 \times 5-10 \mu\text{m}$) with multilateral budding are seen. A few very short pseudohyphae may form.

Note: Characteristic roundish ascospores (one to four per ascus) are best demonstrated when the organism is grown on a special medium, such as V-8 medium, acetate ascospore agar, or Gorodkova medium (p. 399), and stained with ascospore stain (p. 388) or Kinyoun stain (p. 387). (Color Plate 59.) Additionally, ascospores are Gram negative, while vegetative cells are Gram positive.

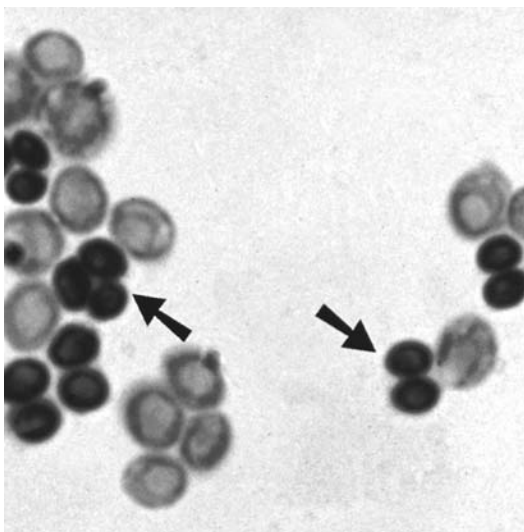
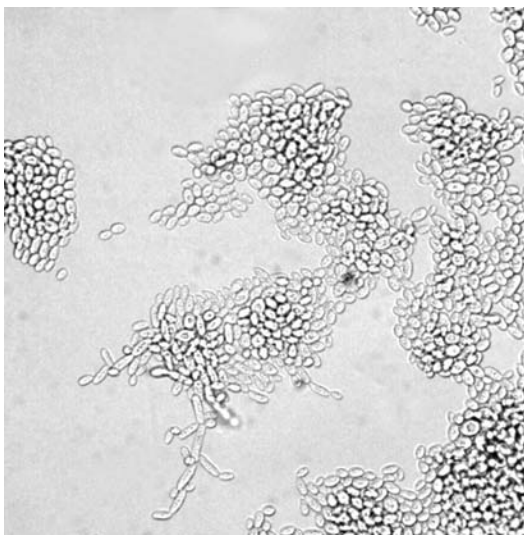
See Tables 4 (p. 128) and 11 (p. 152) for differentiation of yeasts and yeastlike organisms.

(continued on following page)

Saccharomyces cerevisiae (continued)



Asci and ascospores.



Arrows point to ascospores.

For further information, see
Anaissie et al. (ed.), 2009, pp. 259–260
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Enache-Angoulvant and Hennequin, 2005
Thygesen et al., 2012

Wickerhamomyces anomalus (sexual state; formerly *Pichia anomala* and *Hansenula anomala*); *Candida pelliculosa* (asexual state)

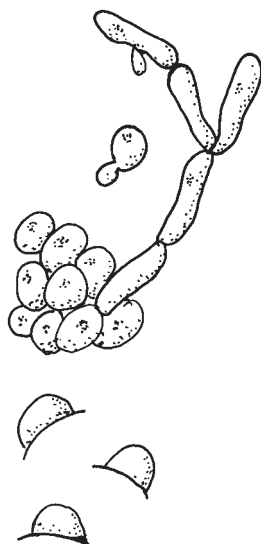
PATHOGENICITY: Occasionally causes infection in predisposed patients. Commonly encountered as a saprobe.

RATE OF GROWTH: Rapid; mature in 3 days.

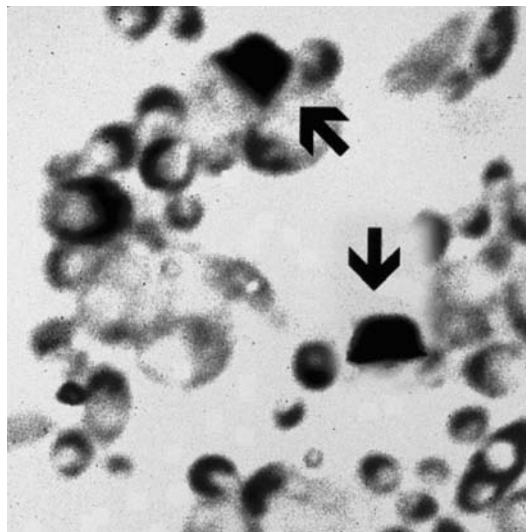
COLONY MORPHOLOGY: Smooth, moist, cream colored.

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, budding yeast cells are seen ($2-4 \times 2-6 \mu\text{m}$). Pseudohyphae form in some isolates. In the sexual state, when cultured on ascospore medium (p. 398) and stained with Kinyoun (p. 387) or ascospore (p. 388) stain, one to four ascospores per ascus are seen. There is a brim that turns downward around each ascospore, giving the impression of a helmet or hat. (Color Plate 60.)

See Tables 4 (p. 128) and 11 (p. 152) for identification of yeasts and yeastlike fungi.



Ascospores.



Arrows point to ascospores.

For further information, see

Anaissie et al. (ed.), 2009, pp. 260–261

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 38

***Malassezia* spp.**

PATHOGENICITY: *Malassezia* spp. are part of the normal skin flora in >90% of adults. Several of the species found on healthy skin are etiologic agents of pityriasis (tinea) versicolor, a superficial infection characterized by pale or dark patches of skin, especially that of the chest, shoulders, and back. They have also been associated with folliculitis, resembling acne, and seborrheic dermatitis, characterized by red, scaly lesions. Catheter-associated sepsis due to *Malassezia* is seen in neonates and adults receiving prolonged intravenous lipids; pulmonary infection may develop in these patients, with respiratory failure in neonates related to propagation of the organism in subendothelial deposits of lipid. The organisms have been isolated from infected areas in a variety of body sites, but their etiologic role is uncertain. The species most commonly associated with infection in humans are *M. globosa* and *M. sympodialis*, and to a lesser extent *M. furfur*, *M. obtusa*, *M. restricta*, and *M. slooffiae*.

RATE OF GROWTH: Rapid; mature in 5 days at 30–35°C. Grow poorly at 25°C, and some species do not grow well at 37°C; they grow on media containing cycloheximide. The organisms require long-chain fatty acids for growth (*M. pachydermatis* [p. 160] is the exception); solid medium overlaid with a thin film of olive oil is the most common method used,* but more-complex media, such as modified Dixon agar (p. 410) or Leeming-Notman agar (p. 414), are required for growth of some of the clinically significant species (*M. restricta* is the most difficult to cultivate). Blood for culture must be taken through the lipid infusion catheter for best recovery of the organism; lysis-centrifugation (ISOLATOR tubes; Wampole Laboratories) is advised as a result of many comparative studies.

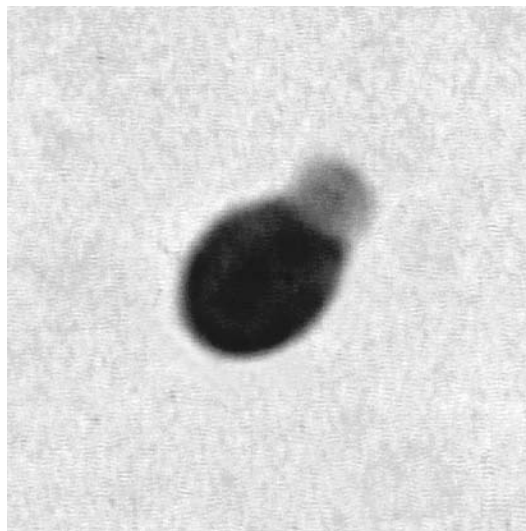
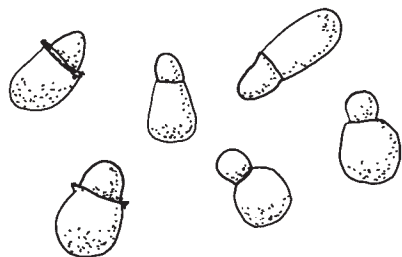
COLONY MORPHOLOGY: Smooth, cream to yellowish brown; often becomes dry, dull, brittle, and wrinkled with age.

MICROSCOPIC MORPHOLOGY: Yeastlike cells ($1.5\text{--}4.5 \times 3\text{--}7 \mu\text{m}$) are actually phialides with small collarettes; the collarettes are very difficult to discern with a routine light microscope. The cells of this genus are unique in being round at one end and bluntly cut off at the other, where wide budlike structures form singly; the buds are usually on a broad base, but narrow in some species. Staining with safranin and examining under oil immersion is a simple and effective way to observe the shape of the organism; also, calcofluor white allows for very clear delineation of the cell wall and distinctive outline. (Color Plate 61.) Hyphal elements are usually absent, but sparse rudimentary forms may occasionally develop.

Identification to species level is not routinely required but may be useful in cases of fungemia. Phenotypic identification can be achieved, but requires unique media not currently commercially available (see de Hoog et al., Kidd et al., and Guého et al. references below). If required, referral to a laboratory specializing in medical mycology and/or performing molecular analyses is suggested.

(continued on following page)

* Olive oil-saturated disks (p. 380) can be used in place of the overlay method.



For further information, see
Anaissie et al. (ed.), 2009, pp. 251–254
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Gaitanis et al., 2012
Guého et al., 1996
Kidd et al., 2016, p. 82

Malassezia pachydermatis

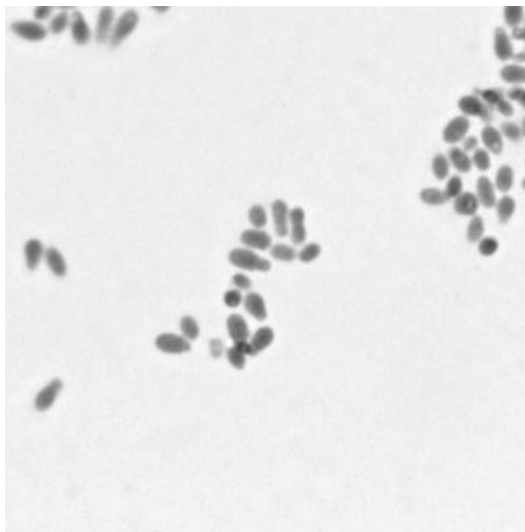
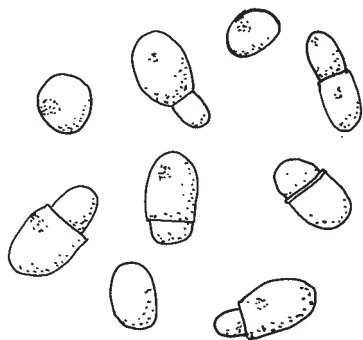
PATHOGENICITY: More often found in lower animals than in humans, it has been associated with inflammation of the ears of dogs. Occasionally reported to cause human infection, particularly in premature neonates receiving intravenous lipid emulsions. Isolates from patients' cases have been found as colonizers in participant health care workers and their pets.

RATE OF GROWTH: Rapid; mature in 5 days. Best growth is at 35–37°C; weak growth is seen at 25°C. Not inhibited by cycloheximide.

COLONY MORPHOLOGY: Creamy, dull, smooth; at first it is cream colored, becoming buff to orange-beige with age. Addition of fatty acids to the medium is NOT required for growth.

MICROSCOPIC MORPHOLOGY: Yeast cells are similar to other species of *Malassezia* (p. 158); they are round to oval (2.5–5.5 × 3.0–6.5 μm); conidia are produced on a broad base at one pole, which develops a collarette. Pseudohyphae and true hyphae are usually absent, occasionally sparsely present. Commercial biochemical yeast identification systems may misidentify this organism as *Candida lipolytica*; examination of microscopic morphology is essential.

See [Tables 4](#) (p. 128) and [11](#) (p. 152) for differentiation of yeastlike fungi.



For further information, see

Anaissie et al. (ed.), 2009, p. 254

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Gaitanis, 2012

Kidd et al., 2016, p. 82

Morris et al., 2005

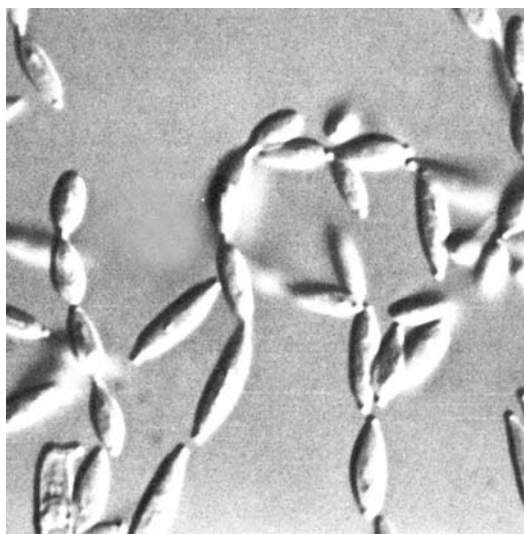
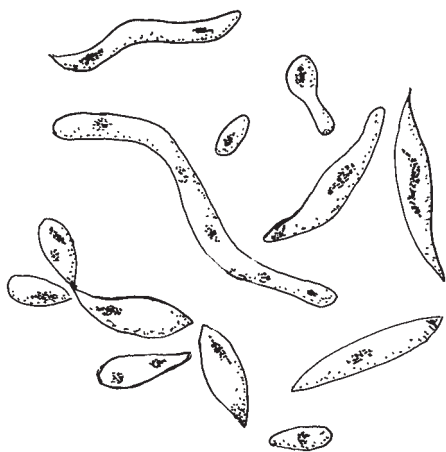
Ustilago sp.

PATHOGENICITY: Parasitic on seeds and flowers of many cereals and grasses. May cause contamination in cultures. It has seldom been implicated in human disease but may be inhaled and subsequently isolated from sputum specimens.

RATE OF GROWTH: Varies; mature in 5–20 days.

COLONY MORPHOLOGY: At first white, moist, pasty, and yeastlike; later it becomes tan to brown, wrinkled, raised, membranous, or velvety. Reverse is light in color.

MICROSCOPIC MORPHOLOGY: Elongate ($2\text{--}3 \times 9\text{--}30\ \mu\text{m}$), irregular, spindle-shaped or beanpod-shaped yeastlike cells. Short hyphae with clamp connections are sometimes observed.



Courtesy of Michael McGinnis.

For further information, see
Kurtzman and Fell, 2000, pp. 796–797
McGinnis, 1980, p. 386

***Prototheca* spp.**

(Commonly classified as achlorophyllous algae; included in this guide because they cause mycosislike infections and are often mistaken for yeasts)

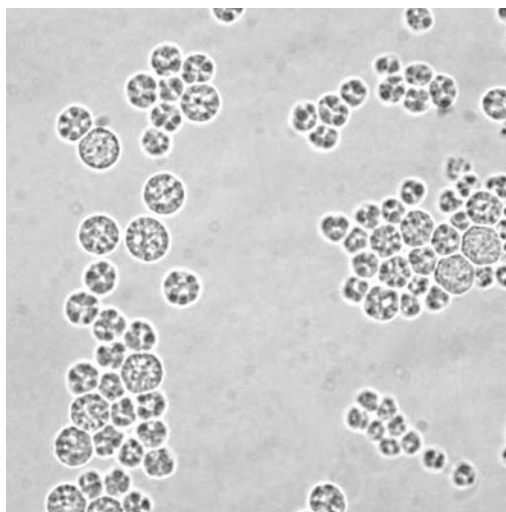
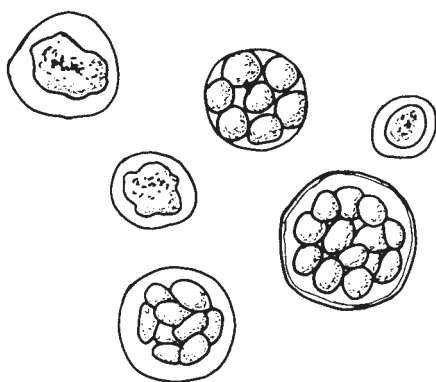
PATHOGENICITY: Can cause protothecosis, which may be cutaneous, subcutaneous, or systemic. Infection may arise through traumatic implantation into subcutaneous tissue. Trauma to the cornea may result in *Prototheca* keratitis. Only *P. wickerhamii* and *P. zopfii* have been involved in human disease, *P. wickerhamii* being the more commonly encountered. The organisms have also been isolated from clinical specimens in the absence of disease. *P. zopfii* is a common cause of bovine fungal mastitis.

RATE OF GROWTH: Rapid; mature in 3 days. Optimum growth is at 30°C; *P. wickerhamii* and *P. zopfii* grow at 37°C, but *P. stagnora* does not. They are inhibited by cycloheximide.

COLONY MORPHOLOGY: Dull white to cream colored; yeastlike in consistency.

MICROSCOPIC MORPHOLOGY: Sporangia of various sizes (7–25 µm in diameter) containing sporangiospores (endospores). Budding does not occur and no hyphae are produced. The sporangia of *P. wickerhamii* are somewhat smaller (4–11 µm in diameter) than those of *P. zopfii* (9–28 µm in diameter), but the size may depend on the substrate and environmental conditions. Endospores are formed by cleavage and are often arranged in a symmetrical, daisylike pattern. *P. stagnora* cells are 7–14 µm in diameter and produce capsules.

See [Tables 4](#) (p. 128) and [11](#) (p. 152) for identification of yeastlike organisms.



For further information, see
 Kurtzman and Fell, 2000, pp. 883–887
 Lass-Flörl and Mayr, 2007
 Minckler et al., 2016
 Todd, 2012

Trichosporon* spp.

PATHOGENICITY: *Trichosporon* is increasingly involved in invasive localized and disseminated disease, including of the kidney, lung, skin, eye, heart, and other tissue sites. Immunocompromised patients with neutropenia are especially susceptible. Some species cause white piedra, a superficial infection of the hair characterized by relatively soft, white nodules located along the shafts of hair. (Black piedra is caused by *Piedraia hortae* [p. 233].) The type and site of infection are somewhat predictive of the involved species (Table 12 [p. 164]). The organisms are also found as normal flora in the skin, nails, and mouth. *Trichosporon asabii* is the cause of summer-type hypersensitivity in Japan. *Trichosporon* spp. are resistant to echinocandins. *T. asabii* is inhibited by amphotericin B but resistant to its fungicidal effect. The cell wall of *T. asabii* shares antigenicity with the capsular polysaccharide of *Cryptococcus neoformans*; this may yield a positive cryptococcal antigen assay on serum of some patients with disseminated *Trichosporon* disease.

RATE OF GROWTH: Moderately rapid; mature in 5–7 days.

COLONY MORPHOLOGY: Yeastlike; at first cream colored, moist, and soft. The surface may become irregularly wrinkled, rather powdery or crumblike; the center may become heaped, and the colony may adhere to, and crack, the agar. The color often darkens to yellowish gray. (A colony of *T. asabii* is seen in Color Plate 62.)

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar at 25°C for 72 h, true hyphae and pseudohyphae with blastoconidia singly or in short chains are seen. Arthroconidia (2–4 × 3–9 μm) form on older cultures. The presence of pseudohyphae and blastoconidia differentiates *Trichosporon* from *Geotrichum* spp.

See Tables 4 (p. 128) and 11 (p. 152) for further differentiation of yeastlike organisms.

See Table 12 (p. 164) for key characteristics of clinically encountered *Trichosporon* spp.

See Table 25 (p. 288) for differential characteristics of fungi in which arthroconidia predominate.

For further information, see

Anaissie et al. (ed.), 2009, pp. 254–258

de Almeida et al., 2016

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Guého et al., 1992

Kidd et al., 2016, pp. 170–173

Liao et al., 2015

(continued on following page)

* The organism known in the older literature as *Trichosporon beigellii* or *Trichosporon cutaneum* (obsolete, invalid names) is now known to consist of numerous distinct species, of which eight appear to be of clinical significance.

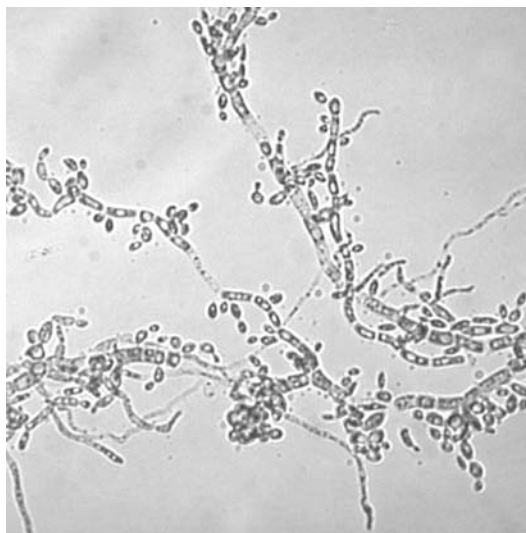
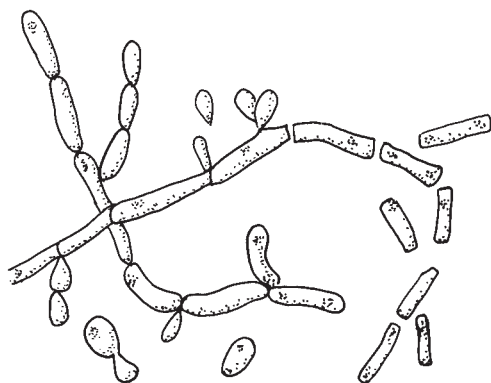


TABLE 12 Key characteristics of the most common clinically encountered *Trichosporon* spp.^a

Species	Infections	Urease	Growth at 37°C	Assimilation of:		
				Inos	Arab	Sorb
<i>T. asahii</i> ^b	Systemic with predilection for hematogenous dissemination	+	+	0	+	0
<i>T. mucoides</i>	Systemic with preference for central nervous system; also infects hair and nails	+	+	+	+	+
<i>T. cutaneum</i>	Rarely skin lesions and white piedra of underarm hairs	+	0	+	+	+
<i>T. inkin</i>	Mostly white piedra of pubic hairs; occasionally systemic	+	+	+	0	0
<i>T. ovoides</i>	White piedra of head hairs; occasional skin lesions	+	V	0	V	0

^a As the species of *Trichosporon* have rather distinct antifungal susceptibility profiles, molecular identification and/or susceptibility testing are essential for isolates from invasive infections. Abbreviations: +, positive; 0, negative; V, strain variation; Inos, inositol; Arab, L-arabinose; Sorb, sorbitol.

^b *Trichosporon asteroides*, a rare cause of superficial and systemic infection, is physiologically indistinguishable from *T. asahii* and is therefore regularly misidentified, but its microscopic morphology is unique in producing hyphae that swell and develop large, multiseptate cells that separate into packets of smaller cells. See de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4.

Blastoschizomyces capitatus*

PATHOGENICITY: Infrequent cause of fungemia and disseminated infection in immunocompromised hosts, most commonly in neutropenic leukemia patients. Lesions have occurred in the lung, kidney, liver, spleen, brain, and other organs; it has also caused endocarditis. The organism is distributed in nature and may be found as normal flora of the skin, respiratory tract, and gastrointestinal tract.

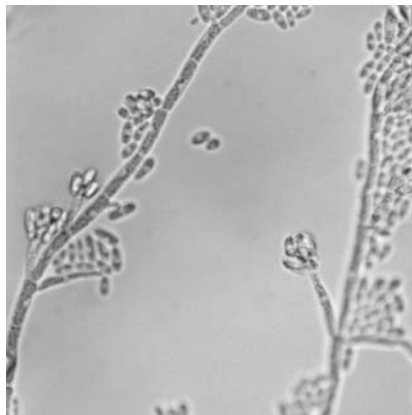
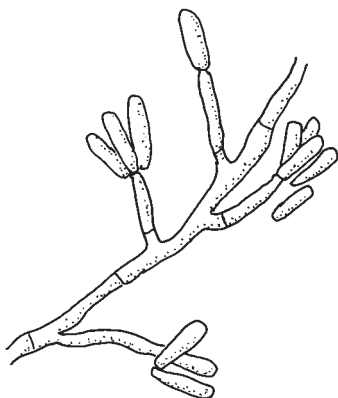
RATE OF GROWTH: Rapid; mature in 5 days.

COLONY MORPHOLOGY: Yeastlike, smooth to wrinkled, radiating edges, developing short aerial hyphae with age; white to cream colored.

MICROSCOPIC MORPHOLOGY: On cornmeal-Tween 80 agar, round to oval budding yeastlike cells, hyphae, and pseudohyphae; few arthroconidia and many annelloconidia are seen. Anellides form along the hyphae or at the ends of hyphal branches; annelloconidia are elongate ($2.5\text{--}3.5 \times 7\text{--}10\text{ }\mu\text{m}$) and flattened at the base and accumulate in clusters at the tips of the anellides. It is difficult to determine that the conidia are annelloconidia and not arthroconidia or blastoconidia; this may account for its confusion with (and sometimes misidentification as) *Trichosporon* spp. or *Candida krusei*. Careful attention to a few biochemical test results is required to differentiate these organisms.

See [Tables 4 \(p. 128\)](#) and [11 \(p. 152\)](#) for differentiation of yeastlike fungi.

See [Table 7 \(p. 137\)](#) for comparison with *C. krusei*.



For further information, see
Anaisie et al. (ed.), 2009, pp. 261–262
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Polacheck et al., 1992
Salkin et al., 1985a
Subramanya et al., 2016

* Some sources may refer to *Blastoschizomyces capitatus* as *Saprochaete capitata* or as the teleomorph *Magnusiomyces capitatus*.

Geotrichum candidum

PATHOGENICITY: Has been isolated from a variety of specimens (lungs, blood, mouth, intestines, vagina, and skin), but its role in infection is uncertain. It is found as normal flora in humans and seems to cause disease (primarily of the lungs) only in severely compromised hosts. Dissemination has been reported, but only rarely.

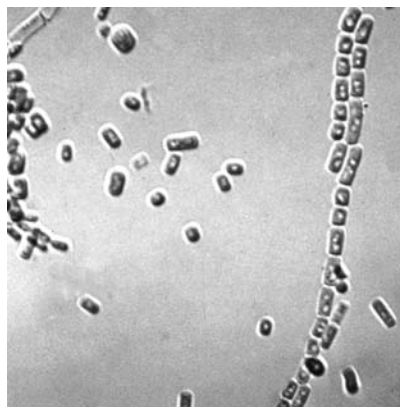
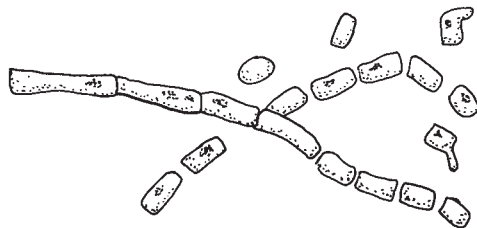
RATE OF GROWTH: Rapid; mature in 4 days.

COLONY MORPHOLOGY: At 25°C, young colonies are white, moist, yeastlike, and easily picked up. Submerged hyphae are later seen at the periphery, giving the appearance of ground glass. Some strains develop a short, white, cottony aerial mycelium. Many strains do not grow at 37°C, but some may have a small amount of surface growth and extensive subsurface growth at this temperature. (Color Plates 63 and 64.)

MICROSCOPIC MORPHOLOGY: Coarse true hyphae (no pseudohyphae) that segment into rectangular arthroconidia that vary in length (most are 4–10 μm) and in the roundness of their ends. Some may become quite round. The rectangular cells characteristically germinate from one corner. The biochemical characteristics and the absence of blastoconidia along the hyphae differentiate this organism from *Trichosporon* spp. (p. 163); the consecutive formation of the arthroconidia (not alternating with empty cells) serves to separate it from *Coccidioides* spp. (p. 287), *Malbranchea* (p. 289), and *Geomyces* (p. 290); and the absence of conidiophores distinguishes it from *Arthrographis* (p. 291).

See Tables 4 (p. 128) and 11 (p. 152) for differentiation of yeastlike organisms.

See Table 25 (p. 288) for differential characteristics of fungi with predominating arthroconidia.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Graeff et al., 2017
 Henrich et al., 2009
 Kidd et al., 2016, pp. 71–72

Thermally Dimorphic Fungi

Introduction to Thermally Dimorphic Fungi 169

Histoplasma capsulatum 170

Blastomyces dermatitidis and *Blastomyces gilchristi* 172

Paracoccidioides brasiliensis 174

Talaromyces (Penicillium) marneffe 176

Sporothrix schenckii complex 178

TABLE 13 Characteristics for differentiating species of the
Sporothrix schenckii complex 180

Introduction to Thermally Dimorphic Fungi

The fungi included here are unique in that they grow as moulds in the environment or when cultured on routine mycology agar (e.g., Sabouraud dextrose agar, inhibitory mould agar, and similar primary media) at 25–30°C and are yeastlike in tissue or when cultured on an enriched medium (e.g., brain heart infusion agar) at 37°C. All of them except *Talaromyces* (*Penicillium*) *marneffei* will grow at 25–30°C on media containing cycloheximide. All of the fungi in this section are known to be pathogenic and must be handled with caution. For laboratory safety, isolates suspected of being *Histoplasma*, *Blastomyces*, *Paracoccidioides*, or *T. marneffei* should be grown on slants rather than on petri plates, and slide cultures should not be performed. Their identifying characteristics are observable on well-prepared wet preparations.

Coccidioides spp. (often placed with these organisms in other texts) do NOT produce yeastlike colonies or cells at 35–37°C on routine mycology agar; therefore, in this guide they are placed with the hyaline hyphomycetes (p. 286).

Cokeromyces recurvatus is thermally dimorphic, but because of its mucormycetous properties, it has been placed within that class (p. 196).

For further information, see:

Chen et al., 2015

Guarro and de Hoog, 2015

Thompson and Gomez, 2015

Histoplasma capsulatum

PATHOGENICITY: Histoplasmosis most commonly develops as an asymptomatic or mildly symptomatic infection in immunocompetent patients. The infection may reactivate years later or may develop as a primary infection as an acute, benign pulmonary disease or may be chronic or progressive and fatal. It may be localized or cause a disseminated, life-threatening infection (primarily of the reticuloendothelial system) and involve various tissues and organs of the body, particularly in immunocompromised patients with impairments of cellular immunity (resulting from, e.g., transplantation or HIV/AIDS). The organism is most often found in bird and bat droppings and is endemic in the Ohio and Mississippi River valleys as well as in other regions of the world, both temperate and tropical. Clusters of histoplasmosis have been classically reported in spelunkers exploring bat dwelling caves.

There are two varieties of *H. capsulatum* that cause disease in humans: *H. capsulatum* var. *capsulatum* and *H. capsulatum* var. *duboisii*. The variety *duboisii* is endemic only in tropical central Africa. It differs from the variety *capsulatum* by producing thick-walled, larger yeast cells (8–15 μm in diameter) in infected tissue; on culture the two varieties are similar.

H. capsulatum var. *capsulatum* elaborates a carbohydrate antigen, which can be identified in the urine for diagnosis of pulmonary and disseminated histoplasmosis.

RATE OF GROWTH: Slow; mycelial forms usually mature within 15–20 days but may take up to 8 weeks. The organism does not survive well in clinical specimens; therefore, when histoplasmosis is suspected, the specimen must be processed immediately.

COLONY MORPHOLOGY: Thermally dimorphic. At 25–30°C, colony is white to brown, or pinkish, with a fine, dense cottony texture. The reverse is white, sometimes yellow or orange-tan. An enriched agar is recommended for initial isolation, but characteristic morphology of the mould phase can be seen on most routine media. (Color Plate 68.)

Upon direct culture of the patient's specimen at 37°C on brain heart infusion (BHI) or other enriched agar, colonies are moist, white, and yeastlike. The yeast phase is inhibited by cycloheximide.

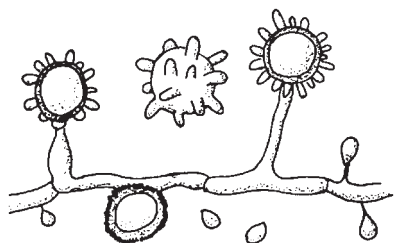
MICROSCOPIC MORPHOLOGY: Slide cultures should not be performed on isolates suspected of being *Histoplasma*; the identifying characteristics will be observable on well-prepared wet mounts.

At 25–30°C in young cultures, septate hyphae are seen bearing round to pear-shaped, smooth or occasionally spiny microconidia (2–5 μm in diameter) on short branches or directly on the sides of the hyphae. At this early stage, *H. capsulatum* might be confused with a rare small form of *Blastomyces dermatitidis* (p. 172). After several weeks, large, thick-walled, round macroconidia (7–15 μm in diameter) form; they are tuberculate, knobby, or have short, cylindrical projections; occasionally they may be smooth. The macroconidia greatly resemble those of *Sepedonium* sp. (p. 330), but *Sepedonium* sp. does not produce microconidia and grows poorly or not at all at 37°C.

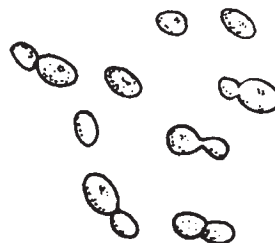
Histoplasma capsulatum (continued)

At 37°C, small, round or oval budding cells (2–3 × 3–5 µm) and occasional abortive hyphae are formed. Conversion from the mould to the yeast phase often requires many generations at 37°C.

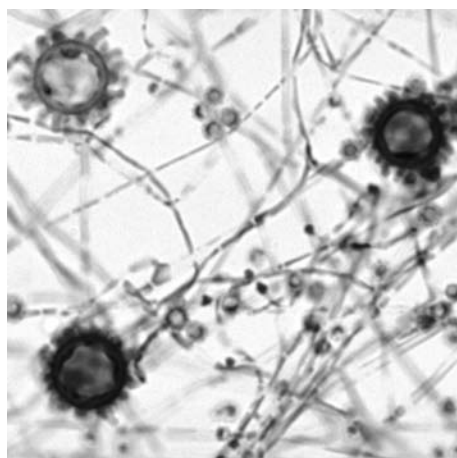
Identification of *H. capsulatum* is readily confirmed by use of a commercially available DNA probe. The conversion of the mycelial form to the yeast phase, although required in the past for morphologic identification (see p. 381), is seldom possible *in vitro* in a reasonable amount of time. Instead, conversion is usually used in a research or educational setting. A test for specific exoantigen is largely only of historic interest, as the reagents are limited in availability.



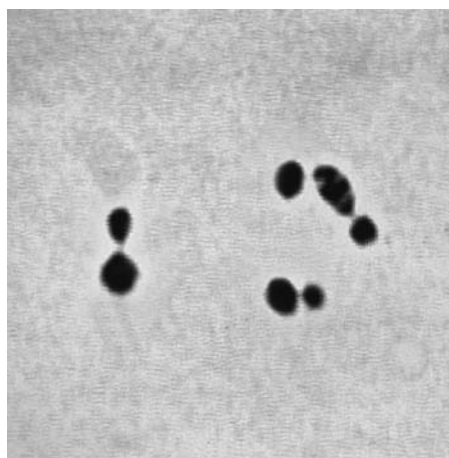
25°C.



37°C.



25°C on Sabouraud dextrose agar.



37°C on BHI agar.

For further information, see

Anaissie et al. (ed.), 2009, pp. 359–363

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Hospenthal and Rinaldi, 2008, pp. 317–329

Kauffman, 2007

Kidd et al., 2016, pp. 75–76

Loulergue et al., 2007

Schuetz, 2013

Blastomyces dermatitidis* and *Blastomyces gilchristi*

PATHOGENICITY: *B. dermatitidis* causes blastomycosis, which may range from a transient pulmonary infection to a chronic infection characterized by suppurative and granulomatous lesions of the lungs, bone, and skin, but potentially of any part of the body; it most commonly begins in the lungs and is disseminated to the skin and bones. Occurs mainly in the mid-Atlantic, southeastern, and north central parts of the United States, as well as the south central regions of Canada.

RATE OF GROWTH: Slow; mycelial forms mature within 14 days. Some strains are slower; cultures should be held for 8 weeks if blastomycosis is suspected. Organism does not survive well in specimens and must therefore be cultured immediately.

COLONY MORPHOLOGY: Thermally dimorphic. At 25–30°C, it is at first yeastlike, then prickly, and finally very cottony with a white aerial mycelium that turns tan or brown with age. Reverse is tan. (Color Plate 69.)

At 35–37°C, it is cream to tan in color, heaped or wrinkled, and waxy in appearance; it grows best on brain heart infusion (BHI) or other enriched agar. Yeast phase is inhibited by cycloheximide.

MICROSCOPIC MORPHOLOGY: Slide cultures should not be performed on isolates suspected of being *Blastomyces*; the identifying characteristics will be observable on well-prepared wet mounts.

At 25–30°C, septate hyphae form with short or long conidiophores; round or pear-shaped conidia (2–10 µm in diameter) develop at the apex of the conidiophore (causing a lollipop-like appearance) or directly on the hyphae. It may resemble *Scedosporium* spp. (p. 222) or *Chrysosporium* spp. (p. 326). Older cultures have thick-walled chlamydoconidia (7–18 µm in diameter).

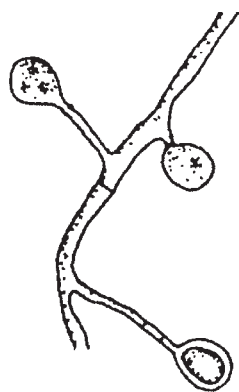
At 37°C on BHI or other enriched agar, forms yeastlike cells (usually 8–15 µm in diameter) that bud on a broad base (4–5 µm wide) and appear to be thick walled and double contoured; the bud often remains attached until it becomes the same size as the parent cell.

Identification of *B. dermatitidis* is readily confirmed by use of a commercially available DNA probe. Identification can also be accomplished by conversion of the mycelial (mould) form to the yeast phase (see p. 381). A test for specific exoantigen is seldom performed largely due to limited availability of reagents.

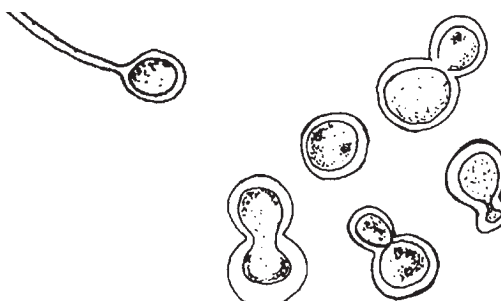
(continued on following page)

* *B. gilchristi* is a cryptic species of *Blastomyces* that has been identified as the most common cause of blastomycosis in children in a major Wisconsin referral center. Identified by molecular sequencing methodology, this species may tend to occur in the more north central region of the United States and the south central part of Canada.

Blastomyces dermatitidis and *Blastomyces gilchristi* (continued)



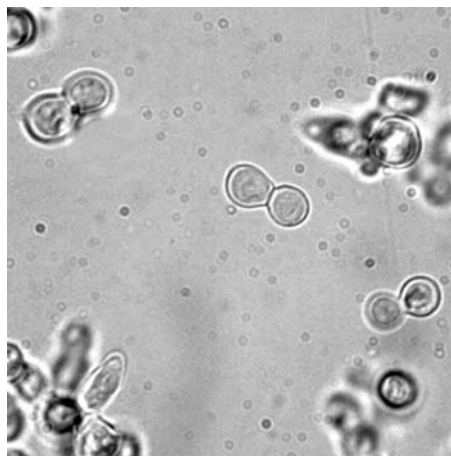
25°C.



37°C.



25°C on Sabouraud dextrose agar.



37°C on BHI agar.

For further information, see
Anaissie et al. (ed.), 2009, pp. 355–359
Castillo et al., 2016
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Hospenthal and Rinaldi, 2008, pp. 277–293
Kidd et al., 2016, p. 19

Paracoccidioides brasiliensis

PATHOGENICITY: Causes paracoccidioidomycosis (South American blastomycosis), a chronic granulomatous disease characteristically beginning in the lungs and spreading to the mucous membranes of the nose, mouth, and occasionally the gastrointestinal tract. Dissemination to skin, lymph nodes, and other internal organs is common. It is endemic in areas from Mexico to Argentina, but is most prevalent in South America, especially northern Brazil, Colombia, and Venezuela. It causes disease predominantly in males due to the role of estrogen in preventing dimorphic transformation in females.

RATE OF GROWTH: Very slow; mycelial forms mature within 21 days.

COLONY MORPHOLOGY: Thermally dimorphic. At 25–30°C, colony is white, heaped, compact, usually folded, and almost glabrous or with a short nap of white aerial mycelium that often turns brown with age. Reverse is light or brownish.

At 37°C on brain heart infusion (BHI) or other enriched agar, colony is heaped, cream to tan, moist, and soft, becoming waxy and yeastlike.

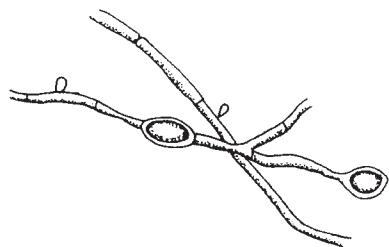
MICROSCOPIC MORPHOLOGY: Slide cultures should not be performed on isolates suspected of being *Paracoccidioides*; the most useful identifying characteristics will be observable on a wet prep of the yeast form at 37°C.

At 25–30°C, usually forms only septate, branched hyphae with some intercalary and terminal chlamydospores; a few microconidia are sometimes observed along the hyphae.

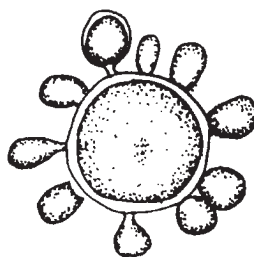
At 35–37°C on BHI or other enriched agar, the characteristic large, round, fairly thick-walled cells (5–50 µm in diameter) with single and multiple buddings (2–10 µm in diameter) are seen. The buds are attached to the mother cell by narrow connections and may almost completely surround the cell, giving the characteristic ship's-wheel appearance. When single-budding cells occur, the narrow necks of attachment should prevent confusion with *Blastomyces dermatitidis*.

Conversion of the mould form to the yeast phase is essential for identification (see p. 381). There are no readily available commercially prepared DNA probes available thus far. Exoantigen testing is usually not feasible due to limited availability of reagents.

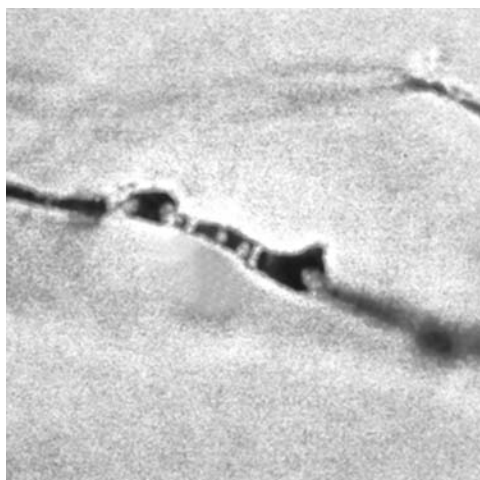
(continued on following page)



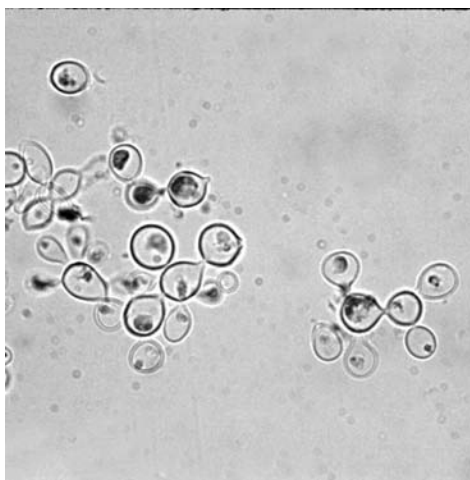
25°C.



37°C.



25°C on potato dextrose agar.



37°C on BHI agar.

For further information, see
Anaissie et al. (ed.), 2009, pp. 366–367
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Hospenthal and Rinaldi, 2008, pp. 331–342
Kidd et al., 2016, p. 107
Teles and Martins, 2011

Talaromyces (Penicillium) marneffei

PATHOGENICITY: Causes deep-seated infection that can be focal or disseminated, predominantly in immunocompromised patients (those with HIV/AIDS, lymphoma, or autoimmune disorders) and occasionally in seemingly immunocompetent individuals who live in, or have traveled in, areas of Southeast Asia where *T. marneffei* is endemic. The organism has been isolated from blood, bone marrow, skin, lung, mucosa, lymph nodes, urine, stool, cerebrospinal fluid, and various internal organs.

RATE OF GROWTH: Rapid; mature within 3 days at 25–30°C. Yeastlike form develops more slowly at 35–37°C. Sabouraud dextrose agar (SDA) supports growth at 25–37°C and demonstrates thermal dimorphism. Cycloheximide inhibits growth.

COLONY MORPHOLOGY: Thermally dimorphic. At 25–30°C on SDA, colony is flat, powdery to velvety, and tan, later becoming reddish yellow with a yellow or white edge; it is often bluish gray-green in the center. A deep-reddish soluble pigment diffuses into the medium after 3–7 days; this is best seen on potato dextrose agar. Some *Penicillium* species may also produce a red pigment but will not convert to a yeastlike form at 35–37°C. Reverse is brownish red. (Color Plates 70 to 72.)

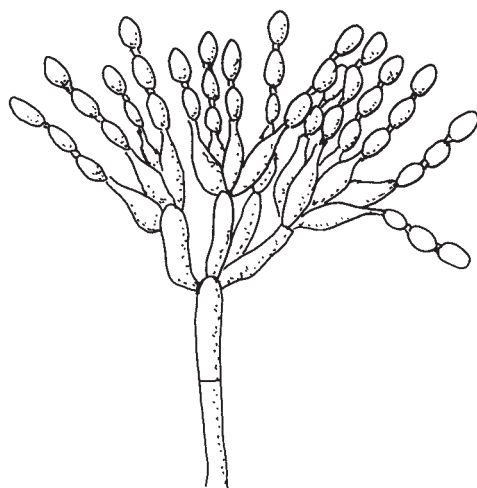
At 35–37°C on SDA, inhibitory mould agar, or brain heart infusion (BHI) or other enriched agar, colony is soft, white to tan, dry, and yeastlike. Conversion from the mould to yeastlike form may take up to 14 days; it is most rapidly accomplished (in ~4 days) by culturing in BHI broth on a shaker; the next-best method is on 5% sheep blood agar.

MICROSCOPIC MORPHOLOGY: Distinction between *T. marneffei* and members of the genus *Penicillium* is important. Slide cultures should not be performed on isolates suspected of being *T. marneffei*; the identifying characteristics will be observable on well-prepared wet mounts of the mould at 30°C and the yeastlike form at 37°C.

At 25–30°C, structures typical of the genus *Penicillium* (p. 309) develop, i.e., smooth conidiophores with four or five terminal metulae, each metula bearing four to six phialides. Conidia are smooth or slightly rough and round to oval (2–3 × 2.5–4 µm) and form chains; short, narrow extensions connect the conidia.

Further distinguishing *T. marneffei* from members of the genus *Penicillium*, when conidia are incubated at 35–37°C, they form hyphal elements that eventually fragment at the septa, producing single-celled, round to oval arthroconidia (3–5 µm in diameter); this is the form commonly referred to as yeastlike cells; buds are not produced. The arthroconidial yeastlike cells of *T. marneffei* continue to reproduce by fission and in so doing may elongate to 8–9 µm.

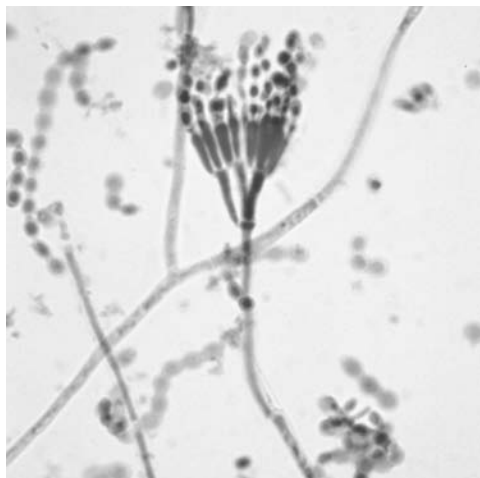
(continued on following page)



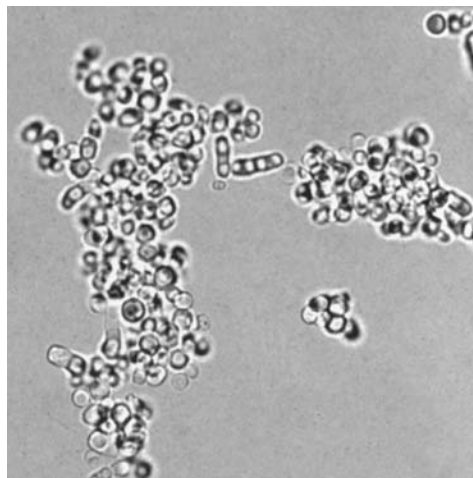
25°C.



37°C.



25°C on SDA. Courtesy of William Merz.



37°C in BHI broth. Courtesy of William Merz.

For further information, see

Anaissie et al. (ed.), 2009, pp. 315–316, 320–321, 369–370, 578

Cooper and Haycocks, 2000

Kidd et al., 2016, p. 110

Samson et al., 2011

Sporothrix schenckii* complex

PATHOGENICITY: Causes sporotrichosis due to *Sporothrix schenckii sensu stricto*, a chronic infection that frequently begins as a lesion of the skin and subcutaneous tissue and then involves the lymphatic channels and lymph nodes draining the area. Initial introduction into the body is usually due to a puncture by contaminated plant material (e.g., wood splinters, thorns, sphagnum moss, or hay). Primary pulmonary sporotrichosis may develop in predisposed individuals after inhaling the fungus. On rare occasions the infection has disseminated with fatal outcome in immunocompromised patients.

By comparison, *Sporothrix brasiliensis* infects predominantly cats that developed ulcerative lesions that serve as a source for infecting owners and those who have contact with the animals. These lesions in humans also may be ulcerative and behave aggressively with loss of skin and subcutaneous tissue.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Thermally dimorphic. At 25–30°C, the colonies are at first small and white or pale orange to orange-gray with no cottony aerial hyphae. Later, the colonies become moist, wrinkled, leathery, or velvety and often darken to a salt-and-peppery brown or black with a narrow white border. Some isolates are black from the beginning; stock cultures may become, and remain, nonpigmented. Colonies with a dark surface have a reverse that is commonly dark in the center and light at the periphery. (Color Plates 66 and 67.)

At 35–37°C, colonies are cream or tan, smooth, and yeastlike. It is best to use brain heart infusion (BHI) agar and transfer several generations to obtain a good yeast phase (p. 404).

MICROSCOPIC MORPHOLOGY: At 25–30°C, hyphae are very narrow (1–2 µm in diameter), septate, and branching, with slender, tapering conidiophores rising at right angles. The apex of the conidiophore is often slightly swollen and bears many small, tear-shaped or almost round hyaline conidia (2–3 × 2–6 µm) on delicate threadlike denticles, forming a “rosettelike” cluster in young cultures; conidia (usually thick walled and brown) form singly along the hyphae. Microscopic features may be best observed on cornmeal agar.

At 35–37°C, round, oval, and fusiform budding cells of various sizes (1–3 × 3–10 µm), commonly called cigar bodies, are seen.

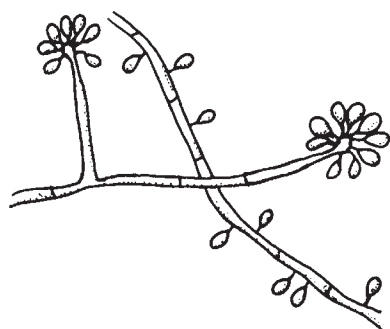
(continued on following page)

* *Sporothrix schenckii* has been found to be a complex of several different species (*S. albicans*, *S. brasiliensis*, *S. globosa*, *S. luriei*, *S. mexicana*, and *S. schenckii sensu stricto*), having similar morphologies. *S. brasiliensis*, *S. globosa*, *S. schenckii*, and very rarely *S. luriei* are known to cause infection in humans.

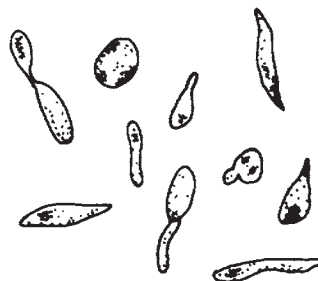
Sporothrix schenckii complex (continued)

The conversion of the mycelial form to the yeastlike form is essential for confirmation of identification (see [p. 381](#)).

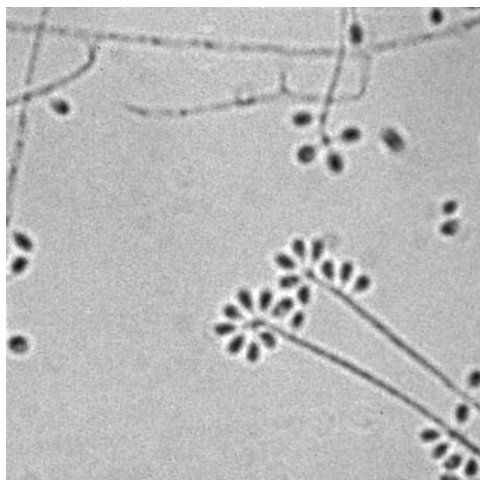
For phenotypic differentiation of species of the *S. schenckii* complex, see [Table 13](#) ([p. 180](#)).



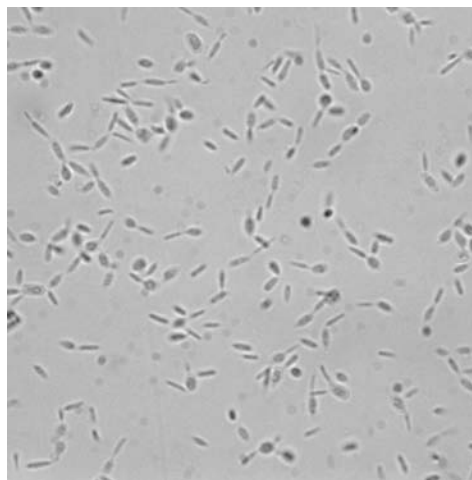
25°C.



37°C.



25°C on Sabouraud dextrose agar.



37°C on BHI agar.

For further information, see

Anaissie et al. (ed.), 2009, pp. 367–369, 518, 537–539

Bastos de Lima Barros et al., 2011

Chakrabarti et al., 2015

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, pp. 186–187

Marimon et al., 2007 (phenotypic characterization of the species of the complex)

TABLE 13 Characteristics for differentiating species of the *Sporothrix schenckii* complex^a

Species	Colony color on CMA ^b	Dark brown conidia on sides of hyphae ^c	Colony diameter on PDA at 30°C in 21 days ^d	Growth at 37°C on PDA ^d	Assimilation of: ^e	
					Sucrose	Raffinose
<i>S. schenckii</i>	Brown	+	<50 mm	+	+	+
<i>S. brasiliensis</i>	Brown	+	<50 mm	+	0	0
<i>S. globosa</i>	Brown	+	<50 mm	–	+	0
<i>S. mexicana</i>	Brown	+	>50 mm	+ (±) ^f	+	+
<i>S. albicans</i>	White	0	>50 mm	+	+	0

^aAdapted from Marimon et al., 2007. Abbreviations: +, positive; 0, negative; ±, slight growth.

^bCMA = cornmeal agar at 30°C (plain; Tween 80 was not incorporated in medium in original study).

^cSeen on slide cultures on CMA after 10–12 days' incubation at 30°C. The growth should be mounted for viewing on slides without the use of cotton (aniline) blue in order to see the natural color of the conidia along the hyphae.

^dInoculated onto center of potato dextrose agar plate with piece of fungus ~1 µm in diameter, placed upside down.

^eCarbohydrate assimilation was tested in the original study by the Yarrow method in microtiter plates. Sucrose (saccharose) and raffinose are both included in the API 20C AUX (bioMérieux, Hazelwood, MO); to date, no data have been published on results of these organisms tested on API 20C, but it is expected that the results would agree with those of the Yarrow method.

^fAt 37°C, *S. mexicana* grows, but not very well.

Thermally Monomorphic Moulds

Thermally monomorphic moulds are fungi that are filamentous at whatever temperatures they are able to grow (25–35°C). Some do not grow at 35–37°C but are filamentous at 25–30°C.

Mucormycetes [183](#)

Dematiaceous Fungi [203](#)

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Mucormycetes

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TABLE 15 Differential characteristics of the clinically encountered *Rhizopus* spp. 187

Rhizopus spp. 188

Mucor spp. 189

Rhizomucor spp. 190

Lichtheimia corymbifera complex (formerly *Absidia corymbifera*) 191

Apophysomyces elegans 193

Saksenaea vasiformis 195

Cokeromyces recurvatus 196

Cunninghamella bertholletiae 198

Syncephalastrum racemosum 199

Basidiobolus sp. 200

Conidiobolus coronatus 201

Introduction to Mucormycetes

The large majority of Mucormycetes that are encountered in the clinical laboratory belong to the order Mucorales. Infections caused by fungi in this order can correctly be described as mucormycosis; however, the misinterpretation that they are all caused by *Mucor* spp. must be avoided. *Rhizopus* spp. are the most common causes of mucormycosis. Among all causes of mucormycosis, the leading four genera are *Rhizopus* spp., *Rhizomucor* spp., *Mucor* spp., and *Lichtheimia corymbifera* complex. As discussed in the section on taxonomy and nomenclature (p. 11), the class Mucormycetes has now replaced the term “Zygomycetes.” As the class of Zygomycetes was found to be polyphyletic (having more than one immediate common ancestor among the included species), the term was discarded and the two phyletically distinct classes of Mucormycetes and Entomophthoromycetes were established.

Mucormycetes are a class of fungi that have broad hyphae (6–15 μm wide) and are nonseptate or almost nonseptate. Septa are most often seen on sporangiophores just below the sporangia and elsewhere in older cultures. Asexual reproduction occurs in a saclike structure called a sporangium, in which the internal contents are cleaved into spores. Some species may rarely form zygospores in culture. Most genera are inhibited by cycloheximide.

The Mucorales are easily recognized by their grayish, floccose colonies that rapidly fill the tube or petri plate in a cotton candy-like fashion. The differentiation of the various genera is based on

- The presence and location (or absence) of rhizoids (rootlike structures along the vegetative hyphae)
- The branching or unbranched nature of the sporangiophores (the stalks bearing the saclike sporangia)

Examining growth on a slant, angled to view the side, on a dissecting microscope can be very helpful in viewing the presence or absence of rhizoids and branching

- The shape of the columella (the small domelike area at the apex of the sporangiophore)

- The appearance of an apophysis (a broadening near the apex of the sporangio-phore, just below the columella)
- The size and shape of the sporangia

See labeled diagram, [p. 192](#).

A few genera (e.g., *Apophysomyces* and *Saksenaea*) require special media ([p. 420](#)) to enhance sporulation.

Some of the members of the order Mucorales can cause severe disease (mucormycosis), predominantly in patients who are predisposed by diabetes mellitus, neutropenia, corticosteroid therapy, hematological malignancies, transplantation, HIV/AIDS, severe burns, intravenous drug abuse, iron overload conditions, and malnutrition. Infections have been reported from a wide range of anatomic sites but are most commonly sino-orbital, rhinocerebral, pulmonary, cutaneous, and disseminated. The organisms are known for their disastrous ability to invade and clot blood vessels.

The Entomophthorales, formerly within the now abandoned class of Zygomycetes, are considered to be an order of fungi that is phyletically distinct from the order of Mucorales. In comparison to mucormycosis, infections caused by members of the order of Entomophthorales occur in otherwise healthy individuals and are less commonly encountered. The two medically important genera of Entomophthorales (*Basidiobolus* and *Conidiobolus*) cause tropical subcutaneous mycoses and are discussed in the latter portion of this section ([pp. 200–201](#)).

For further information, see

Anaissie et al. (ed.), 2009, chapter 12 (for information on mucormycosis)

Garcia-Hermoso et al., 2015

Kwon-Chung, 2012

Roden et al., 2005

Shaikh et al., 2016

TABLE 14 Differential characteristics of similar organisms in the class Mucormycetes^a

Organism	Rhizoids	Sporangiophores	Apophysis	Columellae	Sporangia	Maximum optimal growth temp (°C)
<i>Rhizopus</i>	Present	Single or in tufts; usually unbranched; mostly brownish	Mostly inconspicuous	Almost round or slightly elongated	Round, 50–275 µm	~40–50; species vary
<i>Rhizomucor</i>	Few, usually short	Branched, dark brown	Absent (or very tiny)	Almost round	Round, 40–100 µm	~38–58; species vary
<i>Mucor</i>	Absent	Branched or unbranched; mostly hyaline	Absent	Various shapes	Round, 50–100 µm	≤37
<i>Lichtheimia</i> (<i>Absidia</i>)	Present, but often indistinct	Finely branched; almost hyaline; form in groups of 2–5	Conspicuous, conical	Semicircle; may have projection at top	Pyriform (pear shaped), 20–90 µm	~45–50
<i>Apophysomyces</i>	Present	Generally single and unbranched; grayish brown	Conspicuous, bell shaped	Semicircle; may be elongated	Pyriform, 20–60 µm	≥42

^a Adapted from Scholer et al., 1983, with additions and updates.**TABLE 15** Differential characteristics of the clinically encountered *Rhizopus* spp.^a

Organism	Pathogenic	Maximum growth temp (°C)	Rhizoid length (µm)	Sporangiophore length (µm)	Sporangium diameter (µm)	Columellae	Sporangiospores
<i>R. arrhizus</i> (most common agent of mucormycosis)	+	40–46	150–300	500–2,000	50–250	Almost round	Variable size; average length, 6–8 µm; striated; elongate to lemon shaped
<i>R. microsporus</i> var. <i>rhizopodiformis</i> ^b (second most common agent of mucormycosis)	+	50–52	100–120	200–500	40–120	Slightly elongated: distinct apophysis	Equal in size; average length, 4–6 µm; smooth to slightly striated; almost round to slightly elongated
<i>R. stolonifer</i>	0	30–32	300–350	1,500–4,000	150–350	Almost round	Variable in size; average length, 9–11 µm; very striated; elongate to polyhedric

^a Abbreviations: +, positive; 0, negative.^b Other varieties of *R. microsporus* do not grow at 50°C.

Rhizopus* spp.

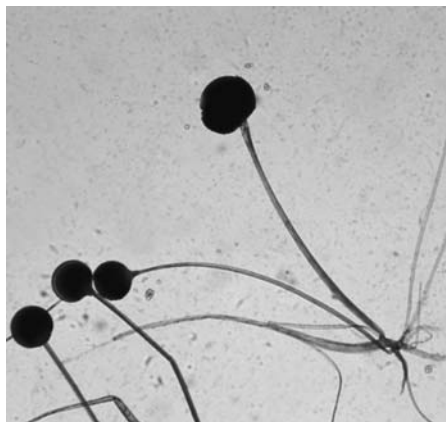
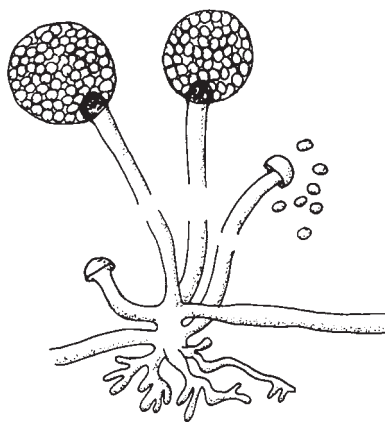
PATHOGENICITY: The most common etiologic agents of mucormycosis (see p. 43 for a description of the disease). They are also found as common contaminants.

RATE OF GROWTH: Rapid; mature within 4 days. The pathogenic species grow well at 37°C. Growth is inhibited by cycloheximide.

COLONY MORPHOLOGY: Quickly covers agar surface with dense growth that is cotton candy-like; colonies are white at first and then gray or yellowish brown. Reverse is white to pale shades of gray or brown. (Color Plate 73.)

MICROSCOPIC MORPHOLOGY: Broad hyphae (6–15 μm in diameter) have no, or very few, septa. Numerous stolons run among the mycelia, connecting groups of long sporangiophores that usually are unbranched. At the point where the stolons and sporangiophores meet, rootlike hyphae (rhizoids) are produced. The sporangiophores are long (up to 3 mm) and terminate with a dark, round sporangium (40–275 μm in diameter) containing a columella and many oval, colorless or brown sporangiospores (4–9 μm in diameter). No collarette remains when the sporangial wall dissolves. Large chlamydospores are sometimes seen. This genus is differentiated from *Mucor* spp. (p. 189) by the presence of stolons, rhizoids, and usually unbranched sporangiophores. It is differentiated from *Lichtheimia* (*Absidia*) spp. (p. 191) by the location of the rhizoids in relation to the sporangiophores and by the size and shape of the sporangia.

See Tables 14 and 15 (p. 187) for identification of genus and species.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Farmakiotis and Kontoyiannis, 2016
 Kidd et al., 2016, pp. 168–169
 Ribes et al., 2000

* *Rhizopus arrhizus* is the most common medically important member of the genus *Rhizopus*. *R. arrhizus* var. *delemar* or *Rhizopus delemar* is a recently described genotypically distinct member of the genus that has been classified phenotypically by organic acid production.

Mucor spp.

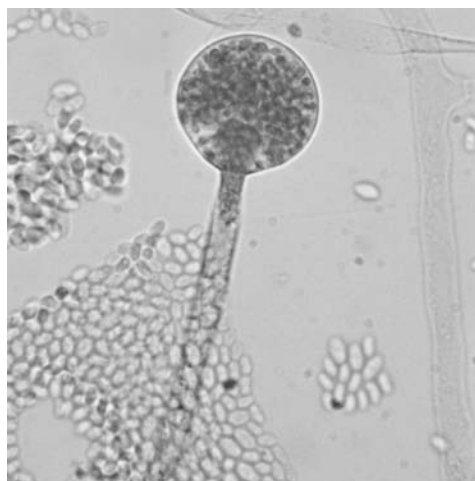
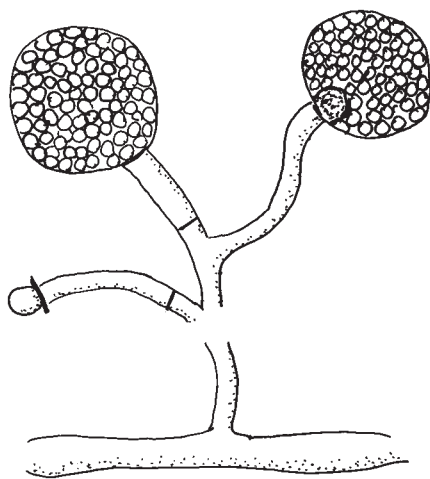
PATHOGENICITY: Occasionally the etiologic agent of mucormycosis (for a description of the disease, see p. 43). This fungus is also known as a common contaminant.

RATE OF GROWTH: Rapid; mature within 4 days. Growth is inhibited by cycloheximide. Some species do not grow well at 37°C.

COLONY MORPHOLOGY: Quickly covers agar surface with floccose colony resembling cotton candy; white, later turns gray or grayish brown. Reverse is white.

MICROSCOPIC MORPHOLOGY: Hyphae are wide (6–15 μm) and practically non-septate. Sporangioophores are long and often branched and bear terminal round, spore-filled sporangia (50–100 μm in diameter). The sporangial wall dissolves, scattering the round or slightly oblong spores (4–8 μm in diameter), revealing the columella and sometimes leaving a collarette at the base of the sporangium. There is no apophysis. No rhizoids are formed. Chlamydospores may form in some species.

See Table 14 (p. 187) for differentiation of similar organisms in the class Mucormycetes.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 131–135
Kwon-Chung and Bennett, 1992, pp. 547–549
Ribes et al., 2000

***Rhizomucor* spp.**

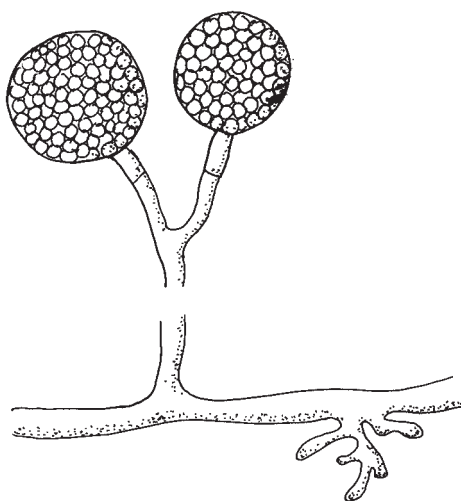
PATHOGENICITY: An occasional etiologic agent of mucormycosis (for a description of the disease, see p. 43), primarily in leukemic patients. Incidence in the clinical laboratory is uncertain, as isolates may have been misidentified in the past.

RATE OF GROWTH: Rapid; mature within 4 days. Growth is inhibited by cycloheximide. Some members of the genus (*R. pusillus* and *R. miebei*) are thermophilic, with a maximum growth temperature of 54–58°C, but *R. variabilis* has a maximum growth temperature of 38°C.

COLONY MORPHOLOGY: Very fluffy growth with texture of cotton candy; gray, becoming dark brownish with age. Reverse is white.

MICROSCOPIC MORPHOLOGY: Appears to be intermediate between *Rhizopus* (p. 188) and *Mucor* (p. 189) spp. Sporangioophores are long, and they branch in a sympodial manner, most often near the top of the sporangioophore. Sporangia are round and are usually 40–100 µm in diameter. A few primitive, short, irregularly branched rhizoids are formed, differentiating the organism from *Mucor* spp. It differs from *Rhizopus* spp. by having branched sporangioophores and by the poor development and location of the rhizoids (may be at the base of the sporangioophore or at points between the sporangioophores).

See Table 14 (p. 187) for differentiation of similar organisms in the class Mucormycetes.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 167
 Kwon-Chung and Bennett, 1992, pp. 546, 549
 Ribes et al., 2000

Lichtheimia corymbifera* complex (formerly *Absidia corymbifera*)

PATHOGENICITY: An infrequent etiologic agent of mucormycosis (for a description of the disease, see [p. 43](#)). *Lichtheimia corymbifera* has been particularly associated with wound infections involving burns, trauma, and surgical interventions. The organism is ubiquitous and may therefore be a contaminant in cultures.

RATE OF GROWTH: Rapid; mature within 4 days. Growth is inhibited by cycloheximide. Maximum growth temperature is 45–50°C.

COLONY MORPHOLOGY: Coarse woolly gray surface; rapidly covers agar slant with fluff resembling gray cotton candy. Reverse is white. ([Color Plate 74](#).)

MICROSCOPIC MORPHOLOGY: Hyphae are wide (6–15 μm in diameter) and nonseptate (or almost so). It is similar in structure to *Rhizopus* spp. ([p. 188](#)) except that the sporangiophores of *Lichtheimia* arise (often in small clusters) at points on the stolon that are between the rhizoids and not opposite them. Also, the sporangiophores (up to 450 μm long) are branched and widen near the top, forming a conical apophysis just below the columella. The columella is typically shaped like a semicircle with a small, domelike projection on top. The sporangia are relatively small (20–90 μm in diameter) and slightly pear shaped instead of round. When the sporangial wall dissolves, a short collarette often remains where the wall met the sporangiophore. The sporangiospores are round to oval (3–5 μm in diameter).

Note: The rhizoids are often difficult to detect; they may be best observed by using a dissecting microscope to examine colonies on a slanted agar surface.

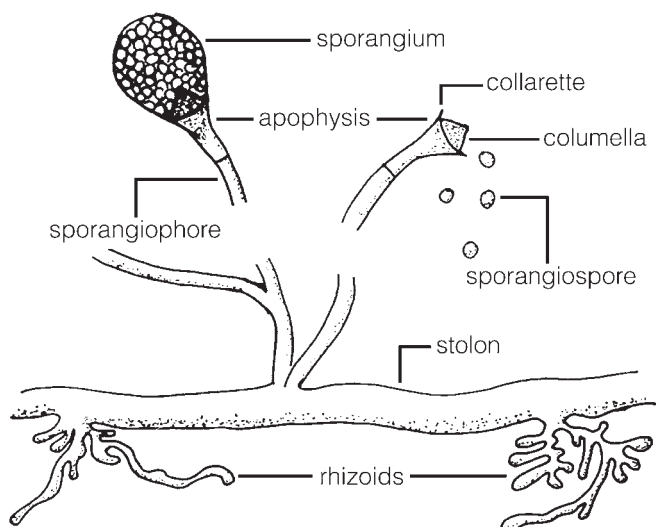
See [p. 193](#) for differentiation from *Apophysomyces elegans*, which it closely resembles.

See [Table 14 \(p. 187\)](#) for differentiation of similar organisms in the class Mucormycetes.

(continued on following page)

* Many isolates identified phenotypically as *L. corymbifera* have been found, by molecular methods, to be *Lichtheimia ramosa* and *Lichtheimia ornata*; together they now compose the complex.

Lichtheimia corymbifera* complex (formerly *Absidia corymbifera*)
(continued)



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 111–112
Schwartz and Jacobsen, 2014

PATHOGENICITY: Occasional agent of mucormycosis (for a description of the disease, see p. 43), Infection is usually acquired by traumatic implantation, such as accidental injuries, surgery, insect bites, and contamination of burns. Systemic cases have also been reported. Some infected patients appear to be otherwise immunocompetent. The organism is found in soil. Mucormycosis following musculoskeletal injuries sustained in dry, arid, or desert soil has been particularly associated with *Apophysomyces elegans*.

RATE OF GROWTH: Rapid; growth fills plate or tube within 4 days; grows at temperatures up to 42°C. It grows on media containing cycloheximide (in contrast to other Mucormycetes).

COLONY MORPHOLOGY: Fluffy, cottony growth that fills tube or plate; surface is white when young, becoming cream to yellow or brownish gray with age. Reverse is white to pale yellow.

MICROSCOPIC MORPHOLOGY: Sporulation does not occur on routine media; only broad, almost nonseptate hyphae form. A special culture method (p. 381) is required to induce sporulation. Hyphae are generally nonseptate and branched (4–8 µm in diameter). Sporangiphores are long (up to 530 µm) and unbranched and arise singly from a hyphal segment that resembles the foot cells seen in *Aspergillus* spp. (p. 293); the apex of the sporangiphore widens to form a funnel-shaped or bell-shaped apophysis (11–40 µm in diameter at the widest part). The columella is a half circle. Sporangia are pear shaped, or pyriform (20–55 µm in diameter), and upon dissolution may leave a small collar at the base of the columella. The sporangiospores are smooth and mostly oblong (5–8 µm in length) and may appear pale brown in mass. Rhizoids may be between the points of origin of the sporangiphores or opposite the sporangiphore, depending on the medium.

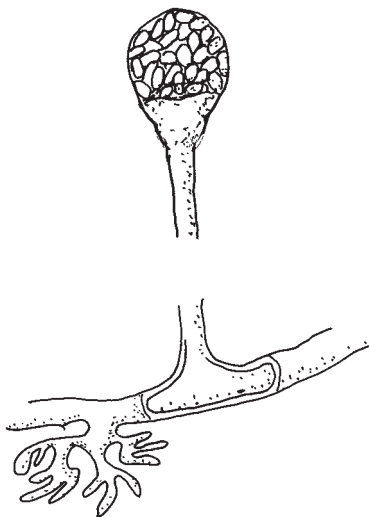
Apophysomyces is similar to *Absidia* but differs by having

- A more pronounced apophysis, which is bell shaped rather than conical
- A “foot cell” at the base of the sporangiphore
- Sporangiphores developing opposite rhizoids on plain agar
- Darkening and thickening of the sporangiphore wall below the apophysis
- Failure to sporulate readily with routine culture methods
- Resistance to cycloheximide

(continued on following page)

* *Apophysomyces* is now considered a complex since the molecular recognition of *A. ossiformis*, *A. trapeziformis*, and *A. variabilis*, which are morphologically indistinguishable from *A. elegans*.

Apophysomyces elegans* (continued)



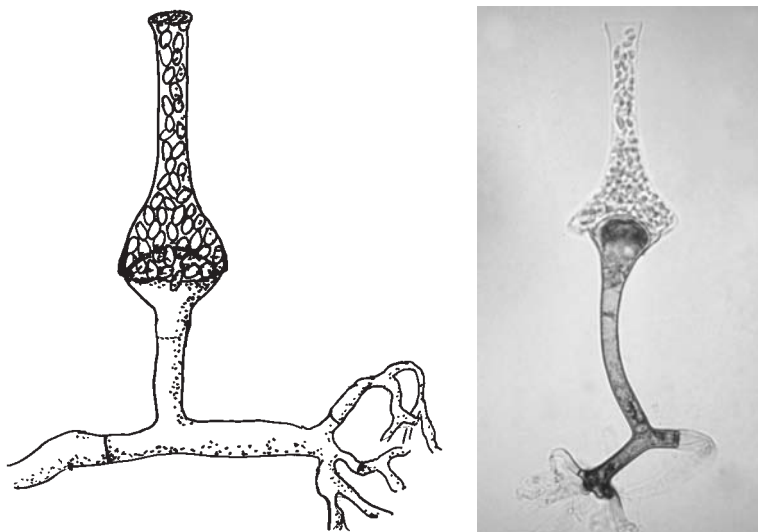
For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 6–8
Kimura et al., 1999

PATHOGENICITY: Occasionally an agent of mucormycosis (for a description of the disease, see p. 43). Cases are usually preceded by traumatic implantation. Infections reported have been cutaneous, subcutaneous, osteomyelitic, rhinocerebral, and cranial; systemic infection is often fatal. Many of the patients appear to be otherwise immunocompetent.

RATE OF GROWTH: Rapid; growth fills plate or tube within 4 days. Maximum growth temperature is 44°C.

COLONY MORPHOLOGY: Very cottony, fluffy white surface. Reverse is white.

MICROSCOPIC MORPHOLOGY: On routine media the organism does not sporulate; only broad, mostly nonseptate, branched, hyaline hyphae form. A special procedure (p. 381) can be used for stimulation of sporulation. Sporulation is also known to occur on Czapek agar after 7 days' incubation at 37°C. Sporangiphores (24–64 μm long) bear sporangia that are flask shaped (50–150 μm long), having a swollen portion near the base and a long neck that broadens at the apex. Sporangiospores are elongate (3–4 μm long) and smooth and are released through the top of the neck. Rhizoids form near the base of the sporangiphore, are dichotomously branched, and darken with age.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 175–176
Ribes et al., 2000

Cokeromyces recurvatus

PATHOGENICITY: The organism has been recovered several times from genitourinary sites (cervix, vagina, and bladder), but there was no tissue invasion demonstrated. It appears to have a predilection for colonization of those sites. It has very rarely been reported in infections at other sites; its possible role as an etiologic agent of disease is uncertain.

RATE OF GROWTH: Moderate; mature in 5–10 days. Growth more rapid at 37°C than at 30°C; growth also occurs at 42°C.

COLONY MORPHOLOGY: At 25–30°C, thermally dimorphic colonies are at first cream to tan, thin, feltlike or powdery, and radially folded; the central area later becomes heaped and dark; entire colony may become brown with age. Reverse is tan to brown. (Color Plate 75.)

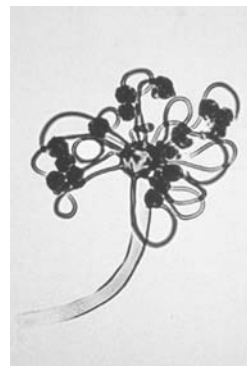
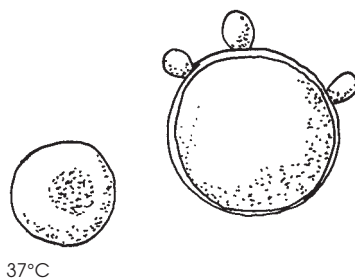
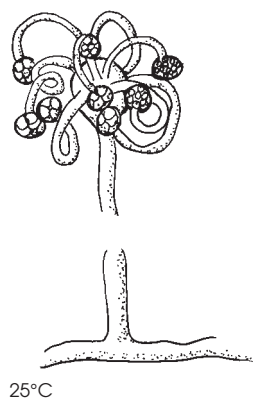
At 35–37°C, preferably on enriched medium such as brain heart infusion agar, in 5–7% CO₂, tan to gray, slightly wrinkled yeastlike colonies develop in 2 days.

MICROSCOPIC MORPHOLOGY: At 25–30°C, broad, sparsely septate hyphae are seen. Sporangiphores (100–500 µm long) terminate in a round vesicle that produces recurving stalks, each bearing a round sporangiole (9–13 µm in diameter). The sporangiole contains 12–20 sporangiospores that are smooth walled and of variable size and shape, mostly oval (average, 2.5 × 4.5 µm). No rhizoids are formed. Zygospores are abundantly produced between pairs of hyphal segments, sporangiophores, or suspensors of mature zygospores. Mature zygospores are round (35–55 µm in diameter), brown, and rough walled.

At 35–37°C in 5–7% CO₂, thin-walled, round yeast cells (15–90 µm in diameter) develop, with single or multiple budding that may resemble the ship's-wheel appearance of *Paracoccidioides brasiliensis* (p. 174).

(continued on following page)

Cokeromyces recurvatus (continued)



Courtesy of Ira Salkin.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kemna et al., 1994
McGough et al., 1990
Ramani et al., 2000

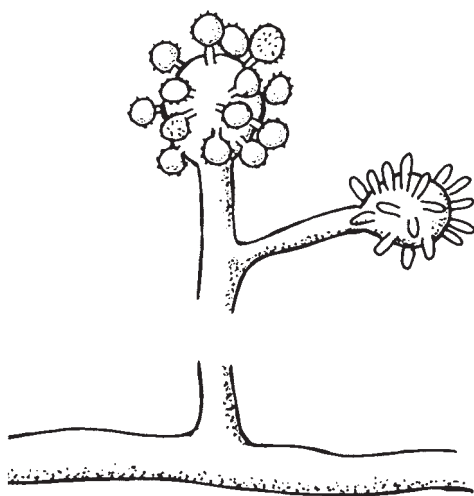
Cunninghamella bertholletiae

PATHOGENICITY: Among the members of the order Mucorales, *Cunninghamella bertholletiae* has been associated with the highest mortality, particularly in causing sino-pulmonary and disseminated infections in patients with hematological malignancies. This property also is reflected by laboratory animal studies demonstrating increased virulence measured by mortality and degree of tissue injury in neutropenic hosts. It is an uncommon cause of cutaneous infections following traumatic inoculation. It also has been identified as a serious pathogen in patients with iron overload conditions.

RATE OF GROWTH: Rapid; mature within 4 days. Can grow at 40–45°C.

COLONY MORPHOLOGY: Floccose, like cotton candy; white, then gray. Reverse is white. (Color Plate 76.)

MICROSCOPIC MORPHOLOGY: Broad hyphae, almost nonseptate. Sporangio-phores are long, branched, ending in swollen vesicles (30–65 μm in diameter); vesicles on lateral branches are smaller (14–35 μm in diameter). The vesicles are covered with spinelike denticles, each supporting a round to oval sporangium (5–8 $\times$ 6–14 μm ; each sporangium yields one spore). The walls of the sporangiola are often encrusted with needlelike crystals. Rhizoids may be seen. Zygosporangia and chlamydospores may also be formed.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Hsieh et al., 2013
 Kidd et al., 2016, pp. 73–74
 Petraitis et al., 2013
 Ribes et al., 2000
 Roden et al., 2005

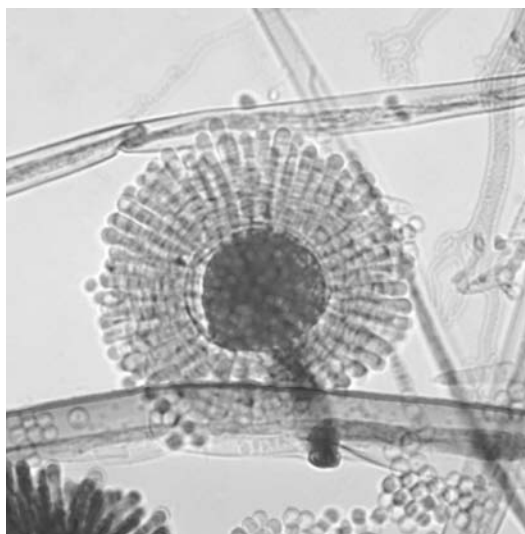
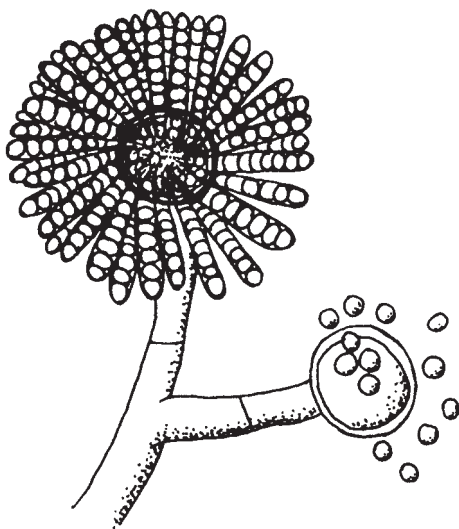
Syncephalastrum racemosum

PATHOGENICITY: Although seldom recovered in a health care setting, *Syncephalastrum racemosum* is occasionally involved in serious infections.

RATE OF GROWTH: Rapid; mature within 3 days. Maximum growth temperature is 40°C.

COLONY MORPHOLOGY: Quickly fills petri plate with white, cotton candy-like fluff, then turns dark gray to almost black. Reverse is white. (Color Plate 77.)

MICROSCOPIC MORPHOLOGY: Hyphae are broad and almost nonseptate (irregular septa may form with age). Sporangioophores are rather short and branched and terminate in a large, round vesicle (30–80 µm). On the vesicle are fingerlike tubular sporangia (4–6 × 12–40 µm) containing chains of round spores. Rhizoids are usually formed. This organism may at first resemble *Aspergillus niger* (p. 297), but careful examination reveals the tubular sporangia and the absence of phialides.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 189
Ribes et al., 2000
Rodríguez-Gutiérrez et al., 2015

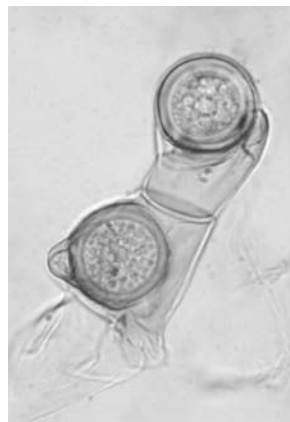
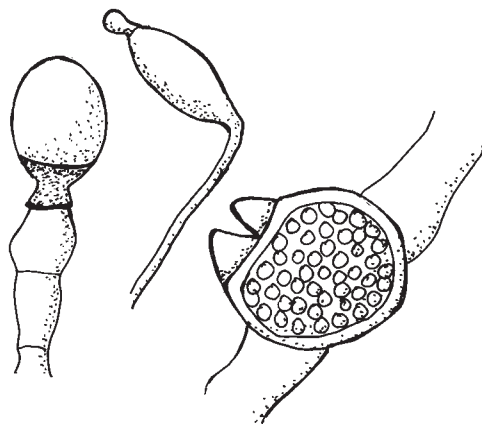
***Basidiobolus* sp.**

PATHOGENICITY: Etiologic agent of entomophthoromycosis basidiobolae (subcutaneous mucormycosis), which is a chronic inflammatory or granulomatous disease generally restricted to the limbs, chest, back, or buttocks. The lesions are characteristically huge, palpable, hard, nonulcerating subcutaneous masses. Gastrointestinal infections are also known to rarely occur, and disseminated disease may follow. Mainly seen in Asia and Africa, but is occasionally reported in other areas, including the United States. Patients infected with *Basidiobolus ramarum* often have a history of exposure to lizards and other small reptiles.

RATE OF GROWTH: Rapid; mature within 5 days. Grows faster at 30°C than at 35–37°C. The organism does not survive well at 4°C.

COLONY MORPHOLOGY: Thin, flat, waxy; buff to gray. Becomes heaped up or radially folded, grayish brown, and covered with white aerial hyphae. Reverse is white. Some strains have an earthy odor similar to that of *Streptomyces* spp.

MICROSCOPIC MORPHOLOGY: Wide (8–20 μm) hyphae having occasional septa that become numerous with the production of spores. Short sporophores enlarge apically to form a swollen area from which a single-celled spore and a fragment of the sporophore are forcibly discharged (these are known as ballistospores). Other sporophores (not swollen) passively release club-shaped spores having a knoblike tip; these spores can function as sporangia, producing more sporangiospores. The organism also produces many round intercalary zygospores (20–50 μm in diameter) with smooth (occasionally rough), thick walls and a prominent beaklike appendage (remnant of a copulatory tube) on one side.



For further information, see

Bigliazzi et al., 2004

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Kidd et al., 2016, pp. 28–29

Ribes et al., 2000

Shaikh et al., 2016

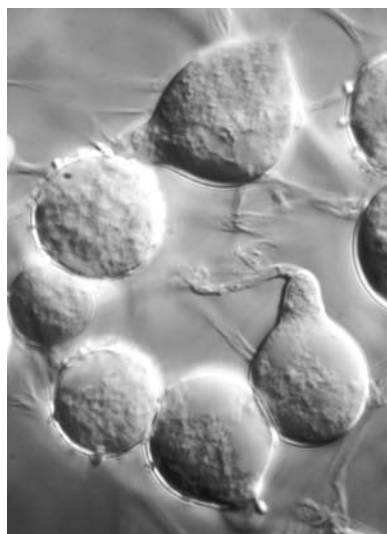
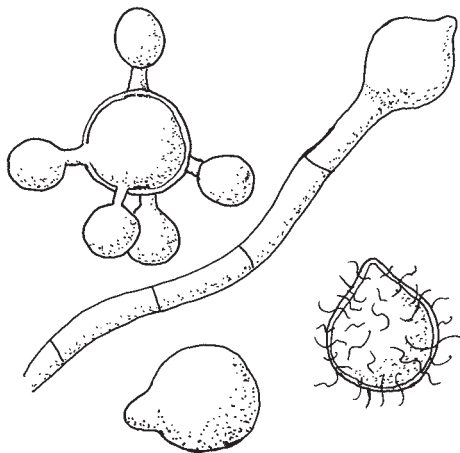
Conidiobolus coronatus

PATHOGENICITY: Etiologic agent of entomophthoromycosis conidiobolae, which is a tropical chronic inflammatory or granulomatous disease usually restricted to the nasal mucosa; it can spread to adjacent subcutaneous tissue and cause disfigurement of the face. The disease is characterized by polyps or palpable subcutaneous masses. On rare occasions, this and other species of the genus have caused deeply invasive, life-threatening infections.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Flat, waxy; buff or gray, becoming sparsely covered with short, white aerial hyphae. With age, colony becomes tan to brown. Reverse is white. Satellite colonies form, and sides of culture tube or lid of plate become covered with spores forcibly discharged by the sporophores.

MICROSCOPIC MORPHOLOGY: Hyphae have few septa; unbranched sporophores bear single-celled, round spores (20–40 μm in diameter). At maturity, the spores (ballistospores) are forcibly ejected and bear a broad, tapering projection at the site of former attachment. The spores may germinate and produce long hyphal tubes that become sporophores, each bearing another spore. A spore may also develop a number of short extensions that give rise to a corona of secondary spores. Some spores may produce short, hairlike, villous appendages.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 63–64
Ribes et al., 2000
Shaikh et al., 2016

Dematiaceous Fungi

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Fonsecaea pedrosoi 207

Fonsecaea compacta (now considered a mutant form of *F. pedrosoi*) 209

Rhinocladiella spp. (some species formerly *Acrotheca* spp.; others *Ramichloridium* spp.) 210

Phialophora verrucosa 211

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Pleurostomophora richardsiae (formerly *Phialophora richardsiae*) 213

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Cladophialophora carrionii (formerly *Cladosporium carrionii*) 219

Cladophialophora bantiana (previously known as *Xylohypha bantiana*, *Cladosporium bantianum*, and *Cladosporium trichoides*) 220

Cladophialophora boppii (formerly *Taeniolella boppii*) 221

Scedosporium spp. complex (also known as *Scedosporium apiospermum* complex) 222

TABLE 18 Differentiating phenotypic characteristics of the clinically encountered members of the *Scedosporium* spp. complex and *Lomentospora prolificans* 224

Lomentospora prolificans (formerly *Scedosporium prolificans* and *Scedosporium inflatum*) 225

Verruconis gallopava (formerly *Ochroconis gallopava* and *Dactylaria constricta* var. *gallopava*) 226

TABLE 19 Differentiation of the clinically encountered *Verruconis* species 227

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Exophiala dermatitidis (*Wangiella dermatitidis*) 229

Hortaea werneckii (*Phaeoannellomyces werneckii*) 230

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Madurella grisea 232

Piedraia hortae 233

Aureobasidium pullulans 234

TABLE 21 Differential characteristics
of *Aureobasidium pullulans* versus

Hormonema dematioides 236

Hormonema dematioides 237

Neoscytalidium dimidiatum (formerly
Scytalidium dimidiatum) 238

Botrytis spp. (*Botrytis cinerea*) 240

Stachybotrys chartarum (*S. alternans*,
S. atra) 241

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Exserohilum spp. 247

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Introduction to Dematiaceous Fungi

Dematiaceous fungi include a large group of organisms that produce dark (olive, brown, gray, or black) colonies due to a melanin pigment in their cell walls.

The diseases produced by some of the dematiaceous fungi are classified according to the clinical presentation and the appearance of the organism in tissue.

- **Chromoblastomycosis:** The fungi are seen in tissue as sclerotic bodies (also known as medlar bodies). These structures are round (5–12 μm in diameter), brownish, usually with a single septum or two intersecting septa. The infection is chronic and causes the development of warty nodules, tumorlike masses, or raised, rough, cauliflowerlike lesions containing the sclerotic bodies that involve the subcutaneous soft tissues but spare muscle, tendon, and bone. The lesions usually develop in the subcutaneous tissue of the lower extremities but are sometimes on other exposed areas, such as the hands, head region, or trunk. On rare occasions, the etiologic agents have been known to spread to the central nervous system, lungs, or muscular tissues.
- **Phaeohyphomycosis:** The etiologic agents occur in tissue as dark, yeastlike cells; pseudohyphalike elements; variously shaped hyphae; or any combination of these forms. The infection may be cutaneous, subcutaneous, or disseminated.
- **Mycetoma:** The infection is a chronic subcutaneous infection involving muscle, tendon, and bone and characterized by swollen tumorlike lesions that yield granular pus through draining sinuses. The granules of eumycotic mycetoma may be dark or light (many of the etiologic agents are not dematiaceous) and contain variously shaped fungal elements. The infection occurs most often in the feet or hands but may occur on any exposed parts of the body.

For more detailed clinical descriptions of these infections, see pp. 40, 51, and 52. For tinea nigra clinical features, see p. 50.

Tinea nigra and black piedra are also caused by dematiaceous fungi. Each disease is caused by one specific fungus; the infections are described on the individual page for each organism.

Many of the infections caused by the dematiaceous fungi are due to traumatic implantation of the organism from the environment into cutaneous or subcutaneous tissue, but pulmonary or disseminated infections have been initiated by inhalation of conidia.

Although the dematiaceous fungi are not highly virulent, some are regularly seen as etiologic agents of disease while others usually are encountered as saprophytes or contaminants and only occasionally act as opportunistic pathogens.

Sporothrix schenckii grows as a dematiaceous fungus when incubated at 25–30°C, but it is yeastlike and relatively nonpigmented at 35–37°C and is therefore placed with the thermally dimorphic fungi (p. 178).

For further information, see

Queiroz-Telles et al., 2017

Revankar and Sutton, 2010

PATHOGENICITY: The most common worldwide cause of chromoblastomycosis (for a description of the disease, see p. 52). The lesions usually develop in the subcutaneous tissue on the lower extremities but sometimes are on other exposed areas. On very rare occasions, the organism has been known to cause internal infections.

RATE OF GROWTH: Slow; mature in 14 days.

COLONY MORPHOLOGY: Surface is dark green, gray, or black, covered with silvery, velvety mycelium; colonies are usually flat and then develop a convex, cone-shaped protrusion in the center. Colony becomes slightly embedded in the medium. Reverse is black. (Color Plates 78 and 79.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, branched, and brown; conidia are dark, $1.5\text{--}3.0 \times 2.5\text{--}6.0 \mu\text{m}$. Conidiation is enhanced on cornmeal agar or potato dextrose agar. Four types of conidial formation may be seen.

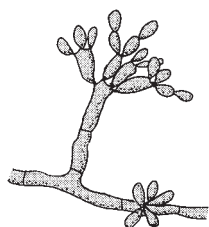
1. *Fonsecaea* type: Conidiophores are septate, erect, and compactly sympodial. The distal end of the conidiophore develops swollen denticles that bear primary single-celled, ovoid conidia. Denticles on the primary conidia support secondary single-celled conidia that may produce tertiary conidia, but long chains are not formed. Elongate conidia often form in verticils at fertile sites along the conidiophore, producing an asterisklike appearance.
2. *Rhinocladiella* type: Conidiophores are septate, erect, and sympodial; swollen denticles bear ovoid conidia at the tip and along the side of the conidiophore. Usually only primary conidia develop; secondary conidia are rare.
3. *Cladosporium* type: Conidiophores are erect and give rise to large, primary, shield-shaped conidia that in turn produce short, branching chains of oval conidia having small, dark hila (scars of attachment).
4. *Phialophora* type: Phialides are vase shaped with terminal cuplike collarettes. Round to oval conidia accumulate at the apex of the phialide. This type of conidiation is often scant or lacking.

Note: In tissues, this organism, as well as the other etiologic agents of chromoblastomycosis, appears as large ($5\text{--}12 \mu\text{m}$ in diameter), round, brownish, thick-walled cells with horizontal and vertical septa. When cultured at 25, 30, or 37°C, the organisms are filamentous.

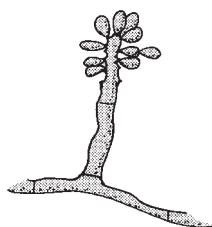
(continued on following page)

* *Fonsecaea monophora* is morphologically similar to *F. pedrosoi* but differs by forming slightly shorter denticles and slightly longer chains of conidia and occurring more commonly as an environmental isolate rather than an infectious agent; the species are best differentiated by molecular methods. *F. monophora* is an occasional etiologic agent of chromoblastomycosis; it has been reported as the cause of several infections in the brain.

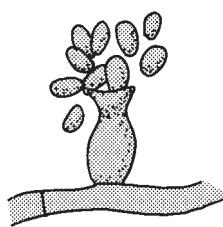
Fonsecaea pedrosoi (continued)



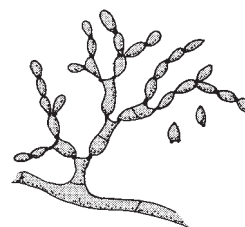
Fonsecaea-type
conidiation.



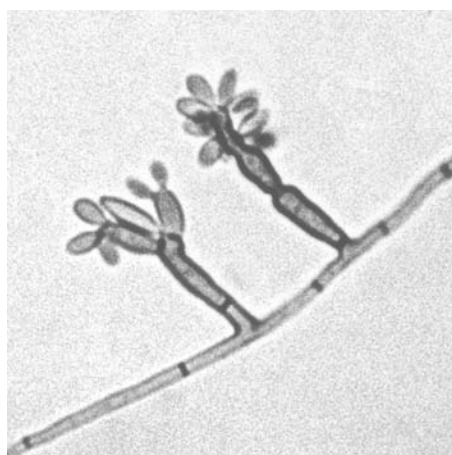
Rhinocladiella-type
conidiation.



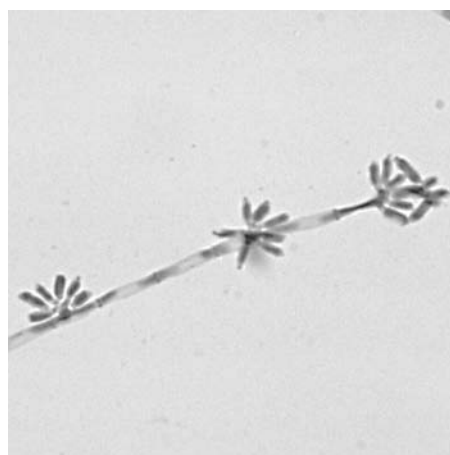
Phialophora-type
conidiation.



Cladosporium-type
conidiation.



Fonsecaea- and *Rhinocladiella*-type conidiation.



"Asterisks" of *Fonsecaea*-type conidiation.

For further information, see

Anaissie et al. (ed.), 2009, pp. 338, 518–519

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 93

Queiroz-Telles et al., 2017

Revankar and Sutton, 2010

Fonsecaea compacta (now considered a mutant form of *F. pedrosoi*)

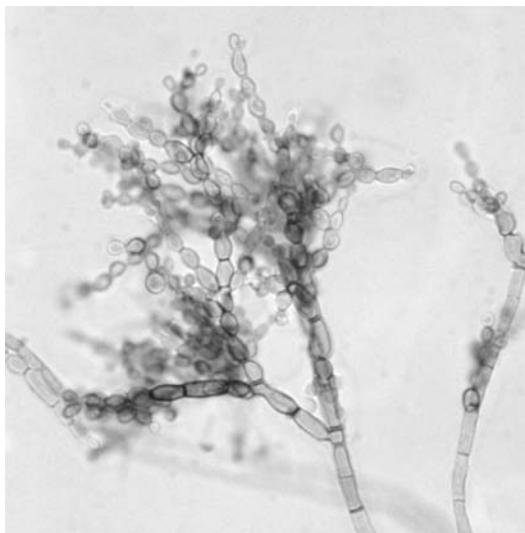
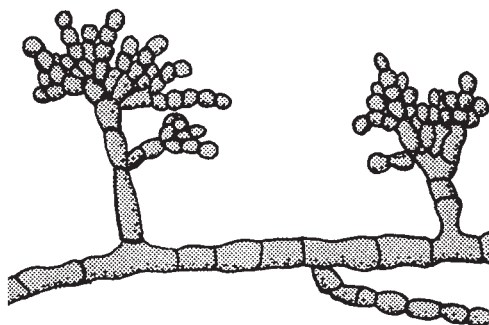
PATHOGENICITY: Causes chromoblastomycosis. For a description of the disease, see p. 52. This organism is rarely encountered.

RATE OF GROWTH: Very slow; mature in 28 days.

COLONY MORPHOLOGY: Surface is dark green to black, heaped, and brittle, with irregular indented border. After 3 or 4 weeks, brownish hyphae appear on the surface with typical conidiophores and conidial formations. Reverse is black.

MICROSCOPIC MORPHOLOGY: On Sabouraud dextrose agar, hyphae are septate, brown, and branched and bear predominantly *Fonsecaea*-type conidiophores that produce short chains and masses of almost round conidia (1.5–3 μm in diameter). The outstanding characteristics of this species are (i) the casklike shape of the conidia with the wide diameter of the septa between conidia and (ii) the compact arrangement of the conidial chains, which are not easily dissociated. *Rhinocladiella*-, *Cladosporium*-, and *Phialophora*-type conidiation (p. 208) may also be seen.

In tissue, the organisms appear as dark, round, septate cells (5–12 μm in diameter).



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Queiroz-Telles et al., 2017

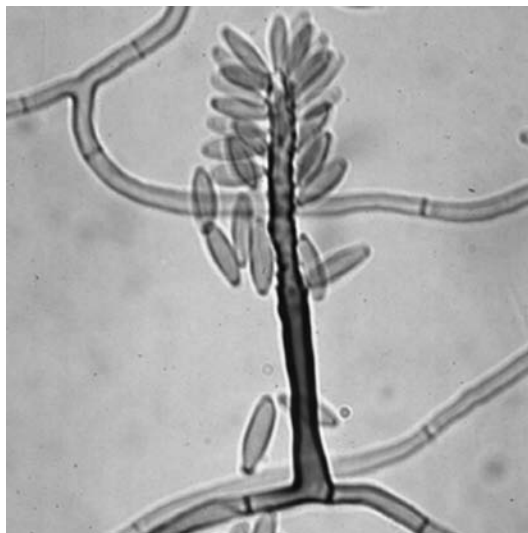
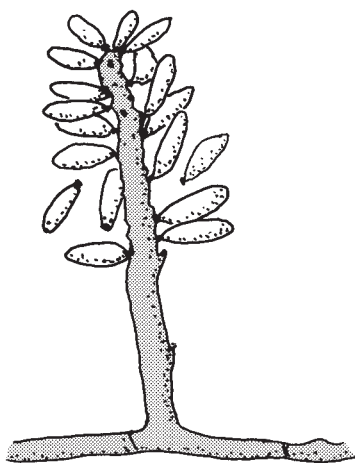
Rhinocladiella* spp. (some species formerly *Acrotheca* spp.; others *Ramichloridium* spp.)

PATHOGENICITY: Several species have very occasionally been involved in chromoblastomycosis and mycetoma. *R. mackenziei* has repeatedly been reported to cause fatal intracranial infections in the Middle East. Members of the genus are also recovered as contaminants.

RATE OF GROWTH: May be moderately rapid to slow (depending on species).

COLONY MORPHOLOGY: Surface of colony may be smooth, velvety, or woolly; dark gray to greenish black. Reverse is dark.

MICROSCOPIC MORPHOLOGY: Hyphae are septate. Conidiophores are septate, erect, and pale brown; may be branched or unbranched; are sympodial (and some geniculate); and may show scars where conidia had been attached. The conidia ($1.7\text{--}2.5 \times 3\text{--}7.5 \mu\text{m}$ or larger in some species) form on the top and along the upper sides of the conidiophore; they are one celled (occasionally two celled) and cylindrical or oval; they may form on denticles, be truncate, and show dark basal scar. Anellidic forms are occasionally seen.



Courtesy of Deanna A. Sutton, doctorfungus.org.

For further information, see
 Anaissie et al. (ed.), 2009, pp. 338–339
 Christini et al., 2010
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Taj-Aldeen et al., 2010

* There is appreciable variation of characteristics among the species of the genus *Rhinocladiella*.

Phialophora verrucosa

PATHOGENICITY: Causes chromoblastomycosis, of which it is the second most common etiologic agent worldwide (*Fonsecaea pedrosoi* is the most common agent). It is also an etiologic agent of phaeohyphomycosis and, on rare occasions, mycetoma. For descriptions of these diseases, see pp. 52, 51, and 40, respectively.

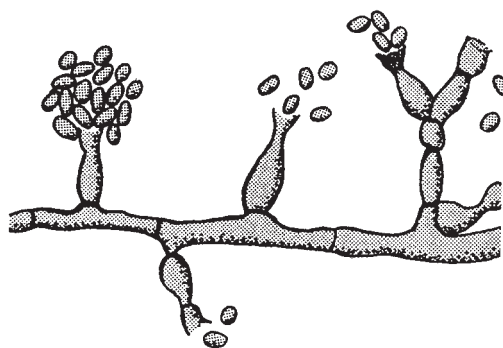
RATE OF GROWTH: Moderate; mature in 7–12 days.

COLONY MORPHOLOGY: Surface is dark greenish brown to black with a close, matlike, olive to gray mycelium. Some strains are heaped and granular; others are flat. Colonies become embedded in the medium. Reverse is black. (Color Plate 80.)

MICROSCOPIC MORPHOLOGY: Hyphae are brown, branched, and septate. Phialides are vase shaped with a flared, cuplike collarette; they usually have a distinct septum at the base. Round to oval conidia (1–3 × 2–4 μm) accumulate at the apex of the phialide, giving the appearance of a vase of flowers.

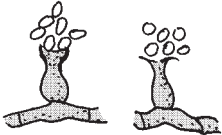
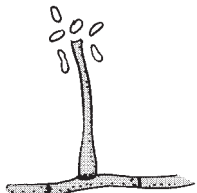
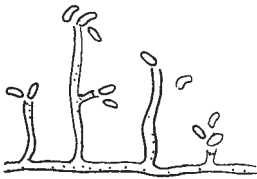
To differentiate *Phialophora* from other similar genera, see Table 16 (p. 212).

Note: In tissue with chromoblastomycosis, the organism appears as dark, round cells, 5–12 μm in diameter, with horizontal and vertical septa.



For further information, see
Anissie et al. (ed.), 2009, pp. 340–341
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Queiroz-Telles et al., 2017
Revankar and Sutton, 2010

TABLE 16 Characteristics of *Phialophora*, *Pleurostomophora*, *Phaeoacremonium*, *Acremonium*, *Phialemonium*, and *Lecythophora*

Organism	Colony	Phialide				Illustration
		Basal septum	Shape	Collarette	Conidia	
<i>Phialophora</i> (p. 211) <i>Pleurostomophora</i> (p. 213)	Dark greenish brown to black	Yes, usually	Flask or vase shaped	Flared, distinct (<i>Pleurostomophora repens</i> is an exception)	Oval or round; clusters	
<i>Phaeoacremonium</i> (p. 214)	Dark olive gray to brown	Yes, usually	Cylindrical; rough at base; tapered toward tip	Almost inconspicuous; tubular or slightly funnel shaped	Oblong; some curved; clusters	
<i>Acremonium</i> (p. 321)	White, gray, or rose; cottony	Yes, usually	Erect, cylindrical; unbranched, tapered	Inconspicuous	Oblong; clusters	
<i>Phialemonium</i> (p. 215)	White, then yellow, gray, light brown, or greenish; may become dark	No, usually not	Short, peg-like or long, cylindrical	Inconspicuous	Tear shaped or oblong; curved or straight; clusters	
<i>Lecythophora</i> (p. 324)	Pink or salmon; moist to slimy	No, usually not	Short; volcano shaped along hyphae	Parallel sided	Oval to oblong; straight or slight curve; no clusters	

Pleurostomophora richardsiae* (formerly *Phialophora richardsiae*)

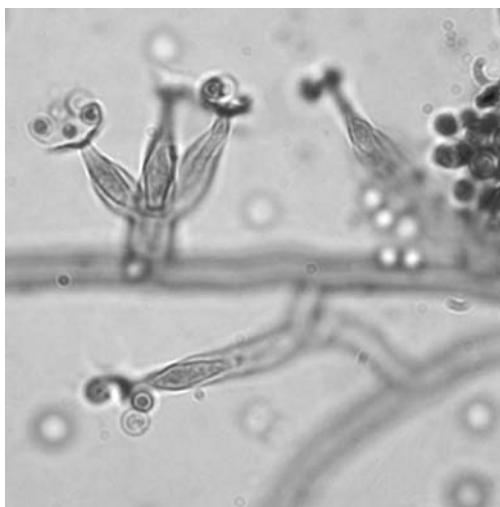
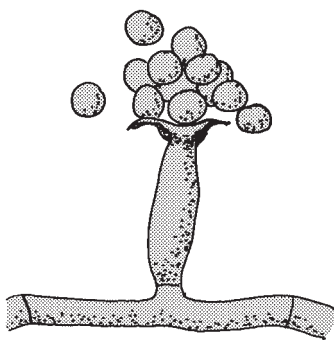
PATHOGENICITY: An uncommon agent of cystic subcutaneous phaeohyphomycosis (see p. 51 for a description of the disease). Most reported cases have been in debilitated or immunocompromised patients. A new species, *Pleurostomophora ochracea*, has recently been reported as a new agent of human mycetoma with yellow grains.

RATE OF GROWTH: Moderate; mature in 6–10 days.

COLONY MORPHOLOGY: Surface is olive brown to brownish gray, woolly to velvety or powdery; brown diffusing pigment may develop with age. Reverse is dark.

MICROSCOPIC MORPHOLOGY: Septate hyphae are at first colorless and become brown. The distinguishing phialides usually (but not always) have a distinct septum at the base and are slightly flask shaped with a characteristic flared, saucer-shaped collarette; they produce conidia that are brown and almost round (2–3.5 μm in diameter). Simple, short, unflared phialides may also form along the hyphae and produce conidia that are hyaline and cylindrical or slightly curved (2–3 $\times$ 3–6 μm).

To differentiate *Phialophora* and *Pleurostomophora* from other similar genera, see Table 16 (p. 212).



For further information, see

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Levenstadt et al., 2012

Mhmoud et al., 2012

* The genus *Phialophora* has been found by molecular analysis to be a complex of many asexual forms; one of the groups has been given the genus name *Pleurostomophora*. *Phialophora repens* has also been transferred to the new genus designation as *Pleurostomophora repens*. *P. repens* is an occasional agent of phaeohyphomycosis. The phialides form singly or in penicillately branched groups; the collarettes are typically unflared and inconspicuous. The organism formerly known as *Phialophora parasitica* is now *Phaeoacremonium parasiticum* (see p. 214).

Phaeoacremonium parasiticum (formerly *Phialophora parasitica*)

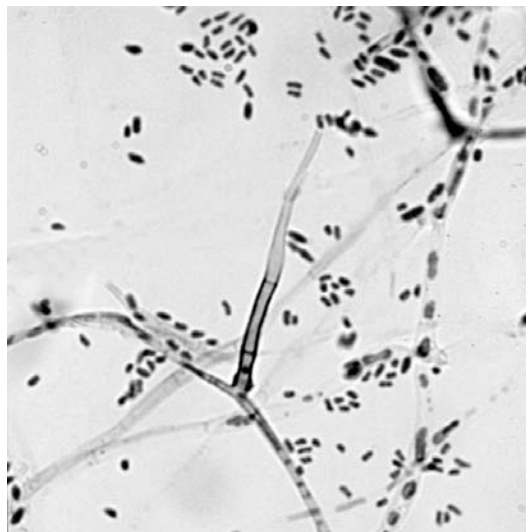
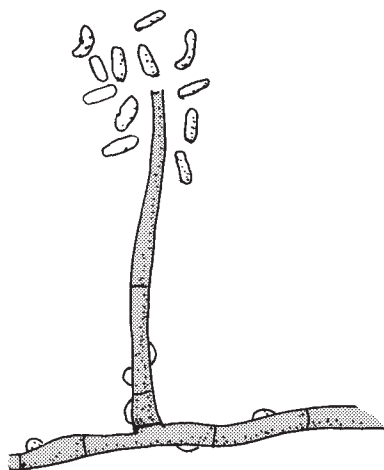
PATHOGENICITY: Has been reported in cases of subcutaneous phaeohyphomycoses, mycetoma, arthritis, endocarditis, and infection of various sites including disseminated infection, particularly in immunocompromised patients.

RATE OF GROWTH: Moderate; mature within 7 days.

COLONY MORPHOLOGY: Surface initially cream colored and velvety, then becomes grayish beige to olive-brown with a lighter edge and develops clusters of aerial hyphae. Reverse tan to brown. (Color Plate 81.)

MICROSCOPIC MORPHOLOGY: Young hyphae appear hyaline, later brown and rough or warty; they may accumulate side by side to form thick bundles (fascicles). Phialides form along the hyphae or on branched or unbranched conidiophores that may be rough walled near the base; phialides are brown, cylindrical ($2-3 \times 15-35 \mu\text{m}$), slightly tapering toward the apex; they usually have a basal septum and a collarette that is small and tubular or vaguely funnel shaped. Conidia are hyaline, oblong ($1-2 \times 3-6 \mu\text{m}$), some curved, gathering in clusters at end of phialide.

To differentiate *Phaeoacremonium* from other similar genera, see Table 16 (p. 212).



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 El-Herte et al., 2014
 Revankar and Sutton, 2010

***Phialemonium* spp.** (genus intermediate between *Phialophora* and *Acremonium*)

PATHOGENICITY: Occasionally isolated from clinical specimens. Has been reported in a subcutaneous phaeohyphomycotic cyst; an endocarditis mixed infection; peritonitis; osteomyelitis; fungemia due to contaminated venous catheters; and infection of cutaneous, subcutaneous, and spleen tissue in a burn patient. All of the involved patients were immunocompromised.

RATE OF GROWTH: Moderately rapid; mature within 5–7 days.

COLONY MORPHOLOGY: Broadly spreading. Surface is at first white or cream, becoming yellow, grayish, or slightly greenish (the pale yellow or green pigment may diffuse into the agar); with age, light brown or gray areas may develop; some colonies produce chlamydoconidia and develop dark centers. Texture may be smooth or slightly fuzzy or may have superficial radiating hyphal strands. Reverse is light with pale wine-buff or brown areas sometimes developing.

MICROSCOPIC MORPHOLOGY: Hyphae are septate and hyaline or very pale yellow-brown; they may accumulate side by side to form thick bundles (fascicles). Phialides form singly along the hyphae, usually with no septum at the base to delimit it from the hypha; they may be short and peglike ($0.5\text{--}1.5 \times 1.0\text{--}9.0 \mu\text{m}$) or longer and cylindrical (up to $30 \mu\text{m}$ long, $1\text{--}2 \mu\text{m}$ wide), usually lacking a visible collarette when grown on routine mycology media (rarely forms a very inconspicuous cylindrical, parallel-walled collarette). Conidia are single celled ($1.0\text{--}2.5 \times 3.0\text{--}6.0 \mu\text{m}$), hyaline, and smooth walled and form clusters. Chlamydoconidia sometimes form in old colonies.

Note: The two major species of *Phialemonium* can be differentiated as follows.

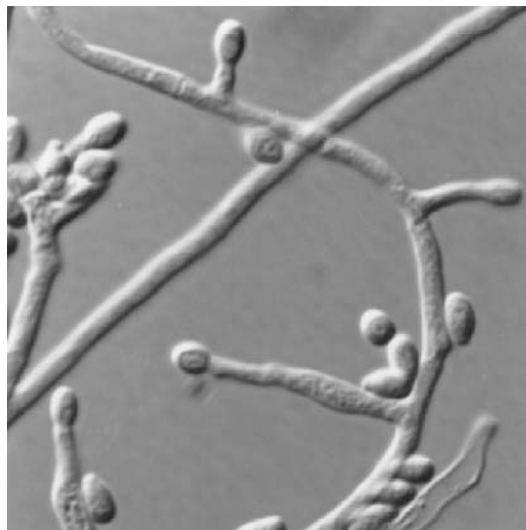
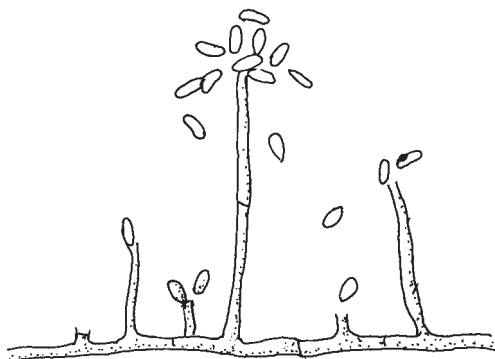
- *P. obovatum* produces conidia that are oval to tear shaped, may appear slightly cut off at the base, and are consistently straight; old colonies become greenish; pale brown chlamydoconidia may develop in old cultures and result in brown spots or a black center on the colony.
- *P. curvatum* (includes *P. dimorphosporum*) produces conidia that are oval, elongate, and curved (some may remain straight). Chlamydoconidia are not formed. Colonies may develop pale wine-buff zones on reverse side.

Phenotypic characteristics of this genus show significant variation depending on culture medium and temperature. It is therefore recommended to standardize conditions, e.g., use potato dextrose agar and incubate at $24\text{--}25^\circ\text{C}$.

To differentiate *Phialemonium* from other similar genera, see [Table 16](#) (p. 212).

(continued on following page)

***Phialemonium* spp.** (genus intermediate between *Phialophora* and *Acremonium*) (continued)



Courtesy of Wiley Schell.

For further information, see
Proia et al., 2004
Revankar and Sutton, 2010

Cladosporium spp.

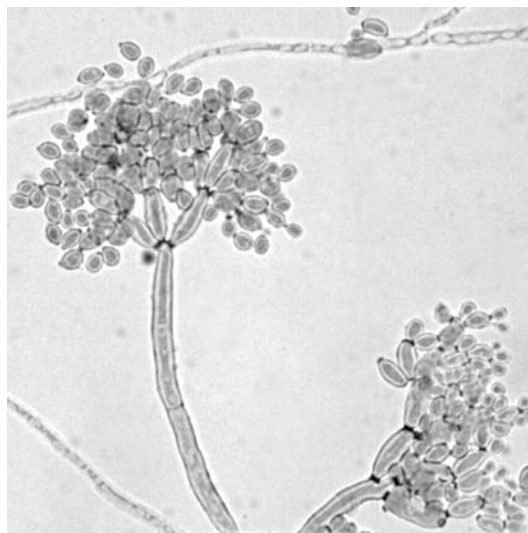
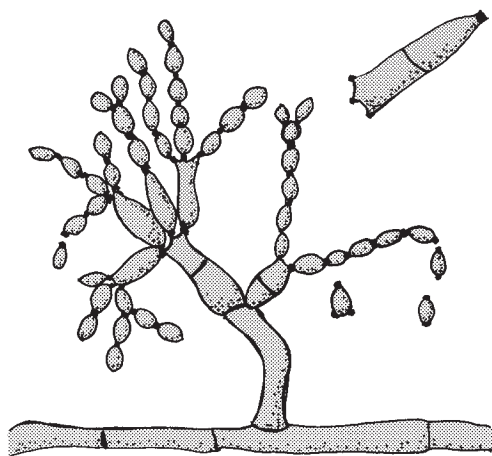
PATHOGENICITY: Commonly considered saprophytic contaminants. They have only occasionally been implicated in infections.

RATE OF GROWTH: Moderately rapid; mature within 7 days at 25°C. Most strains do not grow at 37°C, but some do.

COLONY MORPHOLOGY: Surface is greenish brown or black with grayish velvety nap, becoming heaped and slightly folded. Reverse is black. (Color Plate 82.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate and dark; conidiophores are dark and branched, vary in length, and usually produce two or more conidial chains. Conidia are brown, round to oval ($3\text{--}6 \times 4\text{--}12\ \mu\text{m}$), and usually smooth; they form branching treelike chains and are easily dislodged, showing dark spots (hila) at the point where they were attached to the conidiophore or other conidia. The cells bearing the conidial chains are large, sometimes septate, resemble shields (therefore called “shield cells”), and may be mistaken for macroconidia when seen alone.

See Table 17 (p. 218) for differentiation from *Cladophialophora* spp.



For further information, see

Bensch et al., 2012

de Hoog et al, *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 57

TABLE 17 Characteristics of *Cladosporium* spp. and *Cladophialophora* spp.^a

Organism	Distinct conidio-phores	“Shield cells”	Shape of conidia	Distinct hila on conidia	Conidial chain length	Conidial chain branching	Maximum growth temp (°C)	Gelatin hydrolysis	MDG ^b assimilation	Growth with 15% NaCl	Pathogenicity
<i>Cladosporium</i> spp.	+	+	Oval	+	Short	Frequent	<37 (V) ^c	+ (V) ^d		+	Nonpathogenic
<i>Cladophialophora carrionii</i>	±	±	Oval	±	Medium	Moderate	35–37	–	+	–	Causes chromoblastomycosis
<i>Cladophialophora bantiana</i>	–	–	Oval	–	Long	Sparse	42–43	–	+	–	Causes cerebral phaeohyphomycosis
<i>Cladophialophora boppii</i>	–	–	Round	–	Long	Sparse	<37	–	–		Causes skin infection, chromoblastomycosis, and lung infection
<i>Cladophialophora emmonsii</i>	–	–	Bent	–	Medium	Sparse	37	–	+		Rare cause of subcutaneous phaeohyphomycosis

^aReprinted (updated) by permission of the publisher from Larone, 1989. Copyright by Elsevier Science Inc. Abbreviations: +, positive; –, negative; ±, sometimes difficult to distinguish; V, variable.

^bMethyl- α -D-glucoside (MDG) assimilation test is commonly used in the identification of yeasts. The substrate is included in the API 20C AUX and API ID32 (bioMérieux, Hazelwood, MO) and perhaps other commercial systems; extended period of incubation is required.

^c29% grow at 40°C.

^d86% positive.

Cladophialophora carrionii (formerly *Cladosporium carrionii*)

PATHOGENICITY: Causes chromoblastomycosis, most commonly in Australia, Venezuela, and South Africa (for a description of the disease, see [p. 52](#)).

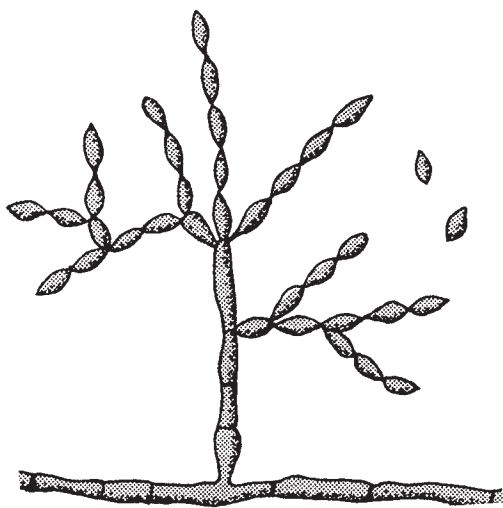
RATE OF GROWTH: Slow; mature within 18 days.

COLONY MORPHOLOGY: Dark surface; flat with slightly raised center; covered with velvety dull gray, gray-green, or purplish brown, short-napped mycelium. Reverse is black. ([Color Plate 83](#).)

MICROSCOPIC MORPHOLOGY: Hyphae are septate and dark with lateral and terminal conidiophores of various sizes. Conidiophores produce long, branching chains of brown, smooth-walled, oval, somewhat pointed conidia ($\sim 2.5 \times 4.5\text{--}7\text{ }\mu\text{m}$) that are easily dispersed with handling. The conidia typically have relatively pale scars of attachment (i.e., hila that are not as dark and prominent as those of *Cladosporium* spp.). Phialides with wide collarettes occasionally form on nutritionally deficient media.

For differentiation from *Cladosporium* spp. and other species of *Cladophialophora*, see [Table 17](#) ([p. 218](#)).

In tissue, the organism appears as large (5–12 μm in diameter), dark, round, septate cells.



For further information, see

Anaissie et al. (ed.), 2009, pp. 339–340, 518

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 55

Queiroz-Telles et al., 2017

Revankar and Sutton, 2010

Cladophialophora bantiana (previously known as *Xylohypha bantiana*, *Cladosporium bantianum*, and *Cladosporium trichoides*)

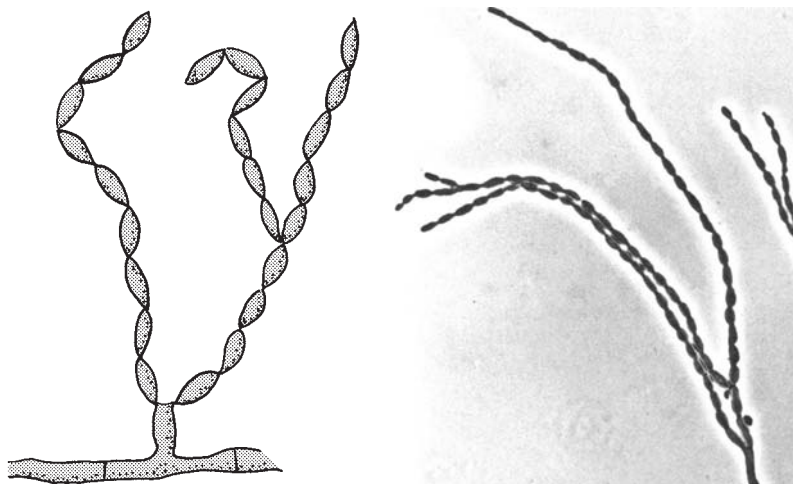
PATHOGENICITY: The organism has a predilection for the central nervous system and consequently causes cerebral phaeohyphomycosis. The infection may present initially as a brain tumor. Cerebral lesions that are solitary and encapsulated carry a more favorable prognosis, while lesions with satellite abscesses, or ones that are poorly encapsulated, carry a worse prognosis. Optimal outcome has been best achieved with combined medical and surgical interventions. Infection might be contracted through inhalation; extreme care and a biological safety cabinet should be used when handling this organism. Slide cultures should NOT be made. This organism is only rarely involved in cutaneous and subcutaneous infections.

RATE OF GROWTH: Slow; mature within 15 days.

COLONY MORPHOLOGY: Surface is olive-gray to brown or black and velvety. Reverse is black.

MICROSCOPIC MORPHOLOGY: Brown, septate hyphae with conidiophores that are similar to the vegetative hyphae; long, sparsely branched, wavy chains of smooth, oval conidia ($2.5\text{--}5 \times 6\text{--}11 \mu\text{m}$); the conidia do not display conspicuous scars of attachment.

For differentiation from similar organisms, see [Table 17](#) (p. 218).



From Michael McGinnis, *Manual of Clinical Microbiology*, 4th ed.

For further information, see

Ajantha and Kulkarni, 2011

Anaissie et al. (ed.), 2009, pp. 339–340

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kwon-Chung and Bennett, 1992, pp. 642–645

McCarthy et al., 2014

Revankar and Sutton, 2010

Cladophialophora boppii (formerly *Taeniolella boppii*)

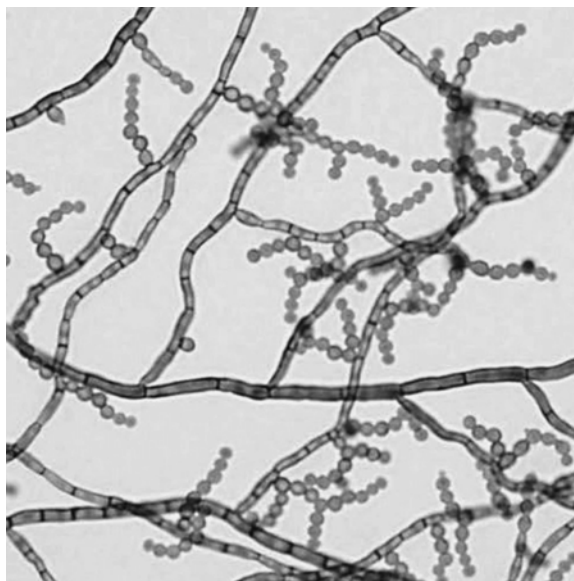
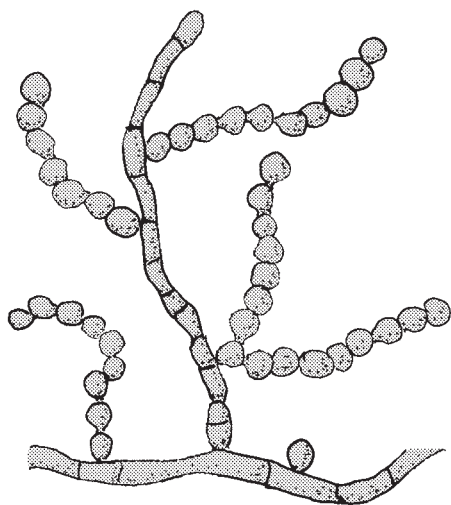
PATHOGENICITY: Has been involved in human skin lesions and in cases of chromoblastomycosis-like infections (but typical sclerotic bodies were not always seen in tissue). It has recently been reported in a lung infection.

RATE OF GROWTH: Slow; mature within 14 days.

COLONY MORPHOLOGY: Surface is olive-gray to black and velvety. Reverse is black.

MICROSCOPIC MORPHOLOGY: Brown, septate hyphae. Conidia are fairly round (3–4 μm in diameter) and form long, mostly unbranched chains directly from the sides of the hyphae; the conidia do not display conspicuous scars of attachment.

For differentiation from *Cladosporium* spp. and other species of *Cladophialophora*, see [Table 17](#) (p. 218).



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

***Scedosporium* spp. complex** (also known as *Scedosporium apiospermum* complex)

Scedosporium was formerly considered the anamorph (asexual) state of *Pseudallescheria* spp., the teleomorph (sexual) state. Consistent with the 2013 change in fungal nomenclature, stipulating that each fungus have only one name, *Scedosporium* is the preferred name for the entire holomorph (sexual and asexual states) of the organism. *Scedosporium apiospermum* is the most common species encountered; cleistothecia (indicative of the sexual state, *Pseudallescheria apiospermum*) seldom develop in routine clinical cultures. *Scedosporium boydii* is more likely to form cleistothecia; its teleomorph is *Pseudallescheria boydii*.

The complex consists of at least four clinically encountered species of *Scedosporium*: *S. apiospermum*, *S. boydii*, *S. aurantiacum*, and *S. dehoogii*. See Table 18 (p. 224) for phenotypic differentiation of the species. “*Graphium*” (see p. 242), as a synanamorph of *Scedosporium*, is simply a descriptive term; the culture report should indicate *Scedosporium* spp. complex or genus and species name, if determined.

PATHOGENICITY: *Scedosporium* spp. are found in soil and polluted or stagnant waters. Although this organism is commonly considered an agent of phaeohyphomycosis, the hyphae are hyaline. Infection occurs most often in the upper and lower extremities but may occur on any exposed parts of the body. *Scedosporium* spp. are the most common cause of eumycetoma in North America. They can infect subcutaneous tissues, muscle, tendon, and bones, as well as lungs, sinuses, eyes, central nervous system, and other body sites. *Scedosporium* spp. are the most common cause of fatal lung and central nervous system infections complicating near-drowning accidents in immunocompetent victims. Disseminated infection is most commonly seen in immunocompromised patients. Invasive *Scedosporium* lung disease in immunocompromised patients may clinically resemble invasive pulmonary aspergillosis. Patients with cystic fibrosis have a high frequency of colonization by *Scedosporium* spp. or chronic infection in the form of fungus balls in the lower respiratory tract, but seldom with progression to locally invasive disease or dissemination. Among the different antifungal agents, members of the *Scedosporium* complex have the lowest MICs to voriconazole. They tend to have high MICs to amphotericin B.

RATE OF GROWTH: Moderately rapid; mature in 7 days. The asexual state grows on media containing cycloheximide, but the cleistothecia of the sexual state are often inhibited.

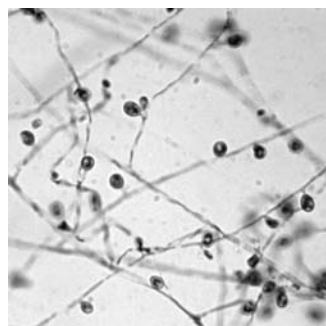
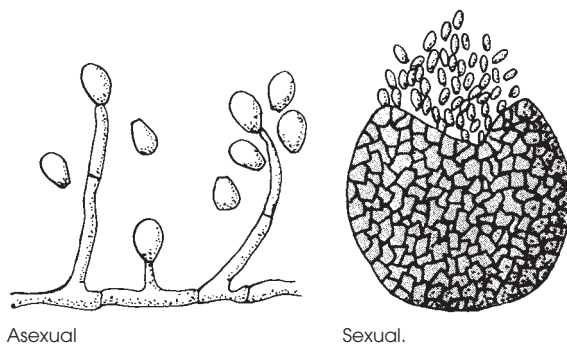
COLONY MORPHOLOGY: Surface has a spreading, white, cottony aerial mycelium that later turns gray or brown. Reverse is at first white but usually becomes gray or black over time. (Color Plates 84 and 85.) *Scedosporium aurantiacum* colonies have a yellow-orange reverse and produce a pale yellow, diffusible pigment.

MICROSCOPIC MORPHOLOGY: *Scedosporium* spp. produce septate hyphae (2–4 μm in diameter), with simple, long or short conidiophores bearing conidia singly or in small groups (may resemble mould phase of *Blastomyces dermatitidis* [p. 172]). The conidia (4–7 $\times$ 5–12 μm) are unicellular and fairly oval or clavate, with the larger end toward the apex, and appear cut off at the base (i.e., truncate); they

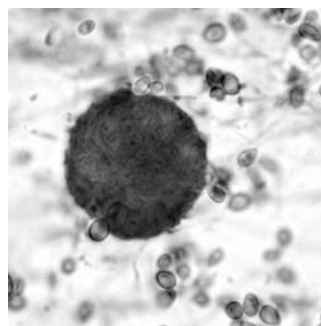
Scedosporium spp. complex (also known as *Scedosporium apiospermum* complex) (continued)

become dark with age. In the sexual state, large, brown cleistothecia (50–200 μm in diameter) are formed and release elliptical ascospores when ruptured. The sexual state may sometimes be induced by culturing on cornmeal agar or potato dextrose agar; the cleistothecia are most likely to form in the center of the colony.

The *Graphium* type of asexual synanamorph conidiation (p. 242) is seen occasionally; it is characterized by septate hyphae with simple, long, dark conidiophores that are cemented together forming synnemata. At the apex of each synnema is a cluster of oval, colorless, single-celled conidia (2–3 $\times$ 5–7 μm), some exhibiting a narrowed, truncate base. Delicate, rhizoidlike structures appear at the base of the synnema.



Asexual stage.



Sexual stage.

For further information, see

Anaissie et al. (ed.), 2009, pp. 316–317, 574, 602, 612 (more in index)

Kantarcioğlu et al., 2012

Katragkou et al., 2007

Lackner et al., 2012

Lackner et al., 2014

Leek et al., 2016

McCarthy et al., 2014

Taj-Aldeen et al., 2015

TABLE 18 Differentiating phenotypic characteristics of the clinically encountered members of the *Scedosporium* spp. complex and *Lomentospora prolificans*^a

Species	Conidiogenous cells	Colony reverse orangish ^b	Yellow diffusible pigment ^b	Assimilates D-ribose	Growth at 40°C	Growth at 45°C
<i>Scedosporium boydii</i> / <i>Pseudallescheria boydii</i> ^c	Cylindrical	0	V	+	+	0
<i>Scedosporium apiospermum</i>	Cylindrical	0	V	0	+	0
<i>Scedosporium aurantiacum</i>	Cylindrical or slightly flask shaped	+	+	+	+	+
<i>Scedosporium dehoogii</i>	Cylindrical or slightly flask shaped	0	0	0	0	0
<i>Lomentospora prolificans</i>	Flask shaped	0	0	0	+	V

^a Source: Gilgado et al., 2008. Abbreviations: +, positive; 0, negative; V, variable.

^b On potato dextrose agar at 25°C.

^c Sexual state (teleomorph).

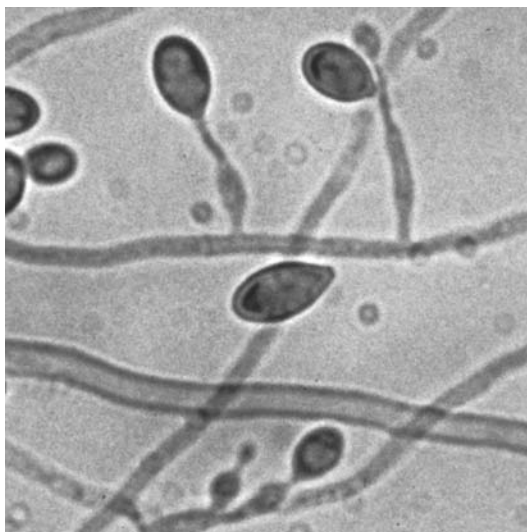
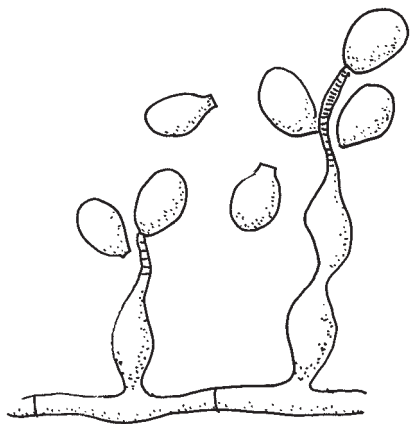
Lomentospora prolificans (formerly *Scedosporium prolificans* and *Scedosporium inflatum*)

PATHOGENICITY: Causes invasive infection that is often characterized by arthritis or osteomyelitis, often occurring by traumatic inoculation, in immunocompetent patients, while disseminated and hematogenous infections occur in immunocompromised hosts. Localized and disseminated infections occur in a variety of sites, especially lung, brain, and bone. The isolates are usually resistant to virtually all available antifungal agents, and disseminated infections are commonly fatal.

RATE OF GROWTH: Rapid; mature within 5 days. Growth is inhibited by cycloheximide.

COLONY MORPHOLOGY: Young colony is cottony or moist (yeasty) and light gray to black. Mature colony becomes dark gray to black and may develop white mycelial tufts with age. Reverse is gray to black. (Color Plates 86 and 87.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with conidiogenous cells (anellides) having a swollen base and elongated “neck”; conidia form small clusters at the apex. Conidia ($2-5 \times 3-12 \mu\text{m}$) are olive to brown, one celled, smooth, and ovoid with a slightly narrowed, truncated (cut-off) base. There is no known sexual state.



For further information, see

Anaissie et al. (ed.), 2009, pp. 316, 574, 612

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Lackner et al., 2012

Lackner et al., 2014

Verruconis gallopava (formerly *Ochroconis gallopava* and *Dactylaria constricta* var. *gallopava*)

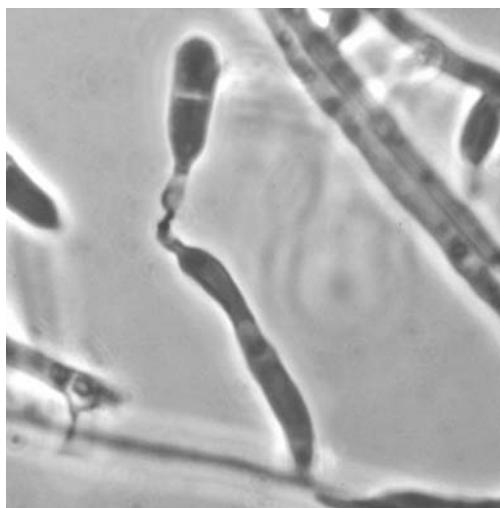
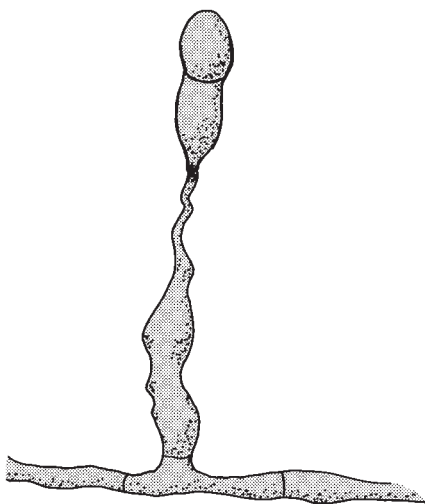
PATHOGENICITY: Has caused subcutaneous and disseminated infections in immunocompromised patients, especially those with solid organ transplantation and hematological malignancies. The organism is known to have a predilection for the central nervous system. It is a relatively common cause of encephalitis in turkeys, chickens, and other birds.

RATE OF GROWTH: Rapid; mature within 5 days. Growth is inhibited by cycloheximide.

COLONY MORPHOLOGY: Surface is feltlike and dark olive-gray, reddish brown, or brownish black. Reverse is dark; a red to brown pigment usually diffuses into the medium. (Color Plate 88.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with conidiophores that are hyaline, erect, and sometimes knobby or bent at the point of conidial formation. Conidia ($2.5\text{--}4.5 \times 11\text{--}18 \mu\text{m}$) form on threadlike denticles; the conidia are brownish, two celled, oval to tear shaped, and typically have a marked constriction at the central septum. A frill of the denticle often remains on the base of the conidium after detachment from the conidiophore. Young conidia may be round and single celled.

See Table 19 (p. 227) for differentiation of species encountered in human specimens.



Courtesy of Dennis Dixon.

For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Giraldo et al., 2014
 Kidd et al., 2016, p. 226
 Revankar and Sutton, 2010
 Shoham et al., 2008

TABLE 19 Differentiation of the clinically encountered *Verruconis* species^a

Organism	Gelatinase at ≤7 days	Growth at 37–42°C	Pathogenic for humans and birds
<i>V. gallopava</i>	0 ^b	+ ^c	+
<i>V. constricta</i>	+	0	0

^a Abbreviations: +, positive; 0, negative.

^b Delayed positive; ≥21 days.

^c Grows more rapidly at 37–40°C than at 30°C.

TABLE 20 Characteristics of some of the “black yeasts”^a

Organism	Decomposition of:		Growth in 15% NaCl	KNO ₃ assimilation	Maximum growth temp (°C)
	Casein (% positive)	Tyrosine (% positive)			
<i>Exophiala jeanselmei</i> complex	0	+ (78)	0	+	≤37
<i>Exophiala (Wangiella) dermatitidis</i>	0	+ (83)	0	0	42
<i>Hortaea (Phaeoannellomyces) werneckii</i>	+ (78)	0 (22)	+	+	<37

^a Reprinted by permission of the publisher from Larone, 1989. Copyright by Elsevier Science Inc. Abbreviations: +, positive; 0, negative. (Updated 2011.)

Exophiala jeanselmei* complex

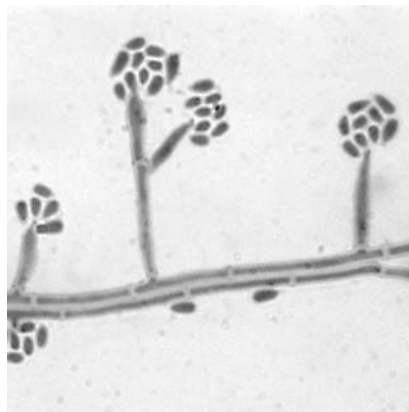
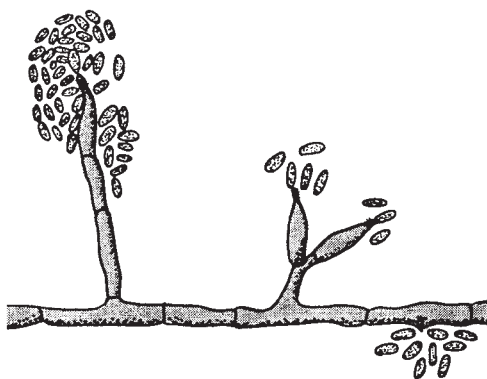
PATHOGENICITY: Causes mycetoma and phaeohyphomycosis. Chromoblastomycosis-like infections have only rarely been reported. For descriptions of diseases, see pp. 40, 51, and 52, respectively. Recent data indicate that posaconazole and itraconazole have the lowest MICs against these members of the *Exophiala jeanselmei* complex.

RATE OF GROWTH: Slow; mature within 14 days when incubated at 25–30°C. Grows more slowly or not at all at 37°C.

COLONY MORPHOLOGY: Surface is at first brownish black or greenish black and skinlike; it then becomes covered with short, velvety, grayish hyphae. Reverse is black. (Color Plates 89 and 90.)

MICROSCOPIC MORPHOLOGY: Young culture consists of many yeastlike budding cells. Eventually, septate hyphae form with numerous conidiogenous cells (annellides) that are slender, tubular, sometimes branched, and characteristically tapered to a narrow, elongated tip. The conidia (1–3 × 2–6 µm) are oval and gather in clusters at the end and sides of the conidiophore and at points along the hyphae. Conidium formation is often best exhibited on cornmeal agar or potato dextrose agar. Chlamydoconidia may be present.

See Table 20 (p. 227) for differentiation from similar organisms.



For further information, see

Badali et al., 2010

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 86

McGinnis, 1980, pp. 211–213, 505–508, 511

Revankar and Sutton, 2010

* Isolates from the United States that in the past had been identified as *E. jeanselmei* based on morphologic and physiologic characteristics have been shown by molecular methods to be mostly two newly named species closely related to *E. jeanselmei*, i.e., *Exophiala oligosperma* and *Exophiala xenobiotica*. A lower percentage were found to belong to several other species of *Exophiala*. The precise species in the complex can be determined only by molecular testing.

Exophiala dermatitidis (*Wangiella dermatitidis*)

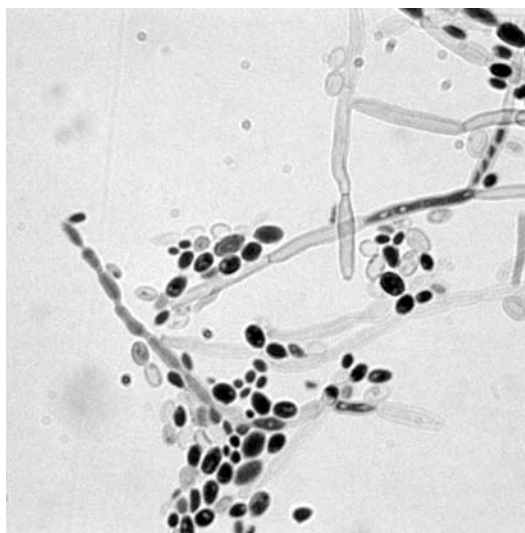
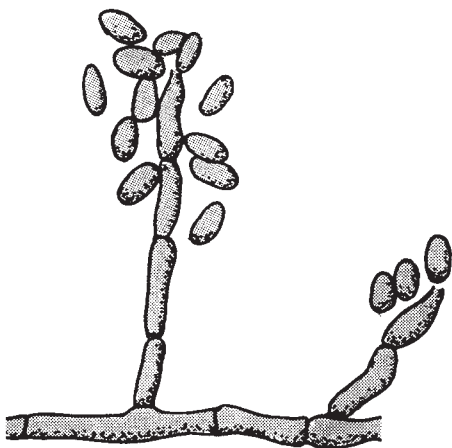
PATHOGENICITY: Causes phaeohyphomycosis (for a description of the disease, see [p. 51](#)). The organism appears to have a predilection for the central nervous system; it has been involved in infections of various sites, including the brain, lung (especially in cystic fibrosis patients), and eye, as well as cutaneous and subcutaneous tissue.

RATE OF GROWTH: Slow; mature and filamentous within 25 days (yeastlike in 10 days).

COLONY MORPHOLOGY: At first the colony is black, moist, shiny, and yeastlike. After 3 or 4 weeks or upon repeated subculture, olive-gray aerial hyphae develop at the periphery and sometimes near the center of the colony. Reverse is dark. ([Color Plates 91 and 92.](#))

MICROSCOPIC MORPHOLOGY: Young cultures are composed of dark (may originally be hyaline), oval to round, budding, yeastlike cells. These cells eventually produce dark, septate hyphae and cylindrical to flask-shaped conidiogenous cells that usually have a somewhat truncate apex without collarette or conspicuous extension. Round to oval, single-celled, pale brown conidia ($2\text{--}3 \times 3\text{--}6 \mu\text{m}$) accumulate at the apex of the conidiogenous cell and down the sides of the conidiophore. Conidia may also be produced at projections along the hyphae. Production of conidia is sometimes sparse.

See [Table 20 \(p. 227\)](#) for differentiation from similar organisms.



For further information, see
Anaissie et al. (ed.), 2009, p. 334
Badali et al., 2010
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Kidd et al., 2016, p. 85
Kondori et al., 2011
Revankar and Sutton, 2010

***Hortaea werneckii* (*Phaeoannellomyces werneckii*)**

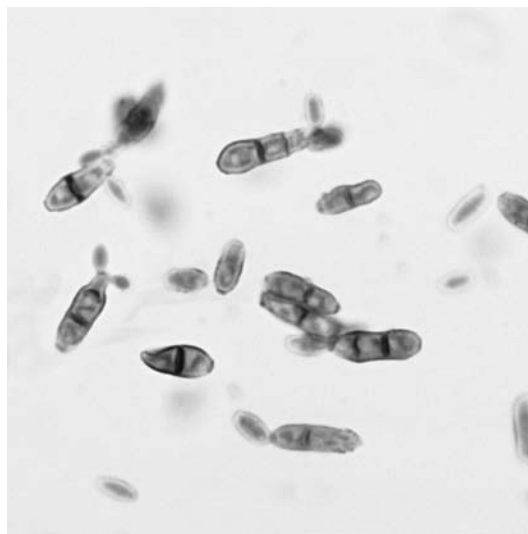
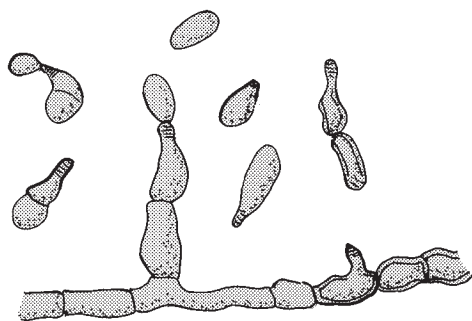
PATHOGENICITY: Etiologic agent of tinea nigra, a superficial, asymptomatic fungal infection of the skin, usually on the palms of the hands and occasionally on other parts of the body. The lesions are flat, smooth, and not scaly and appear as irregularly shaped brown to black spots resembling silver nitrate stains. The palmar and plantar lesions may also resemble melanoma.

RATE OF GROWTH: Develops slowly; mature within 21 days.

COLONY MORPHOLOGY: Surface is at first light colored, moist, shiny, and yeast-like but soon becomes olive-black. Later, grayish green hyphae may form at the periphery, and the center may lose its shine and become olive colored due to thin layer of mycelium. Reverse is black.

MICROSCOPIC MORPHOLOGY: The very early phase consists mainly of pale or dark brown, yeastlike cells. Mature forms are one or two celled ($3-5 \times 7-10 \mu\text{m}$); they are actually annellides, round at one end while tapered and elongated with striations at the other end where conidia are formed. With age, dark, closely septated, thick-walled hyphae develop. Annelloconidia may form and accumulate at annellidic points along the hyphae. Each conidium can function as an annellide and produce new conidia. Chlamydoconidia may develop with age.

See Table 20 (p. 227) for differentiation from similar organisms.



For further information, see
 Anaissie et al. (ed.), 2009, pp. 334-335, 347
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 108
 Schwartz, 2004

Madurella mycetomatis

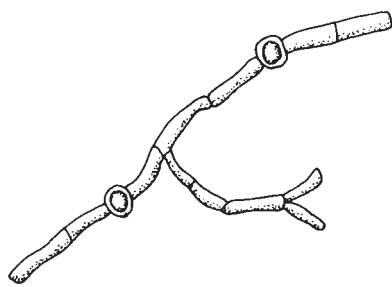
PATHOGENICITY: Causes black grain mycetoma (for a description of the disease, see p. 40) that may result in chronic debilitating deformity and osteoarticular destruction usually of the lower extremities. Most common in South America, India, and East Africa.

RATE OF GROWTH: Moderate; mature in 10 days. Best growth at 37°C; much slower at 25–30°C.

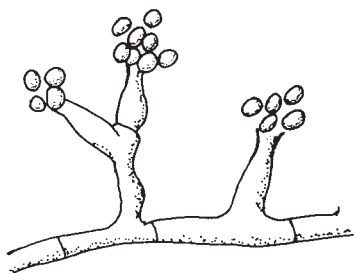
COLONY MORPHOLOGY: Varies greatly; may be smooth or folded and glabrous or powdery and ranges in color from white to yellowish brown. There is usually a brown, diffusible pigment in the agar. Reverse is dark brown.

MICROSCOPIC MORPHOLOGY: On Sabouraud dextrose agar, forms only septate hyphae (1–6 µm in diameter) with numerous chlamydoconidia-like, enlarged cells. On cornmeal agar, some strains produce phialides that bear round or oval conidia at their tips. May form large, black masses of modified hyphae (sclerotia) in old cultures.

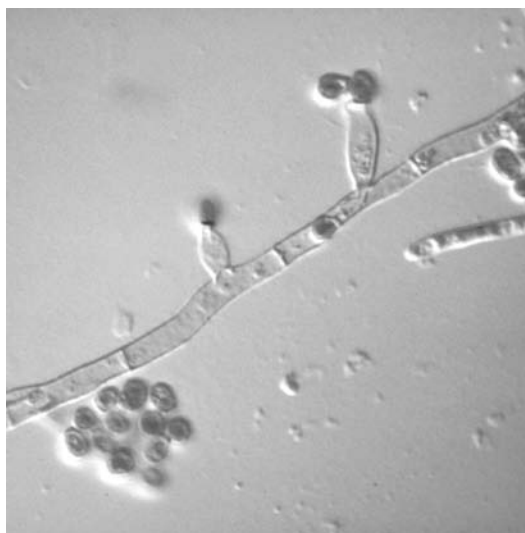
M. mycetomatis differs biochemically from *Madurella grisea* in assimilating lactose but not sucrose.



On Sabouraud dextrose agar.



On cornmeal agar.



On cornmeal agar. Courtesy of Arvind Padhye.

For further information, see
Anaissie et al. (ed.), 2009, p. 343
Kidd et al., 2016, p. 116
Lichon et al., 2006
van Belkum et al., 2013

Madurella grisea

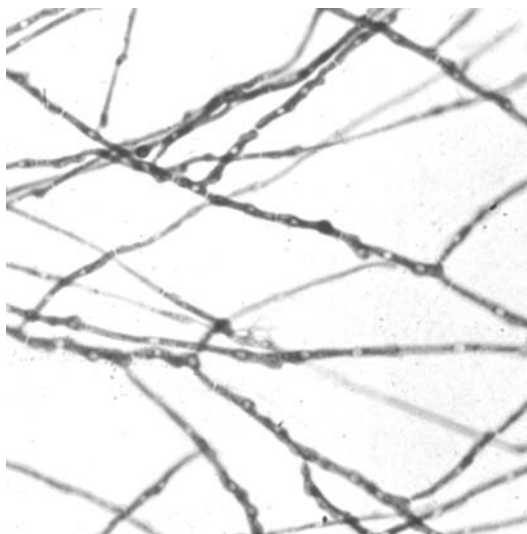
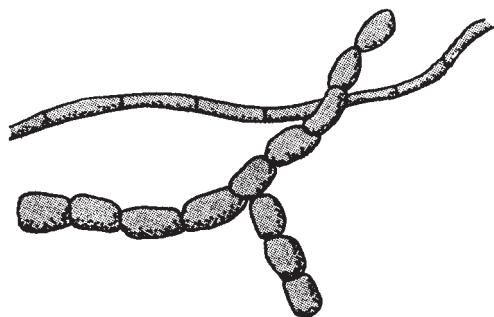
PATHOGENICITY: Causes black grain mycetoma (for a description of the disease, see p. 40). Occurs in India, Africa, Central and South America, and occasionally in the United States.

RATE OF GROWTH: Moderately slow; mature in 12 days. Best growth at 25–30°C; does not grow well, if at all, at 37°C.

COLONY MORPHOLOGY: Surface is somewhat folded in the center with radial grooves toward the periphery. Very short, tan or gray aerial hyphae cover a dark gray or olive-brown mycelial mat. Reverse is dark. May form diffusible pigment, but not as commonly as does *Madurella mycetomatis*.

MICROSCOPIC MORPHOLOGY: Hyphae are septate, mostly wide (3–5 μm), branched, and dark. The hyphae sometimes appear to be made up of chains of rounded cells, suggesting a budding process. Thinner (1–3 μm in diameter), cylindrical, branched hyphae are also present. Conidia are not commonly formed. Some strains may produce pycnidia; chlamydoconidia are occasionally produced.

M. grisea differs biochemically from *M. mycetomatis* in assimilating sucrose but not lactose.



Courtesy of Arvind Padhye.

For further information, see
 Anaissie et al. (ed.), 2009, p. 343
 Kidd et al., 2016, p. 116
 Lichon et al., 2006
 Revankar and Sutton, 2010

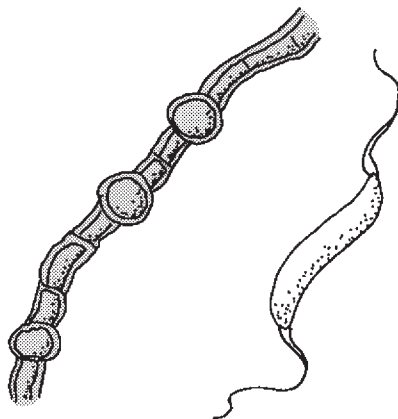
Piedraia hortae

PATHOGENICITY: Causes black piedra, a fungal infection of the hairs of the scalp seen most commonly in the tropics. It is characterized by the formation of small, stony hard, dark nodules along the hair shafts. White piedra is caused by *Trichosporon ovoides* and *Trichosporon inkin* (p. 163).

RATE OF GROWTH: Slow; mature in 21 days.

COLONY MORPHOLOGY: Colonies are small, adherent, compact, somewhat raised, and dark greenish brown to black and may be glabrous or covered with very short aerial hyphae. Reddish brown, diffusible pigment may form. Reverse is black.

MICROSCOPIC MORPHOLOGY: Hyphae are closely septate, dark, and thick walled and vary in diameter, with many intercalary chlamydoconidia-like cells. Asci may be produced in culture. The walls of the asci readily dissolve, releasing single-celled, curved ascospores (5–10 × 30–35 µm) that taper at the ends to form whip-like extensions. The ascospores are more likely to be seen on direct microscopic examination of the specimen than on culture.



For further information, see

Anaissie et al. (ed.), 2009, pp. 347, 516

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Schwartz, 2004

Aureobasidium pullulans

PATHOGENICITY: Relatively rare agent of phaeohyphomycosis; reported cases include corneal, peritoneal, cutaneous, pulmonary, and systemic infections. May also be encountered as a contaminant in clinical specimens.

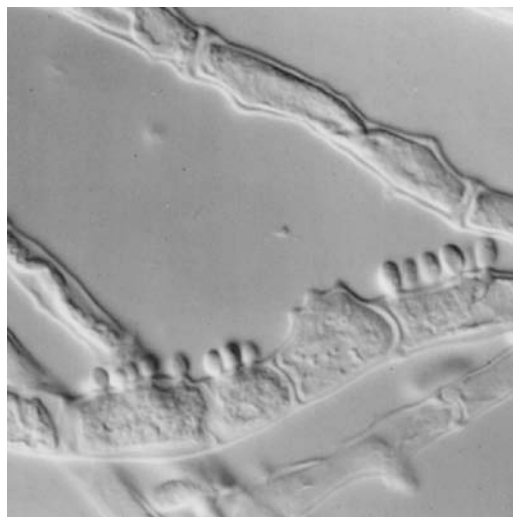
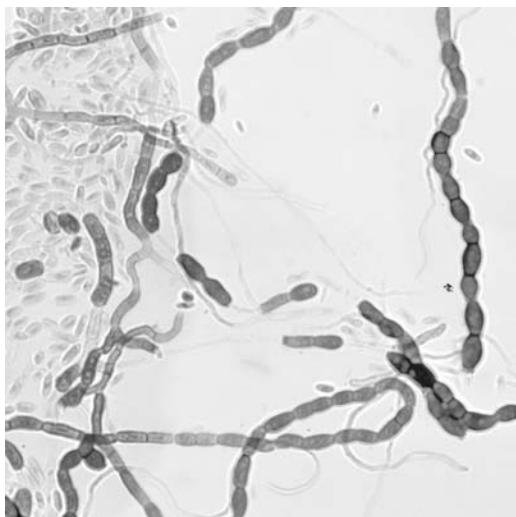
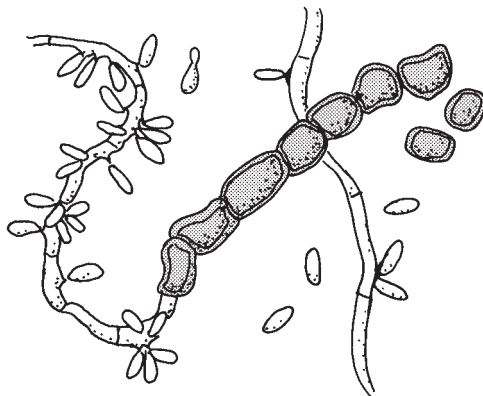
RATE OF GROWTH: Rapid; growth appears within 3–5 days, but pigment production and characteristic morphology may require extended incubation.

COLONY MORPHOLOGY: Colony is at first white, very occasionally pale pink; appears moist and creamy. Eventually develops areas of brown or black (when chlamydoconidia develop) and becomes shiny, with a white to grayish fringe. Reverse is dark when colony is mature. (Color Plate 93.)

MICROSCOPIC MORPHOLOGY: Young colonies consist of unicellular, budding, yeastlike cells. Two types of hyphae (3–12 μm in diameter) develop: (i) hyaline, delicate, and thin walled, producing blastoconidia directly from the walls at certain fertile points, and (ii) thick-walled, dark, and closely septated segments that become chlamydoconidia that may also produce blastoconidia. The blastoconidia are hyaline and oval, vary in size (average, $3\text{--}6 \times 6\text{--}12 \mu\text{m}$), and may continue to multiply by budding; they form synchronously (i.e., simultaneously, each from a separate fertile point), mostly on the hyaline hyphae. The early yeastlike form may be similar to *Candida* spp. or young cultures of *Exophiala dermatitidis* (p. 229) and *Hortaea werneckii* (p. 230), but it can be distinguished by careful examination of growth rate and mature microscopic morphology.

A. pullulans is most likely to be confused with *Hormonema dematioides* (p. 237); for differentiation of these two fungi, see Table 21 (p. 236).

(continued on following page)



Synchronous production of blastoconidia. Courtesy of Wiley Schell.

For further information, see
Anaissie et al. (ed.), 2009, pp. 335–336
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Hawkes et al., 2005
Revankar and Sutton, 2010

TABLE 21 Differential characteristics of *Aureobasidium pullulans* versus *Hormonema dematioides*

Organism	Assimilation of MDG ^a	Formation of hyaline blastic conidia ^b	
<i>Aureobasidium pullulans</i> (p. 234)	Positive	Form mostly on hyaline hyphae Develop in a synchronous fashion (i.e., conidia are formed simultaneously, each from a separate fertile point ; these points are often very close together, producing clusters of conidia)	
<i>Hormonema dematioides</i> (p. 237)	Negative	Form on both hyaline and dematiaceous hyphae Develop in an asynchronous fashion (i.e., conidia are produced in succession from the same single fertile point ; the conidia may form short chains or gather in loose clusters)	

^a Methyl- α -D-glucoside (MDG) assimilation can be tested with the API 20C AUX panel (bioMérieux, Hazelwood, MO); extended incubation is required. This one substrate appears to give credible results with these organisms, but the overall identification profile number cannot be accepted.

^b The microscopic morphology of these organisms is best demonstrated by utilizing the Dalmau method (p. 409) on cornmeal-Tween 80 medium incubated at 25°C for an extended period of time (until dematiaceous hyphae develop).

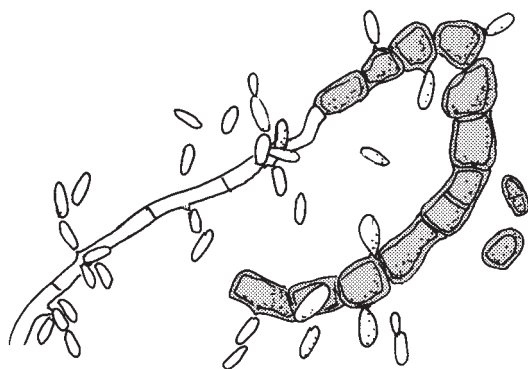
PATHOGENICITY: Has been reported to cause peritonitis, fungemia, and cutaneous phaeohyphomycosis. Exposure to birds has been related to deep infection by *H. dematioides*. It may also be encountered as a contaminant. There is a high probability that this organism has been misidentified as *Aureobasidium pullulans* in the past.

RATE OF GROWTH: Rapid; growth appears within 3–5 days, but pigment production and characteristic morphology may require extended incubation.

COLONY MORPHOLOGY: Colony is initially white to cream, sometimes pinkish; appears smooth and becomes moister as conidia develop; poorly conidiating strains may have a woolly mycelial mat; color eventually becomes brownish black as chlamydoconidia develop. Reverse is dark when colony is mature.

MICROSCOPIC MORPHOLOGY: Young colonies consist of unicellular, budding, yeast-like cells. Two types of hyphae (3–12 μm in diameter) develop: (i) hyaline, delicate, and thin walled, producing blastoconidia directly from the walls at certain fertile points, and (ii) thick-walled, dark, and closely septated segments that become chlamydoconidia. The blastoconidia are hyaline and oval, vary in size (average, 3–6 $\times$ 6–12 μm), and may continue to multiply by budding; they are produced asynchronously (i.e., in succession, from the same single fertile point) on both the hyaline and dematiaceous hyphae.

H. dematioides can easily be mistaken for *Aureobasidium pullulans* (p. 234); for differentiation of these two fungi, see Table 21 (p. 236).



Asynchronous production of blastoconidia.
Courtesy of Wiley Schell.

For further information, see
Anaissie et al. (ed.), 2009, pp. 335–336
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Kent al., 1998
Shin et al., 1998

Neoscytalidium dimidiatum (formerly *Scytalidium dimidiatum*)

PATHOGENICITY: Known to commonly cause nail and skin infections, as well as keratitis; there are also rare reports of more deep-seated infections, such as subcutaneous abscess, sinusitis, endophthalmitis, lymphadenitis, brain infection, empyema, and fungemia predominantly in immunocompromised patients. Infection is principally in individuals who live in or have visited tropical or subtropical areas, while several cases have been reported in individuals who have never left temperate zones.

RATE OF GROWTH: Rapid; mature within 3 days. Growth is inhibited by cycloheximide; therefore, the organism will not grow on most media specifically designed for dermatophytes.

COLONY MORPHOLOGY: Colonies are usually woolly, and growth quickly fills the agar plate or covers the agar slant; some isolates do not spread across the agar as robustly. The surface is gray to brown with a dark reverse or may be white to cream or gray with a buff to yellowish reverse in melanin-deficient mutants. ([Color Plate 94.](#))

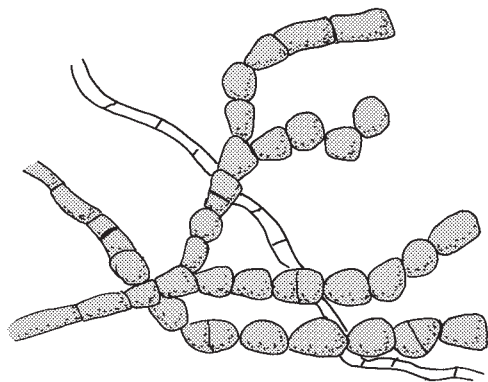
MICROSCOPIC MORPHOLOGY: Hyphae are septate and branched, but no conidiophores are formed. Arthroconidia ($3\text{--}6 \times 5\text{--}13 \mu\text{m}$) develop that have one or two cells, are flattened on the ends, and may be rectangular, square, oval, or roundish and become barrel shaped; they are consecutive; i.e., there are no empty cells between the arthroconidia. The wider hyphae ($6\text{--}10 \mu\text{m}$) and arthroconidia of *N. dimidiatum* are brown, while the narrower side branches of hyphae tend to produce pale arthroconidia. Melanin-free mutants occur (previously known as *Scytalidium hyalinum*); they produce hyphae and arthroconidia that are invariably colorless but are identical to those of *N. dimidiatum* in every other way.

A pycnidial form very occasionally develops in old cultures; it is now considered to be *Fusicoccum dimidiatum* (formerly thought to be *Nattrassia mangiferae*). The pycnidia are large ($100\text{--}300 \mu\text{m}$ in diameter); pycnidial conidia are hyaline when young and with age develop one to five septa and a dark brown central area.

See [Table 25](#) (p. 288) for differential characteristics of fungi in which arthroconidia predominate.

(continued on following page)

Neoscytalidium dimidiatum (formerly *Scytalidium dimidiatum*)
(continued)



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Elinav et al., 2009
Revankar and Sutton, 2010

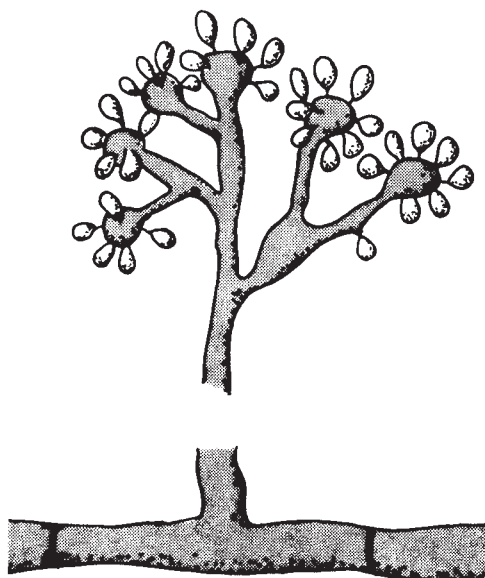
***Botrytis* spp. (*Botrytis cinerea*)**

PATHOGENICITY: The most commonly encountered species is *Botrytis cinerea*. Considered a contaminant when encountered in the mycology lab. Its normal habitat is plants. It spoils strawberries and most other fruits, vegetables, and flowers by forming “gray mould,” but when on grapes under the proper conditions, it is a valuable contributor to the development of the sweetness of some dessert wines.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is at first white and then gray or brown, sometimes with blackish spots; woolly. Reverse is usually dark.

MICROSCOPIC MORPHOLOGY: The term *Botrytis* derives from the ancient Greek word *botrys*, meaning “grapes.” Wide, septate hyphae with dark, septate, long conidiophores that branch only at the apex. The branches have swollen tips that bear round to oval, colorless to pale brown conidia on short denticles. Conidia also form at points along the conidiophore. The swollen apices bearing conidia on short denticles may be suggestive of *Cunninghamella* sp. (p. 198), but close examination of macroscopic and microscopic morphology will provide differentiation of the two.



From Rippon, 1988.

For further information, see
 Barron, 1977, pp. 105–106
 McGinnis, 1980, pp. 192–193, 195
 Rippon, 1988, pp. 760–761

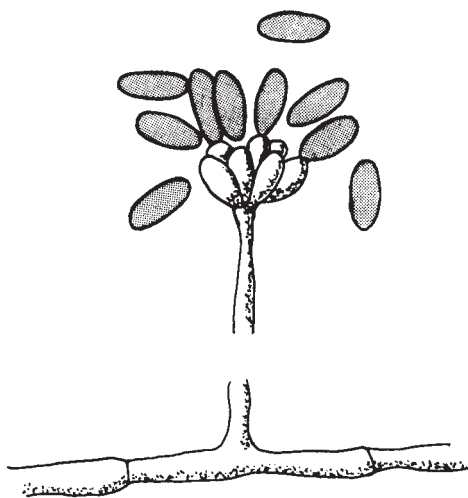
Stachybotrys chartarum (*S. alternans*, *S. atra*)*

PATHOGENICITY: Produces several mycotoxins that appear to have the ability to adversely affect humans and animals after ingestion, inhalation, or percutaneous absorption. The fungus has been associated with pulmonary hemorrhage and hemosiderosis in animals. It has also been implicated in illnesses (with a variety of symptoms), including hypersensitivity pneumonitis, in occupants (of all ages) of water-damaged homes and other buildings. Additional studies are needed to establish a firm causal relationship.

RATE OF GROWTH: Moderately rapid; usually mature within 7 days but can be rather fastidious on routine laboratory media; prefers medium with high cellulose content, e.g., cellulose Czapek agar.

COLONY MORPHOLOGY: Surface is at first white, becoming dark gray to black with age; powdery to cottony, spreading. Reverse is at first light and then dark. (Color Plate 95.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate and colorless to dark. Conidiophores are simple or branched, may become pigmented and rough with age, and bear clusters of 3–10 phialides. The phialides are colorless or pigmented, nonseptate, and cylindrical, with a swollen upper portion. Conidia are dark, oval (average, $4.5 \times 9 \mu\text{m}$), single celled, and smooth or rough walled and usually form in clusters on the phialides.



For further information, see
Anaissie et al. (ed.), 2009, p. 653
Kuhn and Ghannoum, 2003
Vance and Weissfeld, 2007

* The nomenclature of *Stachybotrys* has been confusing; these three species names have been used interchangeably in the literature.

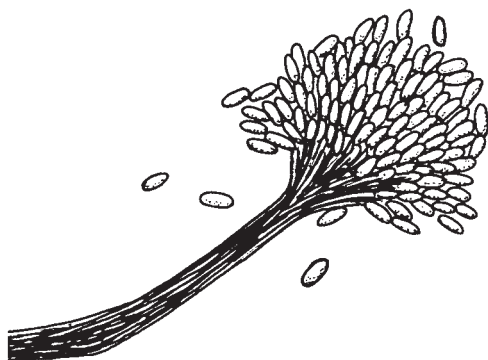
Graphium eumorphum

PATHOGENICITY: May be found as a contaminant but is also an asexual synanamorph of *Scedosporium* spp. (p. 222).

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Surface gray, cottony. Reverse is at first light, turning dark.

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with simple, long, dark conidiophores that are cemented together forming synnemata. At the apex of each synnema is a cluster of oval, colorless, single-celled annelloconidia ($2-3 \times 5-7 \mu\text{m}$), some exhibiting a narrowed, truncate base. Delicate rhizoidlike structures appear at the base of the synnema.



For further information, see
Barron, 1977, pp. 185-188
Kidd et al., 2016, p. 105
McGinnis, 1980, pp. 227-229

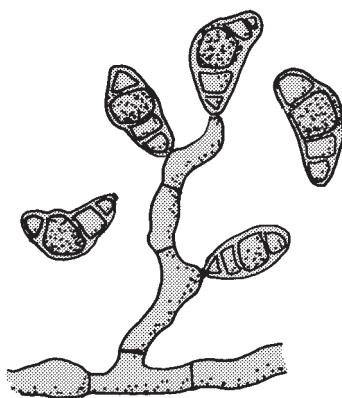
Curvularia spp.

PATHOGENICITY: Etiologic agents of opportunistic infections, most commonly causing keratitis and allergic/chronic sinusitis. May also cause onychomycosis as well as mycetoma and phaeohyphomycosis at various sites, including subcutaneous tissue and deep tissues. Dissemination to the brain is known to occur occasionally, often following sinusitis.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Colony is dark olive-green to brown or black with a pinkish gray, woolly surface. Reverse is dark. (Color Plate 96.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate and dark. Conidiophores are simple or branched and bent or knobby at points of conidium formation (sympodial geniculate growth). Conidia are large ($8\text{--}14 \times 21\text{--}35 \mu\text{m}$), usually contain four cells, and eventually appear curved due to swelling of a central cell. Conidia differ from those of *Bipolaris* spp. (p. 244) by having a central cell that is darker than the end cells, a thinner cell wall, narrower septations between cells, and a distinct curve that develops with age (the curving may take 5 or more days to develop). There are multiple species of *Curvularia* that are best distinguished by molecular methods, including the *GPDH* (glyceraldehyde-3-phosphate dehydrogenase) and *ITS* genes. One should note that the genus *Curvularia* is undergoing active taxonomic revision.



For further information, see
Anaissie et al. (ed.), 2009, pp. 337–338
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Madrid et al., 2014
Manamagoda et al., 2012
Revankar and Sutton, 2010

***Bipolaris* spp.**

PATHOGENICITY: Most commonly cause allergic sinusitis and, in immunocompromised patients, may progress to invade bone and cause lesions in the brain. Known to cause phaeohyphomycosis in a variety of other sites, including the eye, skin, peritoneum, aorta, lung, and central nervous system. Most medically important species are susceptible to anti-mould triazoles and within the range of safely achievable serum concentrations of amphotericin B. The three most common species recovered from human specimens are *B. spicifera*, *B. hawaiiensis*, and *B. cynodontis*.

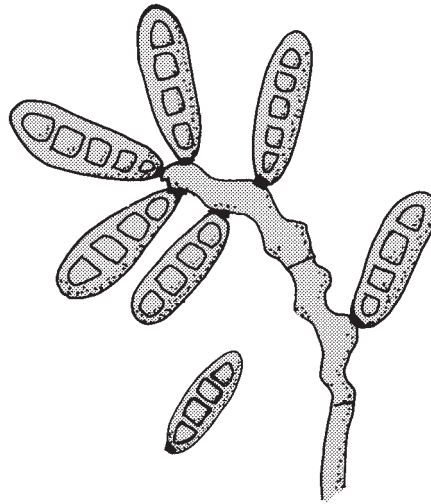
RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is at first grayish brown, becoming black with a matted center and raised grayish periphery. Reverse is dark brown to black. (Color Plates 97 and 98.)

MICROSCOPIC MORPHOLOGY: Dark, septate hyphae. Conidiophores elongate and bend at the point where each conidium is formed (sympodial geniculate growth); this produces a knobby, zigzag appearance. The conidia are brown, oblong to cylindrical ($6\text{--}13 \times 14\text{--}39 \mu\text{m}$), appear thick walled, and have three to five septations and a slightly protruding hilum. When tested for germ tube production (p. 382), germ tubes develop at both poles of the conidia, hence the genus name.

See Table 22 (p. 246) for characteristics that distinguish it from similar organisms.

(continued on following page)

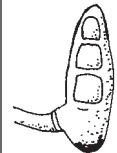
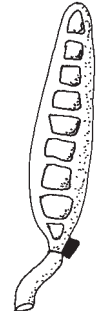


Bipolar germ tubes.

For further information, see
 Anaissie et al. (ed.), 2009, pp. 336–337
 da Cunha et al., 2012
 Kidd et al., 2016, p. 31
 McGinnis et al., 1986
 Revankar and Sutton, 2010

* Before the mid-1980s, most isolates of *Bipolaris* spp. were mistakenly called *Drechslera* spp.

TABLE 22 Characteristics of *Bipolaris*, *Drechslera*, and *Exserohilum* spp.^a

Genus	Reported as pathogen	Conidiation	Conidia			Germ tubes ^b		
			Avg. size (μm)	No. of septa	Hilum	Origin	Orientation from basal cell	Illustration
<i>Bipolaris</i>	+	Profuse	8 × 26	3–5	Protrudes slightly	One or both end cells; adjacent to hilum	Along axis of conidium Goes through hilum	
<i>Drechslera</i>	0	Poor	16 × 65	3–5	Does not protrude	Intermediate and end cells; not adjacent to hilum	Perpendicular to conidial axis	
<i>Exserohilum</i>	+	Profuse	14 × 90 or greater	5–12	Protrudes slightly	One or both end cells; adjacent to hilum; often other cells	Along axis of conidium Displaces hilum	

^a Reprinted by permission of the publisher from Larone, 1989. Copyright by Elsevier Science Inc.^b See p. 382 for instructions on performance of the germ tube test for these organisms.

Exserohilum spp.

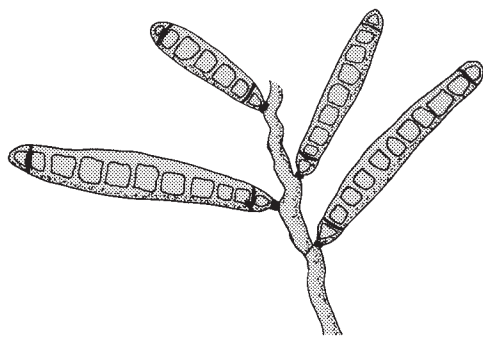
PATHOGENICITY: In 2012, *Exserohilum rostratum* was the cause of epidemic iatrogenic meningoencephalitis, meningoradiculitis, epidural abscesses, and vertebral osteomyelitis following intrathecal or lumbosacral injection of contaminated methylprednisolone solution. Before this outbreak, *E. rostratum* was known to cause other infections, including phaeohyphomycosis (see p. 51 for a description of the disease), sinusitis, subcutaneous infection, and keratitis. Fatal disseminated infections also have been reported to occur in immunocompromised hosts.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is dark gray to black, cottony. Reverse is black. (Color Plate 99.)

MICROSCOPIC MORPHOLOGY: Dark, septate hyphae. Conidiophores elongate and bend (sympodial geniculate growth) at the point where each conidium is formed; this produces a knobby, zigzag appearance. The conidia are brown, long (average, $14 \times 80 \mu\text{m}$ or greater), fusiform, appear thick walled, and usually have 7–11 septa. The hilum (scar of attachment) on each conidium is seen as a dark, conspicuous, square protrusion. The most commonly encountered species, *E. rostratum*, displays a distinctive dark septum at each end cell of the mature conidium.

See Table 22 (p. 246) for differentiation from similar organisms.



For further information, see
Katragkou et al., 2014
Kidd et al., 2016, p. 91
Larone and Walsh, 2013
Lyons et al., 2012

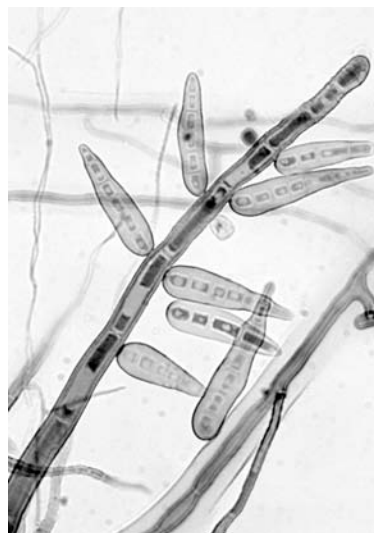
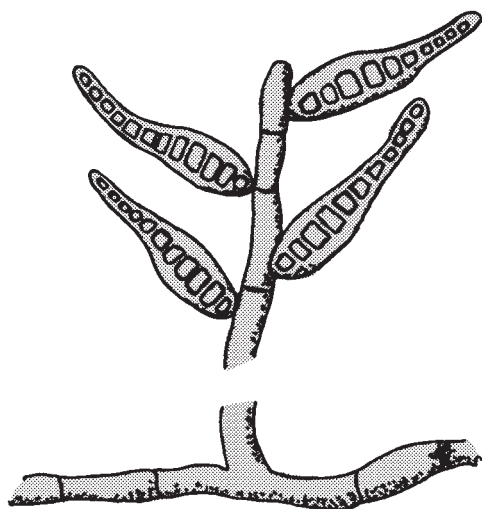
Helminthosporium sp.

PATHOGENICITY: Commonly considered a contaminant. Natural habitat is soil and grass; not known to cause infection.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is dark gray to black, cottony. Reverse is black.

MICROSCOPIC MORPHOLOGY: Hyphae are septate. Conidiophores are brown, determinate (i.e., not elongating at the point of conidium formation), slightly curved, unbranched, and often in clusters. Conidia form along the sides of the conidiophores, frequently in whorls. Conidia are large ($\sim 9 \times 40 \mu\text{m}$), dark, thick walled, club shaped with the broader end toward the conidiophore, and usually contain six or more cells.



For further information, see

Barron, 1977, pp. 199-202

McGinnis, 1980, pp. 228-229, 231-232

Rippon, 1988, p. 769

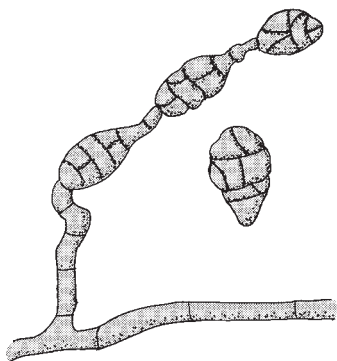
Alternaria spp.

PATHOGENICITY: *Alternaria* spp. are commonly considered saprobic contaminants but are also known to cause phaeohyphomycosis, most commonly in subcutaneous tissue of immunocompromised patients. There have also been reports of onychomycosis, keratitis, and noninvasive rhinosinusitis. Locally invasive sinusitis may occur in hematopoietic stem cell transplant recipients. *Alternaria* spp. also may play an important role in asthma and hypersensitivity pneumonitis.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is at first grayish white and woolly and later becomes greenish black or brown with a light border. May eventually become covered by short, grayish aerial hyphae. Reverse is black. (Color Plates 100 and 101.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate and dark. Conidiophores are septate, of variable length, and sometimes have a zigzag appearance. Conidia are large (usually $8\text{--}16 \times 23\text{--}50 \mu\text{m}$) and brown, have both transverse and longitudinal septations, sometimes produce germ tubes, and are found singly or in chains; they are usually rather round at the end nearest the conidiophore while narrowing at the apex, producing a clublike shape. The nomenclature of *Alternaria* spp. continues to be revised with ongoing studies of their molecular characteristics.



For further information, see

Anaissie et al. (ed.), 2009, pp. 336, 516

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Kidd et al., 2016, p. 3

Kwon-Chung and Bennett, 1992, pp. 630–631, 662–663

Pastor and Guarro, 2008

Woudenberg et al., 2013

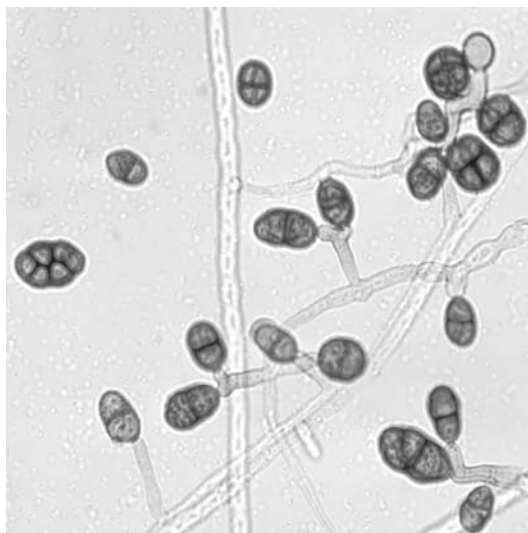
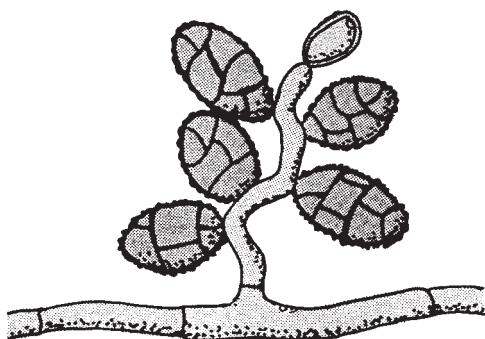
***Ulocladium* sp.**

PATHOGENICITY: Commonly considered a contaminant; very rarely involved in phaeohyphomycosis.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is dark brown to black, cottony. Reverse is black.

MICROSCOPIC MORPHOLOGY: Septate hyphae, light to dark brown. Conidiophores are simple or branched and bent at points of conidial production, giving a zigzag appearance. Conidia are brown to black, smooth or rough, and round to oval or slightly egg shaped ($7\text{--}12 \times 18\text{--}24 \mu\text{m}$), with transverse and longitudinal septations.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 224

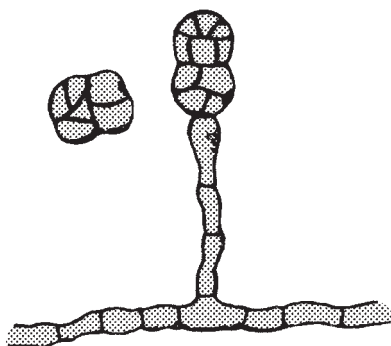
Stemphylium sp.

PATHOGENICITY: Commonly considered a contaminant.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is brown to black, cottony. Reverse is black.

MICROSCOPIC MORPHOLOGY: Septate hyphae, light to dark brown. Conidiophores are simple or occasionally branched, with a dark, swollen terminus bearing individual conidia; the conidiophore develops a nodular or knobby appearance as it ages and produces more conidia. Conidia ($12\text{--}20 \times 15\text{--}30 \mu\text{m}$) are dark, smooth or rough, and round or oval and have transverse and longitudinal septations, often with marked constriction at the central septum.



For further information, see
Barron, 1977, pp. 291–292
Kidd et al., 2016, p. 188
McGinnis, 1980, pp. 283–284

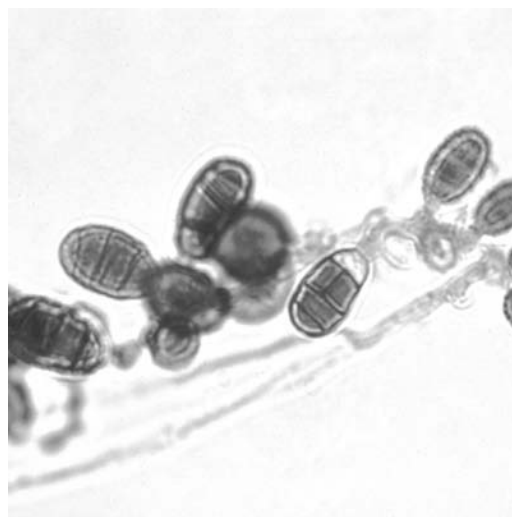
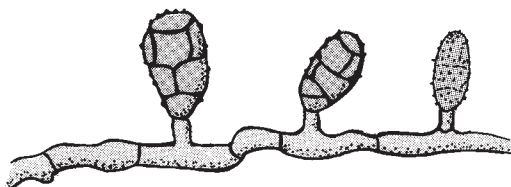
***Pithomyces* spp.**

PATHOGENICITY: Commonly considered a contaminant. *Pithomyces chartarum* causes facial eczema in a variety of animals, but sheep are especially susceptible. *P. chartarum* and *P. sacchari* are the two species of *Pithomyces* that are most commonly recovered from clinical specimens that include cutaneous, respiratory, and nasal samples.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is light to dark brownish black, cottony. Reverse is dark.

MICROSCOPIC MORPHOLOGY: Septate hyphae, pale or light brown. Conidiophores are short, simple, and peglike. Conidia are single, oval (10–20 × 20–30 μm), yellow to dark brown, and usually rough, with transverse and longitudinal septations.



For further information, see
da Cunha et al., 2014
Kidd et al., 2016, p. 158

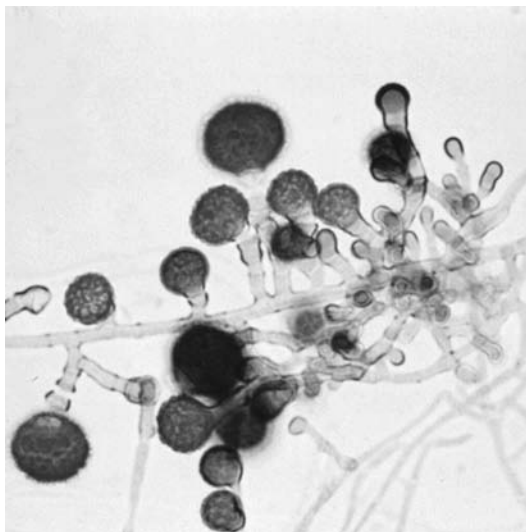
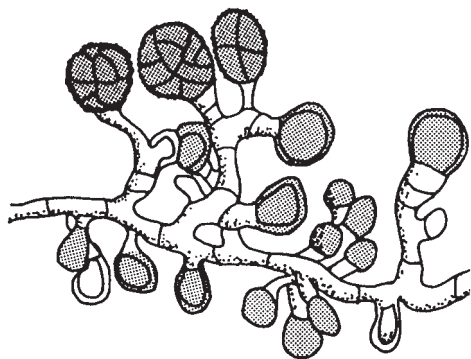
Epicoccum sp.

PATHOGENICITY: Commonly known as a contaminant; not known to cause disease.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Colonies are irregularly cottony and usually yellow to orange at first (due to the color of the hyphae), becoming brown to black where the dark mature conidia eventually form. Reverse is sometimes red or may be dark brown or grayish. A diffusible pigment may color the medium yellow, orange, red, or brown. (Color Plates 102 and 103.)

MICROSCOPIC MORPHOLOGY: Clusters of short conidiophores form on hyphae by repeated branching to form a dense mass (sporodochium) from which conidia arise. Young conidia are round to pear shaped, pale, smooth, and nonseptate. Mature conidia (15–30 μm in diameter) are almost round, multiseptate both longitudinally and transversely, dark brown or black, and often rough and warty. Characteristically, all stages of conidia will be present simultaneously in the clusters.



For further information, see
Kidd et al., 2016, p. 83
McGinnis, 1980, p. 209

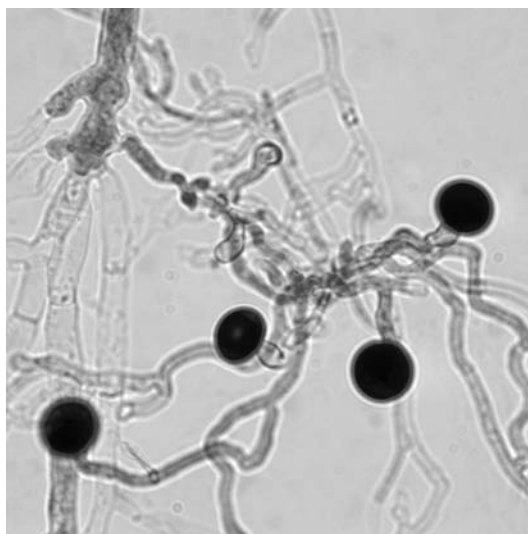
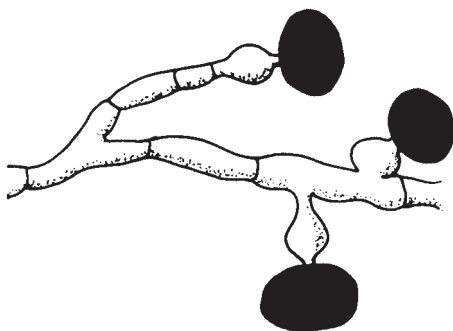
***Nigrospora* sp.**

PATHOGENICITY: Commonly considered a contaminant; possible involvement in disease has very rarely been reported.

RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: Compact, woolly; at first white, then gray. With age, black areas of conidiation appear. Reverse is black. (Color Plate 104.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate with short conidiophores that swell and then taper at point of conidium formation. The conidia are large, densely black, and almost round, slightly flattened ($\sim 14\text{--}20\text{ }\mu\text{m}$ in diameter).



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
McGinnis, 1980, pp. 241–242, 251
Rippon, 1988, pp. 771, 774

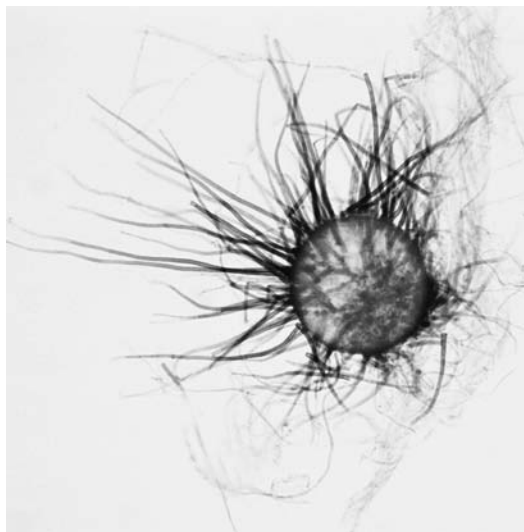
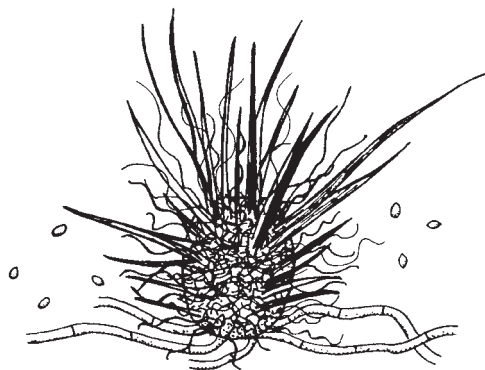
Chaetomium sp.

PATHOGENICITY: Occasionally implicated in infections of the nails and skin and in subcutaneous and systemic phaeohyphomycosis. Several species that grow at 42°C are neurotropic and have caused cerebral phaeohyphomycosis. They more commonly occur as contaminants.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: Surface is cottony, spreading, and usually white, becoming tannish gray, grayish olive, or brown with age. Reverse is usually orange-tan tinted with red but may be brown to black. (Color Plates 105 and 106.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate with large (90–170 × 110–280 µm), round, oval, or flask-shaped perithecia (best seen on potato dextrose agar) that are olive to brown, fragile, and have wavy and/or straight filamentous appendages. Asci are stalked and cylindrical, ovoid, or club shaped; contain four to eight spores; and usually dissolve soon after release from the ostiole (opening) of the perithecium. Ascospores, readily observed, are oval or lemon shaped, single celled, and usually olive-brown but may occur in a variety of sizes, shapes, and colors.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Hubka et al., 2011
Kidd et al., 2016, p. 51

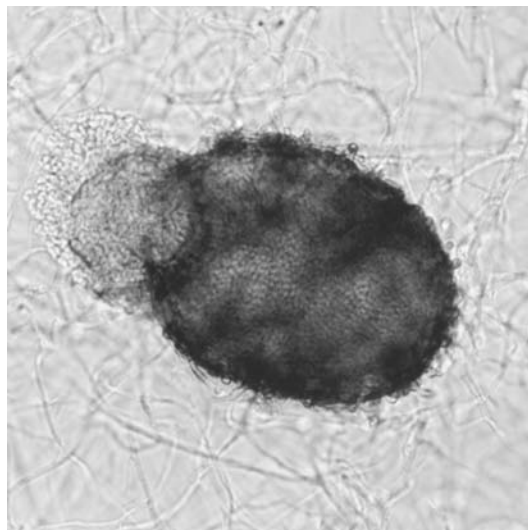
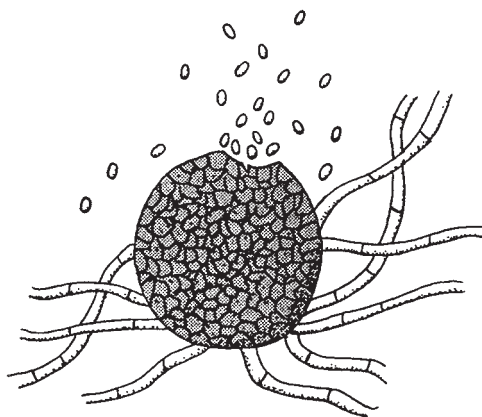
***Phoma* spp.**

PATHOGENICITY: Commonly considered a contaminant. Occasional agents of phaeohyphomycosis.

RATE OF GROWTH: Rapid; mature within 5 days (may take longer for pycnidia to form).

COLONY MORPHOLOGY: Colony is powdery or velvety, spreading, and grayish brown or greenish; some species may develop pink or reddish areas. Reverse is brown to black. A reddish to brown, diffusible pigment is produced by some species. (Color Plate 107.)

MICROSCOPIC MORPHOLOGY: Septate hyphae; large (~60–400 μm in diameter), asexual fruiting bodies (pycnidia). The pycnidia are dark and round or flask shaped and have openings (ostioles) through which conidia are dispersed. The conidia, formed on phialidic cells inside the pycnidia, are mostly one celled, hyaline to pale brown, and may be round, oval, or cylindrical. Chlamydoconidia resembling the conidia of *Alternaria* spp. (p. 249) are produced in some species.



For further information, see
 Anaissie et al. (ed.), 2009, pp. 332, 342
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 155

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Introduction to Dermatophytes

Dermatophytes are filamentous fungi that are able to digest and obtain nutrients from keratin (a relatively insoluble protein; the primary component of skin, hair, and nails). Infection usually involves only the keratinized layer of the skin, as well as hair follicles, hair shafts, and nails. The “disease” known as tinea or ringworm is the result of the host reaction to the cell wall of the fungus and to the enzymes released by the fungus during its digestive process. Dermatophytes are among the few fungi that have evolved a dependency on human or animal infection for the survival of the species. It is therefore not surprising that these fungi are among the most common infectious agents of humans.

The group comprises three genera that can generally be differentiated by their conidium formation.

- Microsporum*: —Macroconidia numerous, thick walled, rough.
—Microconidia usually present.
(*M. audouinii* is an exception; it seldom forms conidia.)
- Trichophyton*: —Macroconidia rare, thin walled, smooth.
—Microconidia numerous.
(Some species do not produce any conidia.)
- Epidermophyton*: —Macroconidia numerous, thin and thick walled, smooth.
—Microconidia are not formed.

Molecular taxonomic studies have led to some disagreement regarding the nomenclature of certain species of dermatophytes. The traditional placement and names of the organisms are utilized on the following pages, as further study appears to be needed to clarify and resolve the evolving understanding of their molecular relatedness.

Molecular and proteomic diagnostics tools are being used increasingly in reference laboratories for rapid identification of dermatophytes to complement existing morphological studies and biochemical profiles.

Latin Terms for Dermatophyte Infections

The clinical dermatophyte diseases are designated in many references by their Latin names. These are constructed by adding to “tinea” the Latin word for the specific body part affected:

Tinea barbae = infection of the bearded areas of the face and neck

Tinea capitis = infection of the scalp and hair shaft

Tinea corporis = infection of the glabrous skin on body parts not otherwise specified (usually the trunk of the body)

Tinea cruris = infection of the groin, perineum, and perianal region

Tinea manuum = infection of the hand

Tinea pedis = infection of the feet

Tinea unguium = infection of the nails (onychomycosis is infection of the nail caused by any fungus, not necessarily caused by a dermatophyte)

For further information, see

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

de Hoog et al., 2017

Elewski et al., 2013

Ellis (<http://www.mycology.adelaide.edu.au/docs/fungus3-book.pdf>)

Hawkins and Smidt, 2014

Ilkit and Durdu, 2015

Rich et al., 2013

Scher et al., 2013

Verrier and Monod, 2017

White et al., 2014

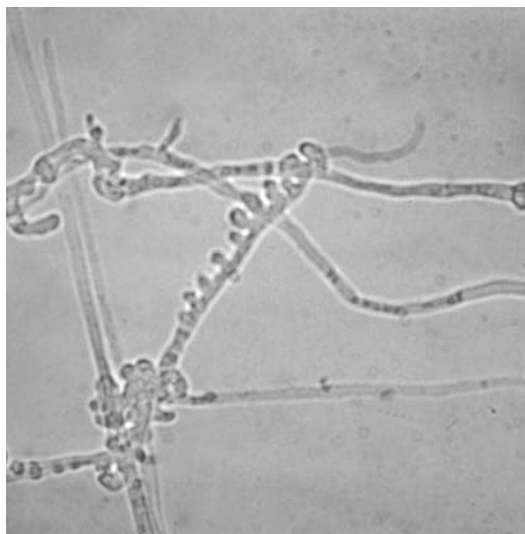
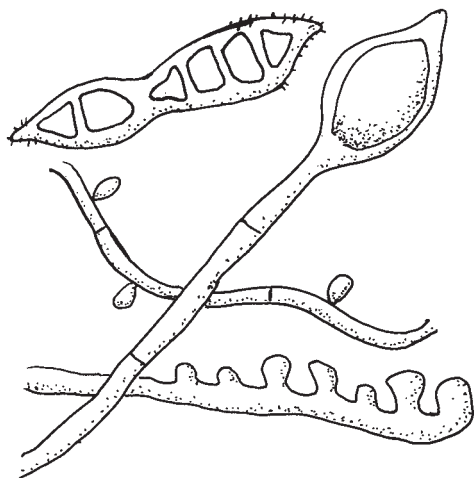
Microsporum audouinii

PATHOGENICITY: This anthropophilic (infecting humans) dermatophyte formerly caused widespread epidemics of tinea capitis in children. The relatively few cases seen now usually have a connection with sub-Saharan Africa. Also known to infect skin on other parts of the body. Very rarely infects adults.

RATE OF GROWTH: Moderate; mature in 7–10 days.

COLONY MORPHOLOGY: Surface is flat, downy to silky, with a radiating edge; it is grayish or tannish white. Reverse is light salmon with reddish brown center (pigment is best seen on potato dextrose agar). (Color Plates 108 and 109.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate with terminal chlamydiconidia that are often pointed on the end. Pectinate (comblike) hyphae are commonly seen. This species is usually almost devoid of conidia but sometimes forms poorly shaped, abortive macroconidia suggestive of *Microsporum canis* (p. 262); in such a case, it is distinguished from poorly sporulating isolates of *M. canis* by its lack of ability to grow on polished rice grains (p. 417). The occasional microconidia that form are identical to those occurring in other species of the genus *Microsporum*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 125
Kwon-Chung and Bennett, 1992, pp. 135–136
Rippon, 1988, p. 241

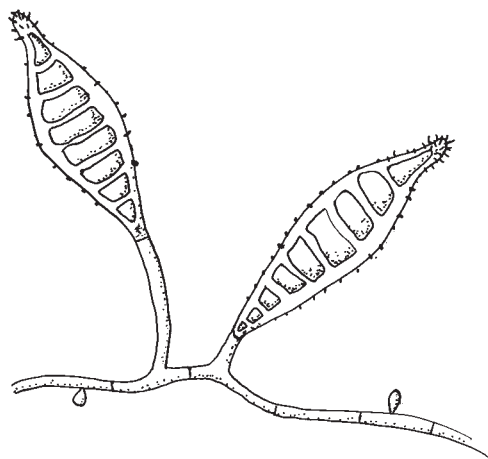
Microsporum canis var. *canis*

PATHOGENICITY: This zoophilic dermatophyte (infecting animals and humans) causes infections of scalp (tinea capitis) and skin (tinea corporis); it is most prevalent in children. This organism is one of the most commonly recognized causes worldwide of tinea capitis and other dermatophytoses. Most infections in humans are acquired from infected dogs or cats or from other animals in an agricultural environment. *M. canis* is a common cause of dermatophytosis in patients who may have lived and worked in a rural or farm setting. This organism is a classical ectothrix pathogen, which destroys the hair shaft from the cuticle inward.

RATE OF GROWTH: Moderately rapid; mature within 4–8 days.

COLONY MORPHOLOGY: Surface is whitish, coarsely fluffy, hairy to silky or fur-like, with yellow pigment at periphery and closely spaced radial grooves. Reverse is deep yellow and turns brownish yellow with age. (Color Plates 110 and 111.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate with numerous macroconidia, which are long (10–25 × 35–110 µm), spindle shaped, rough, and thick walled and characteristically taper to beaklike ends. The rough surface of the macroconidia is seen especially at the beak. Usually more than six compartments are seen in the macroconidia. A few microconidia are sometimes observed; they are club shaped and smooth walled and form along the hyphae.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Hawkins and Smidt, 2014
 Kidd et al., 2016, p. 126
 Rich et al., 2013
 Scher et al., 2013
 White et al., 2014

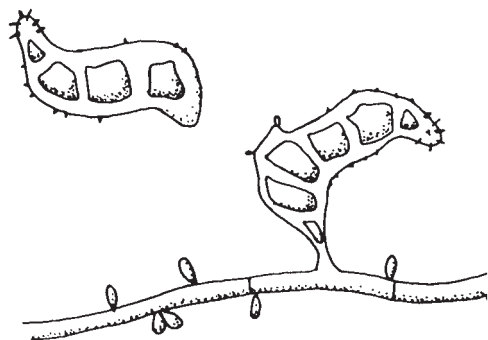
Microsporum canis var. *distortum*

PATHOGENICITY: Occasionally causes tinea capitis and tinea corporis. The patterns of infection are indistinguishable from those of *M. canis* var. *canis*.

RATE OF GROWTH: Moderately rapid; mature within 4–8 days.

COLONY MORPHOLOGY: Surface is fuzzy and flat with raised center; white to buff. Reverse is usually yellow.

MICROSCOPIC MORPHOLOGY: Septate hyphae. Macroconidia resemble those of *Microsporum canis* var. *canis* (p. 262) but are very bent and distorted and have fewer compartments; macroconidia are produced best on potato dextrose agar. Club-shaped microconidia are often abundant (an uncommon finding in *M. canis*).



For further information, see
Kidd et al., 2016, p. 128
Rebell and Taplin, 1970, p. 15

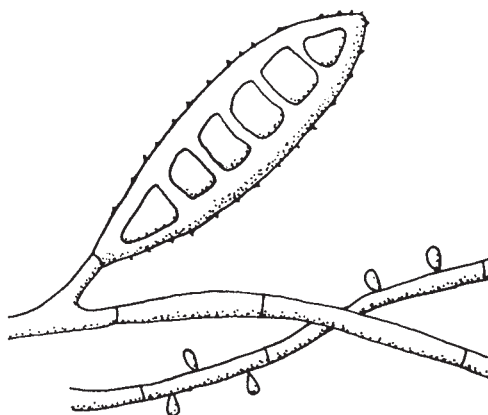
Microsporium cookei (also known as *Paraphyton cookei* and *Lophophyton cookei*)

PATHOGENICITY: This geophilic (recovered from soil) fungus is occasionally involved in infections in humans; it is not known to infect hair *in vivo*.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Surface is coarse, powdery; yellowish or dark tannish central area surrounded by thin, downy, white peripheral zone. Under the aerial mycelium is a characteristic deep grape-red pigment. Reverse is deep purplish red.

MICROSCOPIC MORPHOLOGY: Hyphae are septate and branched. Macroconidia are oval (10–15 × 30–50 µm), thick walled, and rough, with approximately five to eight cells. Thick walls serve to distinguish this species from reddish isolates of *Microsporium gypseum*. Club-shaped microconidia are usually abundant.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kane et al., 1997, p. 206
 Kidd et al., 2016, p. 149
 Kwon-Chung and Bennett, 1992, pp. 142–143
 Rebell and Taplin, 1970, p. 29
 Rippon, 1988, p. 249

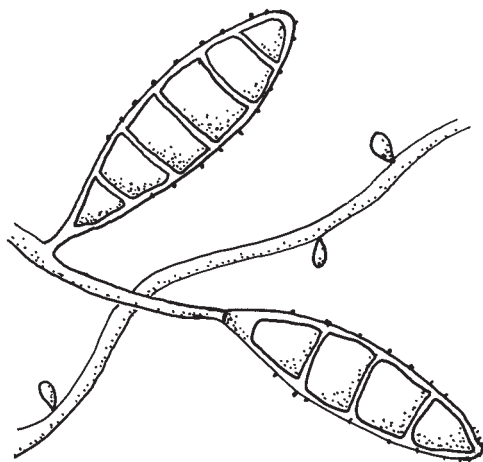
***Microsporium gypseum* complex** (synonymous with *Nannizzia gypsea*)*

PATHOGENICITY: Occasionally infects the scalp and skin on various parts of the body; infections are more common in lower animals than in humans.

RATE OF GROWTH: Moderately rapid; mature within 6 days.

COLONY MORPHOLOGY: Surface is flat and spreading and powdery to granular, developing an irregularly fringed border; it is buff at first, then tan to cinnamon brown. Colony often develops a sterile white hyphal border or cottony white center. Reverse may be yellow, orange-tan, brownish red, or purplish red in spots. (Color Plates 112 and 113.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Macroconidia (8–16 × 22–60 μm) appear in enormous numbers and are symmetric, rough, and relatively thin walled, with no more than six compartments. The ends are rounded, not pointed as in *Microsporium canis*. Microconidia, club shaped, are usually present along the hyphae.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
de Hoog et al., 2017
Kidd et al., 2016, p. 138

* *Nannizzia gypsea* has recently been proposed as the preferred nomenclature for *Microsporium gypseum*.

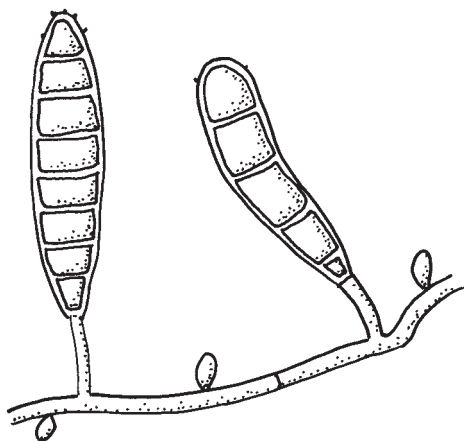
Microsporium gallinae (synonymous with *Lophophyton gallinae*)*

PATHOGENICITY: This zoophilic dermatophyte is a rare cause of tinea capitis and tinea corporis that may be seen as a cause of dermatophytosis in chickens or other fowl.

RATE OF GROWTH: Moderate; mature in 6–10 days.

COLONY MORPHOLOGY: Surface is slightly fluffy or satiny and white, becoming pinkish with age. Reverse is yellow at first and later has a red pigment that diffuses into the medium.

MICROSCOPIC MORPHOLOGY: Hyphae are septate. Macroconidia ($6-8 \times 15-50 \mu\text{m}$) have walls that are relatively thin and usually smooth but sometimes slightly rough at the tip; they contain 4–10 cells, are blunt tipped, and are often distinctively curved with a tapering base. Microconidia are usually abundant.



For further information, see
de Hoog et al., 2017
Kidd et al., 2016, p. 115

* *Lophophyton gallinae* has recently been proposed as the preferred nomenclature for *Microsporium gallinae*.

Microsporium nanum (synonymous with *Nannizzia nanum*)*

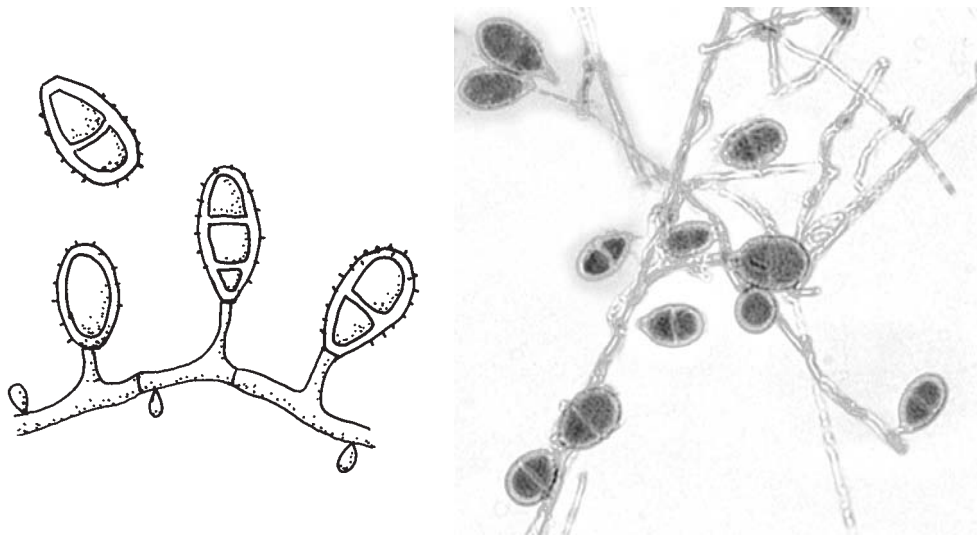
PATHOGENICITY: This zoophilic organism is a rare cause of dermatophytosis in humans; more common in pigs.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Surface is at first white and then yellowish, peachy, or beige; spread thin, downy or powdery, with fringed edges. Reverse is initially orange and later reddish brown. (Color Plates 114 and 115.)

MICROSCOPIC MORPHOLOGY: Septate hyphae; macroconidia ($4-8 \times 12-18 \mu\text{m}$) are rough, fairly thin walled (as in *Microsporium gypseum*), and egg shaped with truncate base, having one to three cells (usually two). Microconidia, club shaped and smooth walled, may also be present.

The short conidiophores, singly formed macroconidia with rough walls and no footlike attachment points, and presence of microconidia serve to distinguish this organism from *Trichothecium roseum* (p. 325).



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
de Hoog et al., 2017
Kidd et al., 2016, p. 139

* *Nannizzia nanum* has recently been proposed as the preferred nomenclature for *Microsporium nanum*.

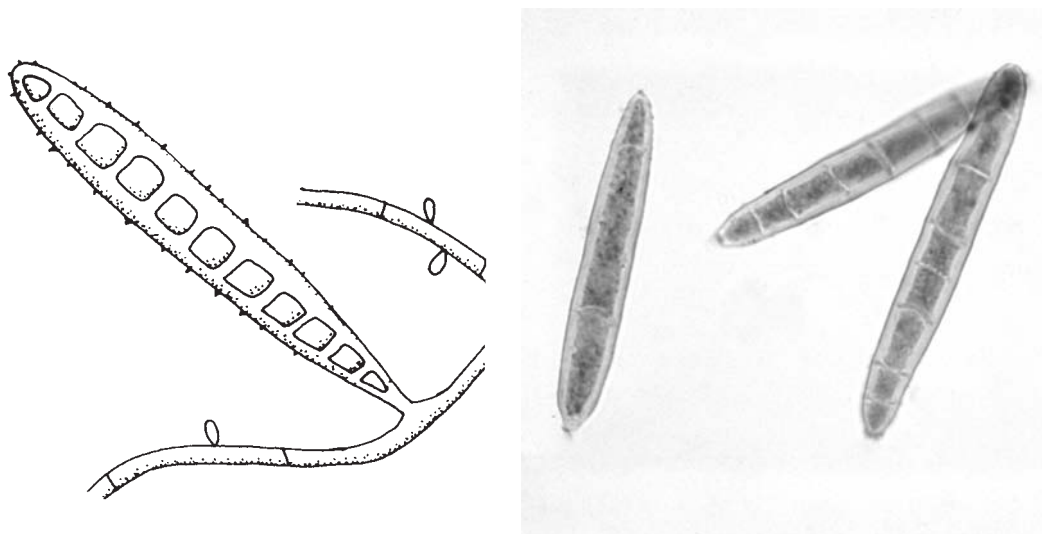
Microsporum vanbreuseghemii

PATHOGENICITY: This zoophilic organism is a rare cause of ringworm in humans and lower animals.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Surface is powdery or fluffy; cream, pink, or tan in color. Reverse is colorless or yellow to orange-tan.

MICROSCOPIC MORPHOLOGY: Hyphae are septate; macroconidia are long (10–12 × 58–62 μm), tapered, thick walled, and usually rough and spiny surfaced, with seven or more cells. The macroconidia are in abundance singly, laterally, or terminally. Microconidia are also present. Care must be taken not to confuse *M. vanbreuseghemii* with *Trichophyton ajelloi* (p. 281).



Courtesy of Glenn Roberts.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Microsporum ferrugineum

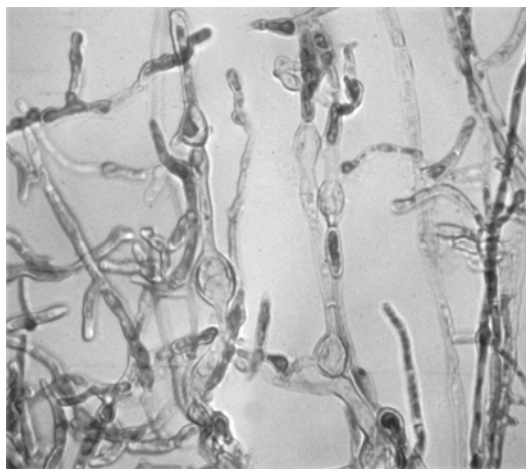
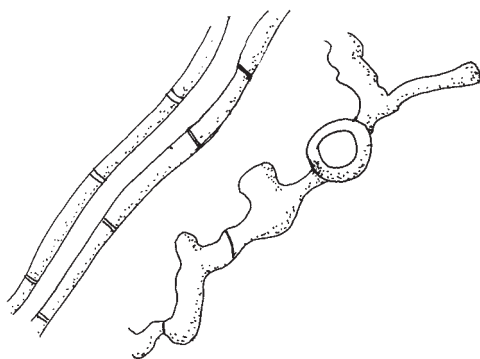
PATHOGENICITY: This species primarily causes tinea capitis in children. It occasionally causes tinea corporis and onychomycosis.

RATE OF GROWTH: Slow; mature in 12–20 days.

COLONY MORPHOLOGY: Isolates from the Far East have a surface that is usually yellow to rusty orange, smooth, waxy, and heaped; on repeated subculture, the pigment is often lost. Another colony type (typical in the Balkans) is also waxy, but white to pale yellow and flatter. Either form may develop a fine velvety overgrowth. Reverse is cream to brownish.

MICROSCOPIC MORPHOLOGY: Hyphae are septate; some are characteristically long and straight with prominent cross walls; these are called “bamboo” hyphae. Other hyphae are irregularly branched, clubbed, and fragmented and may have intercalary chlamydoconidia-like cells. Macroconidia, rarely produced, resemble those of *Microsporum canis* (p. 262) and *Microsporum cookei* (p. 264).

Note: The orange form of this organism may be differentiated from *Trichophyton soudanense* (p. 276), which it somewhat resembles, by the formation of light yellowish colonies on Lowenstein-Jensen medium; *T. soudanense* will form dark reddish-brown-to-blackish colonies.



Courtesy of Michael Rinaldi.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 129
Rich et al., 2013
Scher et al., 2013

Trichophyton mentagrophytes

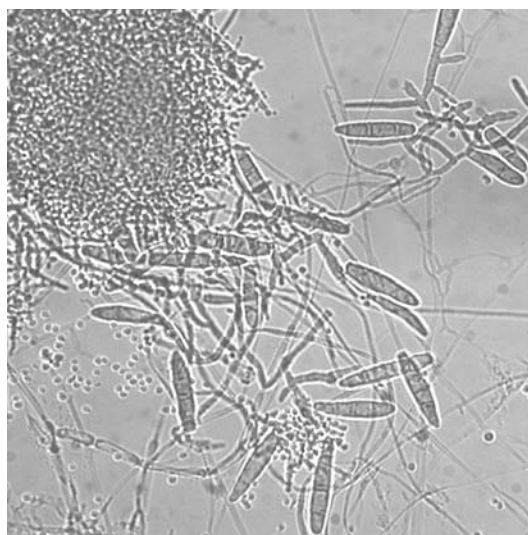
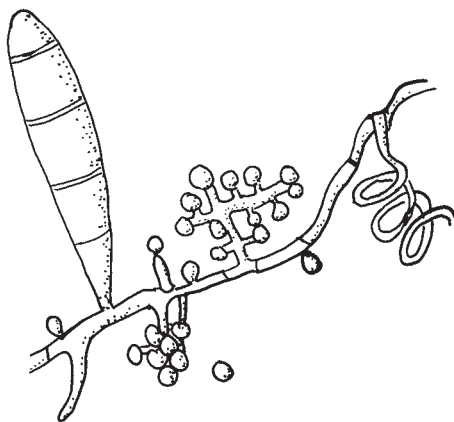
PATHOGENICITY: This zoophilic dermatophyte commonly invades all parts of the body surface, including hair and nails. It is a common cause of tinea pedis and onychomycosis. It also infects a wide range of domestic and agricultural animals.

RATE OF GROWTH: Moderate; mature in 6–10 days.

COLONY MORPHOLOGY: Varies greatly; surface may be buff and powdery or white and downy. May become pinkish or yellowish. Powdery form exhibits concentric and radial folds. Colonies rapidly develop a dense fluff with little or no conidiation. Reverse is usually brownish tan but may be colorless, yellow, or red. (Color Plates 116 and 117.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Microconidia in powdery cultures are very round (4–6 μm in diameter) and clustered on branched conidiophores or, in fluffy strains, are smaller, fewer in number, tear shaped, and more easily confused with those of *Trichophyton rubrum* (p. 272). Macroconidia (4–8 $\times$ 20–50 μm) are sometimes, but not always, present; they are cigar shaped and thin walled, have narrow attachments to hyphae, contain one to six cells, and are more readily found in young primary cultures 5–10 days old. Coiled spiral hyphae are often seen. Nodular bodies are seen in some strains.

See Table 23 (p. 271) to differentiate *T. mentagrophytes* from similar species of *Trichophyton*.



For further information, see

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Ilkit and Durdu, 2015

Kidd et al., 2016, pp. 203–204

Rich et al., 2013

Scher et al., 2013

TABLE 23 Differentiation of similar conidia-producing *Trichophyton* spp.^a

Organism	Growth on <i>Trichophyton</i> agars		Urease (7 days)	<i>In vitro</i> hair perforation	Red pigment on cornmeal with 1% dextrose	Growth at 37°C	Growth on <i>Trichophyton</i> agars ^b	
	No. 1 (casein base)	No. 4 (casein + thiamine)					No. 6 (NH ₄ NO ₃ base)	No. 7 (NH ₄ NO ₃ + histidine)
<i>T. mentagrophytes</i>	4+	4+	+	+	0	+	4+	2+
<i>T. rubrum</i>	4+	4+	0 or W	0	+	+	3+	4+
<i>T. tonsurans</i>	± or +	4+	+	0 ^V	0	+	±	±
<i>T. terrestre</i>	4+	4+	+	+	V	0	2+	2+
<i>T. megninii</i>	4+	2+	+ ^{V, c}	0		+	0	4+

^a Abbreviations: +, positive; 0, negative; W, weak; ±, trace; 4+, maximum growth; V, variable.^b As *T. megninii* is the only dermatophyte that requires histidine, *Trichophyton* agar no. 6 and 7 are used only when it is suspected.^c Use urea broth, not agar, to ensure positive result in 7 days with *T. megninii*.

*Trichophyton rubrum**

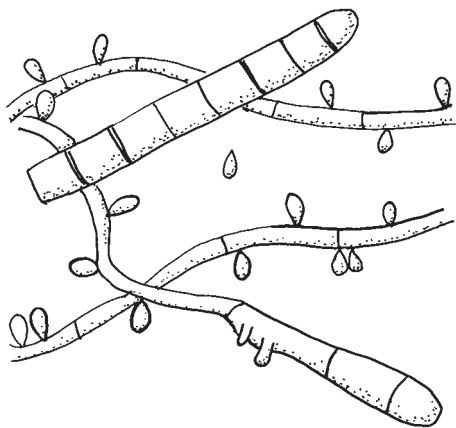
PATHOGENICITY: Infects the skin and nails and only rarely the beard, hair, or scalp. *T. rubrum* is one of the most commonly recognized causes of dermatophytosis and onychomycosis in humans worldwide. The most common sites of infection are the feet (tinea pedis) and the nails (onychomycosis).

RATE OF GROWTH: Moderately slow; mature within 14 days.

COLONY MORPHOLOGY: Surface is granular or fluffy, white to buff. Reverse is deep red or purplish; occasionally it is brown, yellow-orange, or even colorless. The pigment production is best seen on potato dextrose agar (p. 417) or cornmeal dextrose agar (p. 408). (Color Plates 118 and 119.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Tear-shaped microconidia ($2\text{--}3.5 \times 3\text{--}5.5 \mu\text{m}$) usually form singly all along the sides of the hyphae (not clustered like *Trichophyton mentagrophytes* [p. 270]). Macroconidia ($4\text{--}8 \times 40\text{--}60 \mu\text{m}$) may be abundant, rare, or absent; when present, they are long, narrow, and thin walled, with parallel sides (pencil-like), and have 4–10 cells. Macroconidia may form directly on ends of thick hyphae singly or in groups. Microconidia characteristically form directly on macroconidia. Arthroconidia tend to form from both hyphae and macroconidia. Granular cultures have more macroconidial formation and larger, rounder microconidia than the fluffy form.

See Table 23 (p. 271) for differentiation from similar species of *Trichophyton*.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, pp. 207–210
 Rich et al., 2013
 Scher et al., 2013

* Molecular studies have shown *T. rubrum* to be a complex of many species; the above description is for *T. rubrum sensu stricto*.

Trichophyton tonsurans

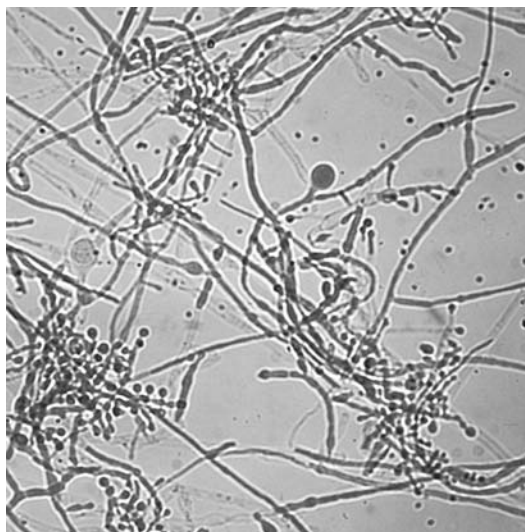
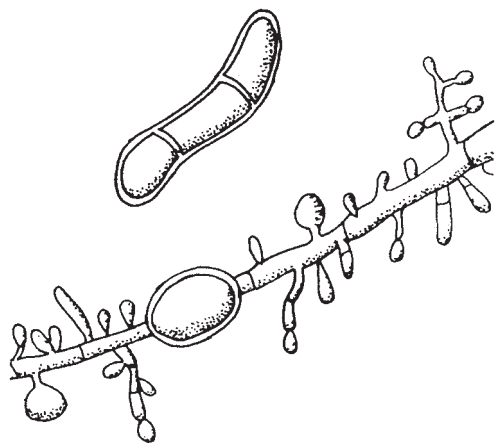
PATHOGENICITY: This is the principal etiologic agent of tinea capitis in the United States; it also infects the skin and nails. *T. tonsurans* causes tinea capitis principally in children who live in large urban areas.

RATE OF GROWTH: Moderately slow; mature in 12 days.

COLONY MORPHOLOGY: Highly variable. Surface may be white, grayish, yellow, rose, or brownish. Surface is usually suedelike, with many radial or concentric folds. Reverse is usually reddish brown (pigment may diffuse into the medium); sometimes it is yellow or colorless. (Color Plates 120, 121, and 122.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with many variably shaped microconidia along the hyphae or on short conidiophores that are perpendicular to the parent hyphae. Microconidia are usually teardrop or club shaped but may be elongate or enlarge to round “balloon” forms. Intercalary and terminal chlamydospores are common in older cultures. Macroconidia are rare, irregular in form, and a bit thick walled. The organism may have spiral coils and arthroconidia. This species has a partial requirement for thiamine.

See Table 23 (p. 271) for differentiation from similar species of *Trichophyton*.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Hawkins and Smidt, 2014
Kane et al., 1997, pp. 174–177
Kidd et al., 2016, pp. 213–214
Kwon-Chung and Bennett, 1992, pp. 148–149

Trichophyton terrestre (synonymous with *Arthroderma insingulare*)

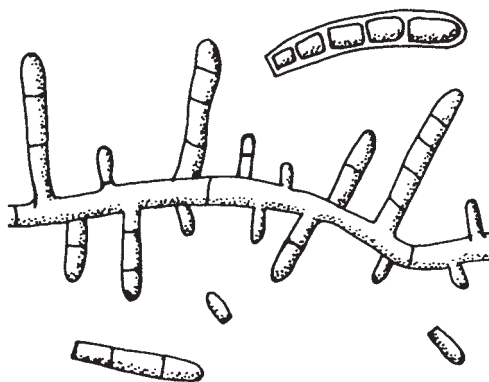
PATHOGENICITY: Not known to cause infection in humans but may be confused with other *Trichophyton* spp.

RATE OF GROWTH: Moderate; mature within 8 days. It does not grow at 35–37°C.

COLONY MORPHOLOGY: Surface is white to yellow and velvety or granular. Reverse is colorless, yellow, reddish, or brown. (Color Plates 123 and 124.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with club-shaped microconidia (often on short stalks) or characteristic larger, peg-shaped microconidia that usually exhibit transition forms to rather numerous smooth, thin-walled macroconidia (4–5 × 8–50 µm). The conidia often stain more intensely with lactophenol cotton blue than do the hyphae and are cut off on a relatively broad base.

See Table 23 (p. 271) for differentiation from similar species of *Trichophyton*.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kane et al., 1997, pp. 172–174
 Kidd et al., 2016, p. 9
 Rebell and Taplin, 1970, pp. 33–34

Trichophyton megninii

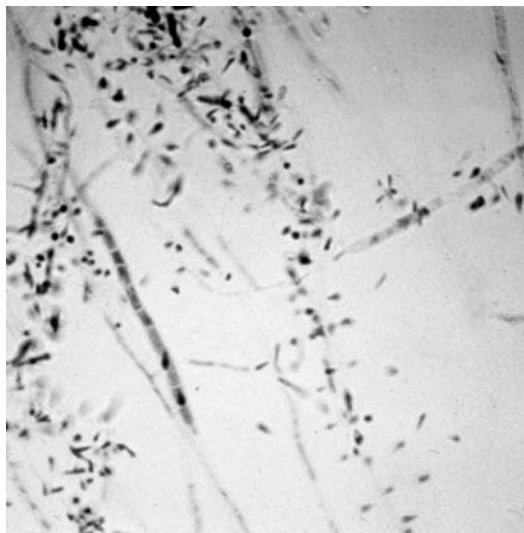
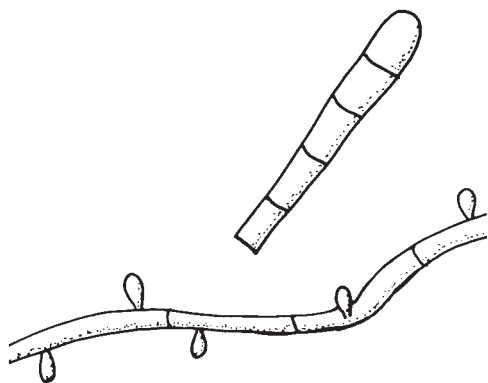
PATHOGENICITY: This anthropophilic organism primarily infects the beard, but it also infects the scalp and the skin on various parts of the body. Very rarely encountered in the Americas; it is endemic in parts of southern Europe and in north and east central Africa.

RATE OF GROWTH: Moderate; mature within 6–10 days.

COLONY MORPHOLOGY: Surface is suedelike, at first white and then pink to violet with widely spaced radial grooves. Reverse is red.

MICROSCOPIC MORPHOLOGY: Hyphae are septate, with teardrop-shaped microconidia along the sides. Macroconidia, infrequently produced, are long, narrow, thin walled, and pencil shaped. There is a close resemblance to *Trichophyton rubrum* (p. 272), but *T. megninii* differs by requiring histidine (in *Trichophyton* agar no. 7) and often giving a positive test for urease within 7 days.

See Table 23 (p. 271) for differentiation from similar species of *Trichophyton*.



Courtesy of Michael Rinaldi.

For further information, see
Kane et al., 1997, pp. 145–149
Kwon-Chung and Bennett, 1992, p. 151
Rippon, 1988, p. 263

*Trichophyton soudanense**

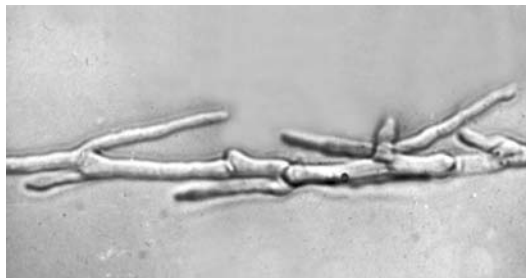
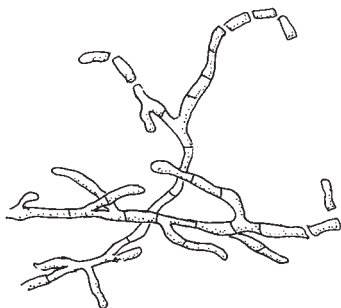
PATHOGENICITY: Primarily infects the scalp and hair as an endothrix infection and may spread to other parts of the body. It is endemic in Central and West Africa and has occasionally been reported in Europe, the United States, and South America.

RATE OF GROWTH: Slow; mature within 15 days. Growth factor requirements are variable.

COLONY MORPHOLOGY: Surface is yellow to orange, suedelike, and flat to folded, with a radiating fringe. Purplish red variants exist. Reverse is similar in color to the surface. (Color Plate 125.)

MICROSCOPIC MORPHOLOGY: Septate hyphae that often break up to form arthroconidia. Characteristically, branches form at both forward and backward angles to the parent hypha (i.e., in directions that are both the same as and opposite to that of the elongating hypha). This is known as “reflexive” branching; when adjacent branches point in opposite directions, they often give the appearance of barbed wire. The unique characteristic microscopic formations are most likely to be found in the radiating fringe of the colony. Teardrop-shaped microconidia may also form along the hyphae; no macroconidia are seen. Chlamydoconidia may develop in older cultures.

Note: The orange form of this organism may be differentiated from *Microsporum ferrugineum* (p. 269), which it somewhat resembles, by the formation of dark reddish-brown-to-blackish colonies on Lowenstein-Jensen medium; *M. ferrugineum* forms pale yellow colonies on this medium.



Courtesy of Stanley Rosenthal.

For further information, see
 Kane et al., 1997, pp. 168–172
 Kidd et al., 2016, p. 212
 Kwon-Chung and Bennett, 1992, p. 152
 Rippon, 1988, pp. 263–264

* Some molecular studies have found *T. soudanense* to be closely related to (and considered synonymous with) *Trichophyton violaceum*, while others have shown it to be more closely related to (and a subtype of) *Trichophyton rubrum*.

TABLE 24 Growth patterns of *Trichophyton* species on nutritional test media^a (p. 422)

Organism	Percent	Growth on <i>Trichophyton</i> agar no.						
		1	2	3	4	5	6	7
USUALLY NO CONIDIA ON ROUTINE MEDIA; INCUBATE <i>TRICHOPHYTON</i> AGARS AT 37°C								
<i>T. verrucosum</i>	84	0	±	4+	0			
	16	0	0	4+	4+			
<i>T. schoenleinii</i>		4+	4+	4+	4+			
<i>T. concentricum</i> ^b	50	4+	4+	4+	4+			
	50	2+	2+	4+	4+			
<i>T. violaceum</i> ^c		± or 1+			4+ (in 3 weeks)			

USUALLY PRODUCES MICROCONIDIA AND SOMETIMES MACROCONIDIA ON ROUTINE MEDIA; INCUBATE *TRICHOPHYTON* AGARS AT ROOM TEMPERATURE

<i>T. tonsurans</i>		± or 1+			4+			
<i>T. rubrum</i> ^d		4+			4+			
<i>T. mentagrophytes</i> ^d		4+			4+	4+		
<i>T. equinum</i> ^e		0				4+		
<i>T. megninii</i> ^f		4+			2+		0	4+
<i>T. terrestre</i> ^g		4+			4+			

^a Abbreviations: 1, casein agar base (vitamin free);

2, casein + inositol;

3, casein × inositol and thiamine;

4, casein + thiamine;

5, casein + nicotinic acid;

6, ammonium nitrate agar base;

7, ammonium nitrate + histidine;

0, no growth;

+, growth;

1+, ¼ as much growth as 4+;

2+, ½ as much growth as 4+;

3+, ¾ as much growth as 4+;

4+, maximum growth.

^b For more information, see Rebell and Taplin, 1970 (p. 61), or Kane et al., 1997 (pp. 135–136).

^c Usually has distinct pigment on primary isolation.

^d Differentiation of *T. rubrum* and *T. mentagrophytes* is by morphology, urease test, *in vitro* hair perforation test, and pigment production on cornmeal dextrose agar. See Table 23 (p. 271).

^e Commonly found in horses; has been confused with *T. mentagrophytes*, but *T. equinum* usually requires nicotinic acid. For more information, see Rebell and Taplin, 1970 (p. 45), or Kane et al., 1997 (pp. 136–138).

^f No other dermatophyte shows this regular requirement for histidine.

^g Not known to cause infections, but may be confused with some pathogenic species. See Table 23 (p. 271).

Trichophyton schoenleinii

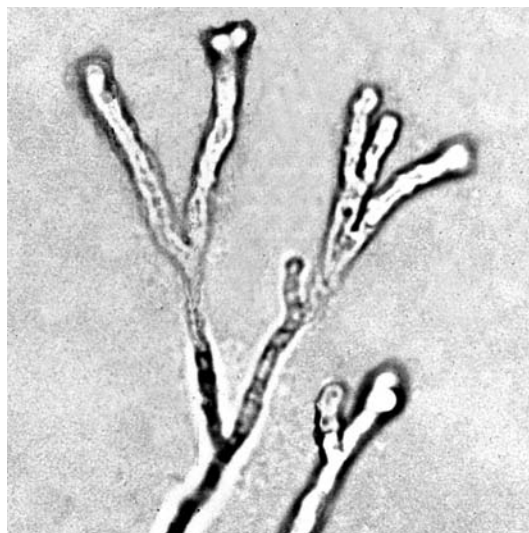
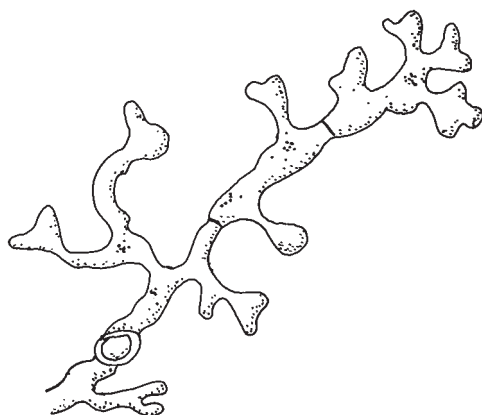
PATHOGENICITY: Causes favus, a severe, chronic, scarring scalp infection that results in permanent hair loss; sometimes infects the nails and skin.

RATE OF GROWTH: Slow; mature within 15 days.

COLONY MORPHOLOGY: Colony is whitish, waxy, or slightly downy; heaped or folded; primary isolates are sometimes yeastlike. Growth is often submerged and splits the agar medium. Reverse is colorless or pale yellowish orange to tan.

MICROSCOPIC MORPHOLOGY: Hyphae are septate, highly irregular, and knobby. The subsurface hyphae usually form characteristic antlerlike branching structures commonly called favic chandeliers; they have swollen tips that resemble nail heads. Chlamydoconidia are numerous. Microconidia and macroconidia are absent. Initial growth from clinical specimen may resemble yeast both macroscopically and microscopically.

See [Table 24 \(p. 277\)](#) for growth pattern on *Trichophyton* agars.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kane et al., 1997, pp. 164–166
 Kidd et al., 2016, p. 211
 Kwon-Chung and Bennett, 1992, pp. 149–150
 Rippon, 1988, pp. 260–262

Trichophyton verrucosum

PATHOGENICITY: This zoophilic pathogen, which is usually contracted from cattle, infects the scalp, beard, nails, and skin on various parts of the body.

RATE OF GROWTH: Slow; mature in 14–21 days. Unlike other dermatophytes, this fungus grows best at 37°C.

COLONY MORPHOLOGY: Usually small, heaped, and buttonlike but sometimes flat. Texture skinlike, waxy, or slightly downy. Usually white, but can be gray or yellow. Reverse varies from nonpigmented to yellow.

MICROSCOPIC MORPHOLOGY: On routine mycology media at 37°C, forms hyphae with many intercalary chlamydoconidia (typically in chains) and some antlerlike branches. On enriched media with thiamine, produces many small, delicate, single microconidia and occasional long, thin, irregular macroconidia shaped like string beans or rats' tails.

See Table 24 (p. 277) for growth patterns on *Trichophyton* agars. This species requires thiamine and usually inositol as well.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kane et al., 1997, pp. 177–181
Kidd et al., 2016, p. 215–216
Kwon-Chung and Bennett, 1992, pp. 148–149
Rippon, 1988, pp. 259–260

Trichophyton violaceum (synonymous with *Trichophyton yaoundei*)

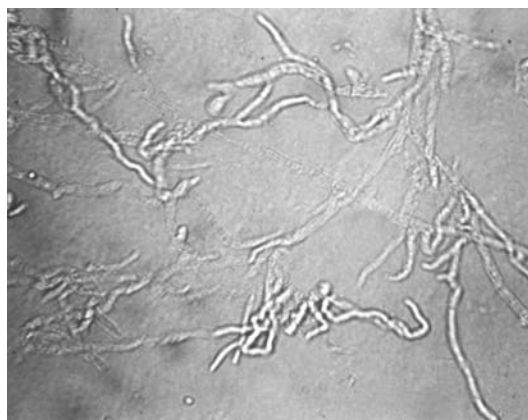
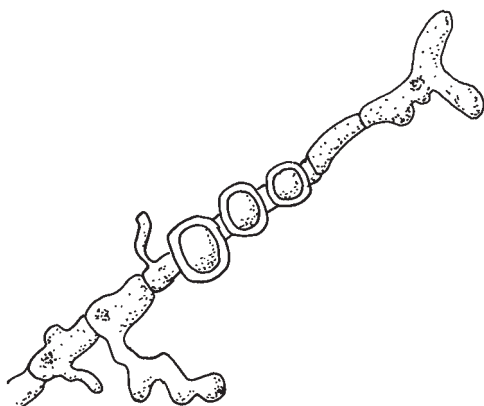
PATHOGENICITY: This endothrix anthropophilic organism most commonly infects the scalp and hair but also causes infection in skin and nails. It is one of the most common causes of tinea capitis in West Asia and North Africa.

RATE OF GROWTH: Slow; mature in 14–21 days.

COLONY MORPHOLOGY: Original cultures are waxy, wrinkled, heaped, and deep purplish red. Subcultures are more downy, and they decrease in color. Reverse is lavender to purple.

MICROSCOPIC MORPHOLOGY: Hyphae are tangled, branched, irregular, and granular, with intercalary chlamydoconidia. Microconidia and macroconidia are not usually seen on routine media, but a few may form on thiamine-enriched media.

See Table 24 (p. 277) for growth pattern on *Trichophyton* agars. This species has a partial requirement for thiamine.



Courtesy of Michael Rinaldi.

For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 217
 Kwon-Chung and Bennett, 1992, pp. 150–151
 Rebell and Taplin, 1970, p. 54
 Rippon, 1988, p. 259

* Some mycologists consider *Trichophyton soudanense* to be a form of *T. violaceum*.

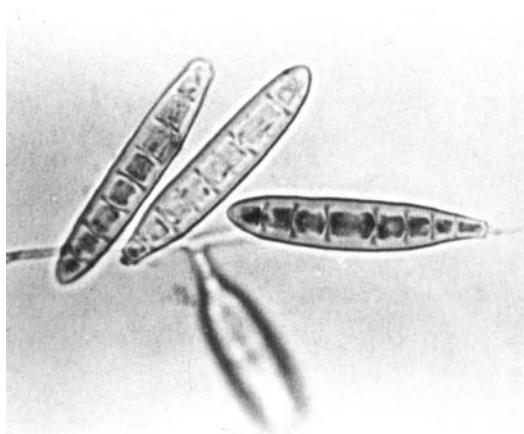
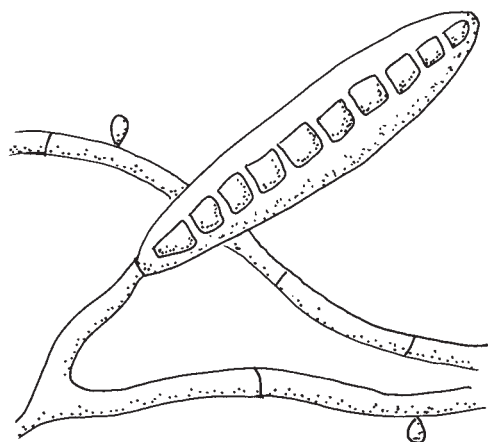
Trichophyton ajelloi (synonymous with *Arthroderma uncinatum*)

PATHOGENICITY: Has only rarely been reported as a possible cause of infections in humans.

RATE OF GROWTH: Moderate; mature within 7 days.

COLONY MORPHOLOGY: Surface is cream to orange-tan, rather powdery, and flat or folded. Reverse may be colorless or have a reddish purple or bluish black pigment that diffuses into the medium.

MICROSCOPIC MORPHOLOGY: Hyphae are septate with many macroconidia that are long (5–10 × 20–65 μm) and cigar shaped or cylindrical with tapering ends. They are smooth surfaced and moderately thick walled and contain 5–12 cells. Microconidia are sparse in most isolates but may be absent in others. Care must be taken not to confuse this organism with *Microsporum vanbreuseghemii* (p. 268) or *Epidermophyton floccosum* (p. 282).



From Murray et al. (ed.), 1999, *Manual of Clinical Microbiology*, 7th ed., chapter 100.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kane et al., 1997, pp. 133–135
Kidd et al., 2016, p. 10
Rebell and Taplin, 1970, p. 31
Rippon, 1988, pp. 263–265

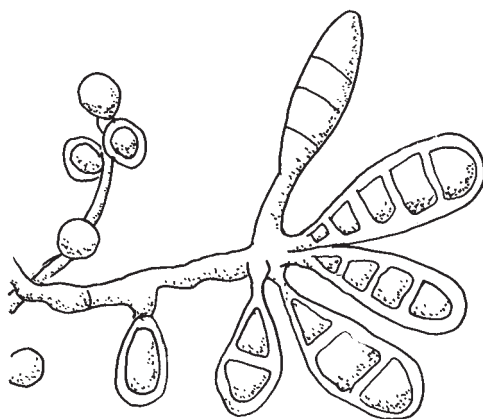
Epidermophyton floccosum

PATHOGENICITY: *Epidermophyton floccosum* causes tinea corporis, tinea cruris, and onychomycosis. It does not infect hair.

RATE OF GROWTH: Moderate; mature within 10 days.

COLONY MORPHOLOGY: Surface is brownish yellow to olive-gray or khaki; it is at first lumpy and sparse and then folded in the center and grooved radially, becoming velvety. After several weeks, fluffy white sterile mycelium covers the colony. Reverse is orange to brownish, sometimes with a thin yellow border. (Color Plates 126 and 127.)

MICROSCOPIC MORPHOLOGY: Septate hyphae; no microconidia. Macroconidia (7–12 × 20–40 μm), seen best in young cultures, are smooth, both thin and slightly thick walled, and club shaped with rounded ends; they contain two to six cells and are found singly or in characteristic clusters. With age, macroconidia often transform into chlamydoconidia; it is therefore imperative to observe the microscopic morphology before the culture passes its prime. Arthroconidia are also commonly formed with age. Microconidia are not formed. Older cultures commonly develop white sterile hyphae. Stock cultures may be best maintained on Sabouraud dextrose agar containing 3–5% sodium chloride.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 84
 Rich et al., 2013
 Scher et al., 2013

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Introduction to Hyaline Hyphomycetes

This section contains the fungi that have not been discussed earlier in this guide. These moulds have colorless, septate hyphae and produce conidia that may be colorless or pigmented. Their colony surfaces are white, gray, tan, yellow, pink, or green; the reverse is white or lightly pigmented. Some of these organisms are obligate pathogens (e.g., *Coccidioides immitis* and *Coccidioides posadasii*), but most of them are opportunistic and cause disease only in immunocompromised patients.

Fungi were previously categorized as pathogens or saprophytes; however, organisms that were once considered to be saprobic have been identified as important pathogens in immunocompromised patients. Almost any fungus isolated from a clinical specimen might be an etiologic agent of infection in a predisposed individual. All fungal isolates should be identified; most of those commonly considered as saprobic are identified in many laboratories only to genus level, as it often requires additional expertise or molecular methods to differentiate the species.

In the medical laboratory, the saprophytic fungi are frequently encountered as contaminants. The source of the organism may be the transport container, the medium, or the laboratory environment, but may also be the result of specimen handling. Every effort should be made to instruct clinicians on proper techniques for specimen collection.

***Coccidioides* spp.**

What was previously known only as *Coccidioides immitis* has been found to consist of two separate species, *C. immitis* and *C. posadasii*. *C. immitis* is primarily located in the San Joaquin Valley of southern California and has occasionally been recovered in Mexico; *C. posadasii* is more widespread, found in areas of endemicity throughout the Americas (but seldom in the San Joaquin area). Their only known phenotypic difference is the ability of *C. immitis* to grow more rapidly than *C. posadasii* in high salt concentrations, but overlap in growth rates renders this property nondiagnostic. The confirmatory tests mentioned below do not differentiate between the two species. More-advanced molecular methods are required for identification to species level, but it is not usually necessary, as there appears to be no difference in their clinical presentation or response to therapy.

PATHOGENICITY: Causes coccidioidomycosis, which is a highly infectious process that may be an acute but benign, self-limiting respiratory disease or a chronic, malignant, sometimes fatal infection involving the skin, bone, joints, lymph nodes, adrenals, and central nervous system. It is endemic in the arid southwestern United States and in dry regions of Mexico, Central America, and South America. These areas are known as the Sonoran life zones, which are characterized by arid climate punctuated by occasional rainfall; this type of climate allows for the life cycle of *Coccidioides* spp. to be completed between a hyphal stage in the soil, acquisition of arthroconidia by a host, and transformation to spherules in mammalian tissue.

RATE OF GROWTH: Moderate; mature within 10 days. Growth occurs in 3–5 days, but production of arthroconidia may take 1–2 weeks.

COLONY MORPHOLOGY: There may be great variation in colony morphology. On Sabouraud dextrose agar at 25 or 37°C, the colony often is at first moist, grayish, and membranous and soon develops a white, cottony aerial mycelium, which becomes gray or tan to brown with age; may also be pinkish or yellow. Reverse is white to gray, sometimes yellow or brownish. Only when grown on special Converse medium with increased CO₂ at 37–40°C is the spherule, or tissue phase, formed *in vitro* (this is not routinely performed in clinical laboratories).

MICROSCOPIC MORPHOLOGY: Cultures exhibit coarse, septate, branched hyphae that produce thick-walled, barrel-shaped arthroconidia (3–4 × 3–6 μm) that alternate with empty cells (disjuncter cells). The walls of the empty cells break and are characteristically present on either end of the freed arthroconidia. Racquet hyphae are formed in young colonies and can serve as a sign, before development of arthroconidia, that *Coccidioides* may be present. Careful microscopic examination should prevent confusion with *Geotrichum candidum* (p. 166). *Coccidioides* is more likely to be confused with *Malbranchea* (p. 289), which also forms alternating arthroconidia along the hyphae but has a more narrow, delicate appearance.

See Table 25 (p. 288) for differential characteristics of fungi in which arthroconidia predominate.

To confirm the identity of *Coccidioides* and definitively differentiate it from organisms that it may closely resemble, it is necessary to perform one of the following

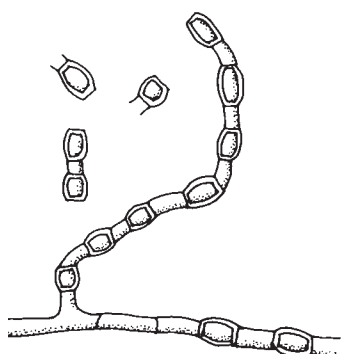
Coccidioides spp. (continued)

tests: (i) specific DNA probe (commercially available; be aware that isolates killed by suspension in formaldehyde will yield false-negative reactions with the AccuProbe [Hologic Inc., Marlborough, MA] system, while heat-killed isolates remain positive), (ii) immunodiffusion test for exoantigen (may be performed by a few reference laboratories), (iii) cultivation of spherules in special synthetic Converse medium in increased CO₂ at 37–40°C, or (iv) animal inoculation for *in vivo* production of spherules (seldom performed).

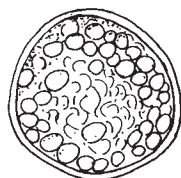
In tissues or body fluids, *Coccidioides* exists as large, round, thick-walled spherules (10–80 µm in diameter), which contain endospores (2–5 µm in diameter). Immature nonendospore-forming cells may resemble nonbudding forms of *Blastomyces dermatitidis* (p. 172). When cultured on routine media, whether incubated at 25, 30, or 37°C, the organism is filamentous (for that reason, it is not placed with the thermally dimorphic fungi in this guide).

Note: Because the arthroconidia are highly infectious, the cultures must be handled with great care and grown in tubes only, not petri plates. The tubed growth should be submerged under sterile water before being handled. A biological safety cabinet must be used. Slide cultures should NOT be made. The characteristic arthroconidia can be readily seen with a wet prep.

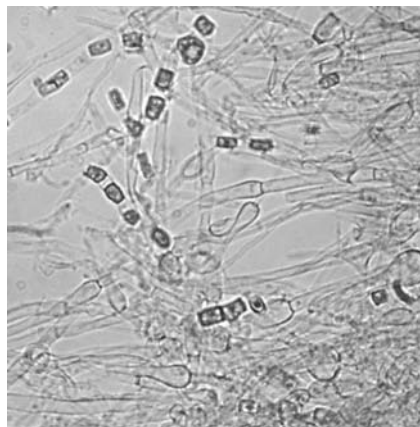
In 2012, *Coccidioides* spp. were removed from the list of select agents of bioterrorism in the United States. Select agents regulations can be found at <http://www.selectagents.gov>.



Cultured at 25 or 37°C.



In tissue (spherule).



For further information, see

Anaissie et al. (ed.), 2009, pp. 363–364, 576

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

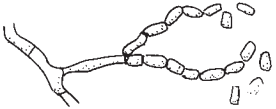
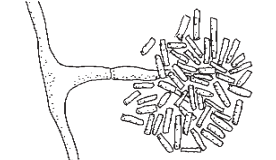
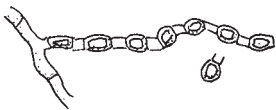
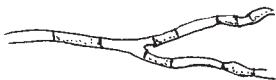
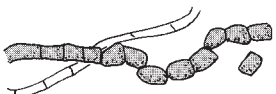
Fisher et al., 2002

Galgiani et al., 2016

Kidd et al., 2016, pp. 60–61

Nguyen et al., 2013

TABLE 25 Differential characteristics of fungi in which arthroconidia predominate^a

Genus	Dark pigment	Pseudohyphae	Conidiophores	Alternating arthroconidia ^b	Arthroconidia barrel shaped	Blastoconidia	Illustration of microscopic morphology
<i>Geotrichum</i> (p. 166)	0	0	0	0	0	0	
<i>Trichosporon</i> (p. 163)	0	+	0	0	0	+	
<i>Arthrographis</i> (p. 291)	0	0	+	0	0	+ ^v	
<i>Hormographiella</i> (p. 292)	0	0	+	0	0	0	
<i>Geomyces</i> (p. 290)	0	0	+	+	+	0	
<i>Coccidioides</i> (p. 287)	0	0	0	+	+	0	
<i>Malbranchea</i> (p. 289)	0	0	0	+	0	0	
<i>Neoscytalidium</i> (p. 238)	+ ^v	0	0	0	+ ^v	0	

^a Abbreviations: +, positive; 0, negative; V, variable.^b Empty cells appear between the arthroconidia.

Malbranchea spp.

PATHOGENICITY: Commonly considered a contaminant. At least one case of sinusitis has been reported.

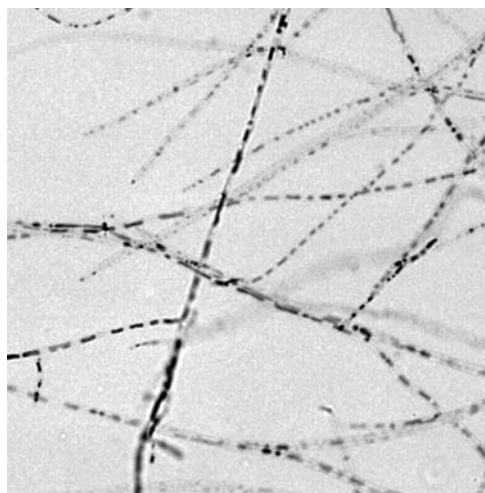
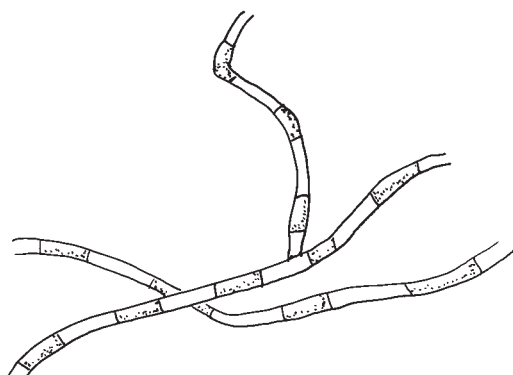
RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: Surface may be white, yellow, beige, orange, pinkish, or brownish; texture is granular, powdery, or woolly. Reverse is light. (Color Plates 130 and 131.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, hyaline; no conidiophores are formed. Straight or curved arthroconidia alternating with empty cells develop in the hyphae. The arthroconidia may be of various lengths but are the same width as the fairly narrow hypha (usually $<3\ \mu\text{m}$). Arthroconidia are released by breakage of the empty cells, and a portion of the adjacent empty cell often remains attached to separated arthroconidia. The arthroconidia are not swollen and not thick walled, differentiating them from those of *Coccidioides* (p. 287). In its sexual state (which is rarely seen in routine culture), large, round, asci-producing fruiting bodies (gymnothecia) are formed; they have no opening and are surrounded by loosely organized hyphae. One species of *Malbranchea* forms large, round, dark masses of cells (sclerotia).

If the identification is questionable, and there is suspicion that the isolate could be *Coccidioides*, safety precautions must be diligently observed, wet preps should be made rather than slide cultures, and an exoantigen test or nucleic acid probe test specific for *Coccidioides* spp. should be performed.

See Table 25 (p. 288) for differential characteristics of fungi in which arthroconidia predominate.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4; Kane et al., 1997, pp. 306–309

Geomyces pannorum

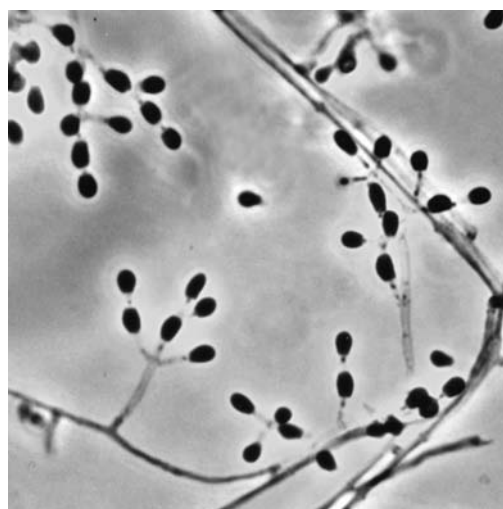
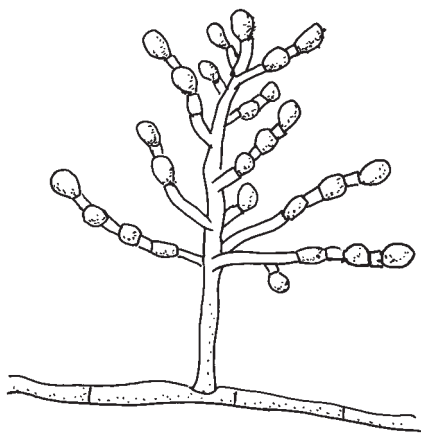
PATHOGENICITY: Commonly considered a contaminant; encountered in specimens of skin and nails; only rarely reported as an etiologic agent of infection in humans. There are rare reports of it causing cutaneous infection in dogs. *Geomyces destructans* has been identified as a cause of white nose disease in bats, which has caused widespread decimation of bat populations in the eastern United States.

RATE OF GROWTH: Moderate to slow; mature in 10–21 days. Grows well at 5–25°C; no growth at 37°C. Grows on media with cycloheximide.

COLONY MORPHOLOGY: Surface is white, pale yellow, gray, or tan; usually cottony or powdery. May be flat or elevated in the center; radial grooves sometimes form. Reverse can be colorless, but yellow is more typical, and a yellow or tan pigment may diffuse into the agar.

MICROSCOPIC MORPHOLOGY: Hyphae are septate. Conidiophores are erect, narrow, and measure 10–100 μm long; branches form in whorls around the central structure. Conidia (2–4 $\times$ 2–6 μm) are smooth or rough and form at the tips of the branches and also in intercalary positions separated by short empty spaces to create chains of two to four alternating, barrel-shaped arthroconidia. Conidia may also form along the sides of the conidiophore. Terminal and lateral conidia are somewhat clavate, having a rounded apex and truncate (cut-off, flattened) base.

See [Table 25](#) (p. 288) for differential characteristics of fungi in which arthroconidia predominate.



Courtesy of Lynne Sigler.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Gianni et al., 2003

Arthrographis kalrae

PATHOGENICITY: Reported rarely as a cause of mycetoma, septic arthritis, pulmonary infection, fungemia, and keratitis, as well as onychomycosis. It also has been isolated from various sites with questionable etiologic significance. It is commonly considered a contaminant in skin and nails.

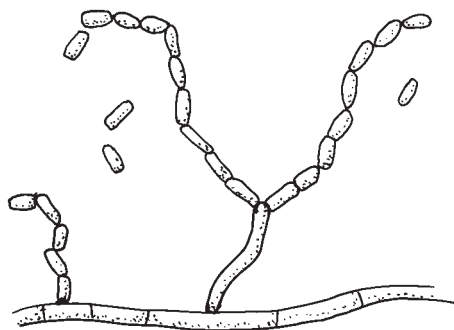
RATE OF GROWTH: Slow at 25–30°C; mature in 10–21 days. Growth is enhanced at 37°C; also grows at 45°C. Grows on media containing cycloheximide. *Arthrographis cuboidea* is a rapid grower, reaching maturity in 3–5 days.

COLONY MORPHOLOGY: Surface at first smooth and yeastlike, later velvety; cream to pale yellow or tan. Reverse pale yellow, may become tan. (Color Plate 132.)

MICROSCOPIC MORPHOLOGY: Yeastlike cells seen on early growth; later hyphae are septate and colorless. Conidiophores, sometimes in bundles, are simple or branched in a treelike fashion; they produce chains of consecutive arthroconidia that are mostly rectangular, some oval ($2\text{--}3.5 \times 3\text{--}7 \mu\text{m}$). Intercalary arthroconidia also form. Round blastoconidia ($3.5\text{--}5.5 \mu\text{m}$ in diameter) may develop directly on the hyphae (most commonly on submerged hyphae).

See Table 25 (p. 288) for differential characteristics of fungi in which arthroconidia predominate.

Note: *A. cuboidea* differs from *A. kalrae* by growing rapidly (3–5 days) at 25–30°C but not growing at 45°C and by producing a pink-to-lavender pigment at 35–37°C that may form slowly and diffuse into the medium. It is not known to cause infection.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Sigler and Carmichael, 1983

Hormographiella aspergillata

PATHOGENICITY: Has only occasionally been reported in immunocompromised patients to cause fatal pulmonary infections, cases of endocarditis following valve replacement, and skin and eye infections.

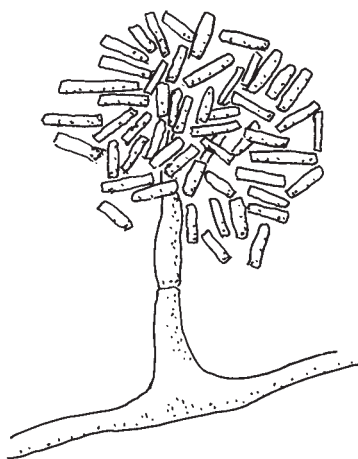
RATE OF GROWTH: Rapid; mature within 4 days. Growth is inhibited by cycloheximide.

COLONY MORPHOLOGY: Surface is white to cream colored, may become tan. Texture is dense, suedelike with cottony tufts; the edges are irregular. Reverse is pale.

MICROSCOPIC MORPHOLOGY: Hyphae septate, 2–5 μm wide. Conidiophores are broad based, septate, and may be branched or unbranched; at the apex or along the sides are fertile points from which are produced short hyphae that break at their septa, forming clusters of thin-walled, rectangular arthroconidia (1.5–3.0 $\times$ 2.5–6.5 μm). The arthroconidia are much narrower than the conidiophores; they are cylindrical, most with flat ends, but the terminal cells are usually rounded at their distal end.

See Table 25 (p. 288) for differential characteristics of fungi in which arthroconidia predominate.

Note: Rapid growth and susceptibility to cycloheximide further differentiate this organism from *Arthrographis kalrae*.



For further information, see

Conen et al., 2011

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Kenyon et al., 2013

Ong et al., 2015

The genus *Aspergillus**

PATHOGENICITY: Members of the genus *Aspergillus* cause a group of diseases known as aspergillosis; the diseases may be in the forms of invasive infection, colonization, toxicoses, or allergy. The organisms are opportunistic invaders, the most common moulds to infect various sites in patients with lowered resistance due to neutropenia and/or treatment with high-dose corticosteroids or immunomodulatory drugs. *Aspergillus* species are the most common causes of acute fungal pneumonia and sinus infection in immunocompromised patients with neutropenia, iatrogenic immunosuppression, or following transplantation. Acute pulmonary aspergillosis may be complicated by dissemination to multiple sites including brain, liver, and bone. Chronic pulmonary aspergillosis may develop in less immunocompromised patients as a progressive necrotizing cavitory process or as an aspergilloma in a preexisting lung cavity. *Aspergillus* spp. may also cause allergic bronchopulmonary aspergillosis, particularly in patients with cystic fibrosis. *Aspergillus* keratitis is an important worldwide cause of mycotic corneal infection. *Aspergillus flavus* in agriculture produces aflatoxin, which is a potent environmental carcinogen and hepatotoxic agent. *Aspergillus* spp. are widespread in the environment and are commonly found as contaminants in cultures. Approximately 180 species of *Aspergillus* are known, but less than a quarter of them have been found to cause human disease. The species included on the following pages are the most commonly encountered in clinical specimens.

RATE OF GROWTH: Usually rapid; mature within 3 days; some species are slower growing.

COLONY MORPHOLOGY: Surface is at first white and then any shade of green, yellow, orange, brown, or black, depending on species. Texture is velvety or cottony. Reverse is usually white, golden, or brown. (Color Plates 133 to 147.)

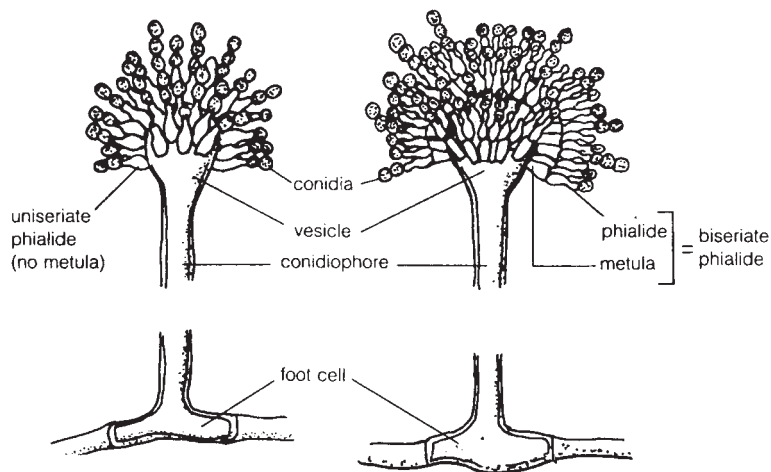
MICROSCOPIC MORPHOLOGY: Hyphae are septate (2.5–8.0 μm in diameter); an unbranched conidiophore arises from a specialized foot cell. The conidiophore is enlarged at the tip, forming a swollen vesicle. Vesicles are completely or partially covered with flask-shaped phialides (formerly referred to as sterigmata), which may develop directly on the vesicle (uniseriate form) or be supported by a cell known as a metula (biseriate form). The phialides produce chains of mostly round, sometimes rough conidia (2–8 μm in diameter).

See Table 26 (p. 299) for differentiation of species most commonly encountered in the clinical laboratory.

(continued on following page)

* Each *Aspergillus* species described on the following pages may in fact be a species complex, i.e., a group of organisms that are morphologically indistinguishable and require molecular testing if precise species identification is needed.

The genus *Aspergillus* (continued)



For further information, see

Anaissie et al. (ed.), 2009, pp. 271–296, 561–570
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, pp. 12–25
 Kousha et al., 2011
 Kwon-Chung and Bennett, 1992, pp. 201–247
 Patterson et al., 2016
 Segal, 2009
 van der Linden et al., 2013

Aspergillus fumigatus complex

(For general information about the genus *Aspergillus*, see [p. 293](#).)

PATHOGENICITY: It is the most common species of *Aspergillus* to cause invasive pulmonary aspergillosis, disseminated aspergillosis, allergic bronchopulmonary aspergillosis, fungal sinusitis, and mycotic keratitis. The organism is usually susceptible to antifungal triazoles and amphotericin B, which are considered first-line therapies for treatment of aspergillosis. However, recent studies indicate the emergence of resistance of *A. fumigatus* to triazoles.

RATE OF GROWTH: Rapid; mature in 3 days. Differs from the other common *Aspergillus* species by its ability to grow at 45°C.

COLONY MORPHOLOGY: Surface is velvety or powdery, various shades of green at prime with a narrow white border; colony turns dark gray with age. Reverse is white to tan. ([Color Plates 133](#) and [134](#).)

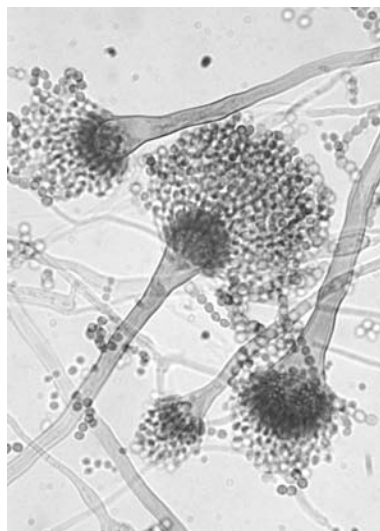
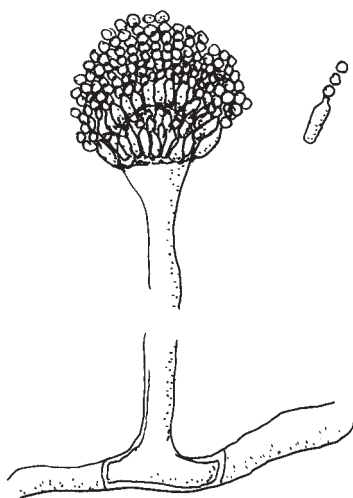
MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, relatively short (usually <300 µm long), and 5–10 µm in diameter. The phialides are uniseriate, close together (compact), forming only on the upper two-thirds of the vesicle, parallel to the axis of the conidiophore (known as columnar formation). Conidia are round, smooth, or slightly rough and 2–3.5 µm in diameter.

See [Table 26 \(p. 299\)](#) for differential characteristics of the most commonly encountered *Aspergillus* species.

Note: *Aspergillus lentulus*, which also causes pulmonary aspergillosis, phenotypically resembles *A. fumigatus*; however, it is slower sporulating, tends to be more resistant, does not grow well at 48°C (*A. fumigatus* does so), and occurs uncommonly among clinical isolates. *A. lentulus* is best distinguished from *A. fumigatus* by molecular methods.

(continued on following page)

***Aspergillus fumigatus* complex** (continued)



For further information, see

Anaissie et al. (ed.), 2009, pp. 271–296, 561–570
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, pp. 16–20
Kosmidis and Denning, 2015
Latgé and Steinbach, 2009
McCarthy et al., 2017
Raper and Fennell, 1973, pp. 238–268
Verweij et al., 2016

Aspergillus niger complex

(For general information about the genus *Aspergillus*, see [p. 293](#).)

PATHOGENICITY: Ubiquitous in nature. Most commonly isolated from the external ear, causing otomycosis; also a frequent agent of aspergilloma (fungus balls) in preexisting pulmonary cavities. It occasionally causes acute and chronic invasive pulmonary and disseminated disease.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface is black with white border; thallus is deep, visibly composed of long, white to cream, erect hyphae with clusters of black conidia at the apices. Reverse white to cream. ([Color Plates 135](#) and [136](#).)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Conidiophores long (400–3,000 × 12–17 μm), smooth, may be brownish near top. Vesicles 30–75 μm in diameter. Phialides radiate around entire vesicle and are biseriate, with the metulae twice as long as the phialides. Conidia are 3.5–4.5 μm in diameter, rough, and dark.

See [Table 26 \(p. 299\)](#) for differentiation of species most commonly encountered in the clinical laboratory.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 23
Raper and Fennell, 1973, pp. 293–344

Aspergillus flavus complex

(For general information about the genus *Aspergillus*, see [p. 293](#).)

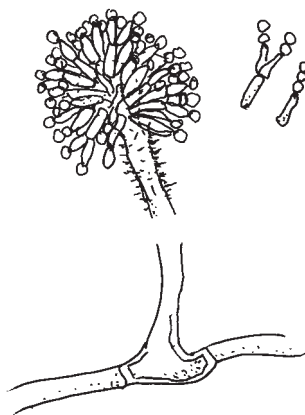
PATHOGENICITY: It is the second most common species of *Aspergillus* to cause invasive aspergillosis. *Aspergillus flavus* has a stronger propensity among the *Aspergillus* species to cause invasive sinus disease. It also is the most common species causing invasive sinusitis in immunocompetent patients in the area of Sudan and in the Middle East. It is the most commonly reported food-borne fungus; many of the isolates produce aflatoxins. Aflatoxins constitute a group of highly potent carcinogens and hepatotoxins that are formed in stored grains, corn, and legumes that are subsequently ingested by humans and farm animals.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface yellow-green to olive, often with specks of yellow; white border may be present. Texture is velvety to cottony. Reverse usually yellowish to tan ([Color Plates 137](#) and [138](#).)

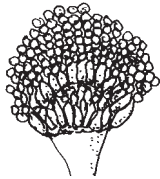
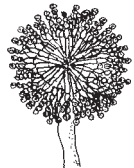
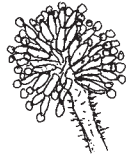
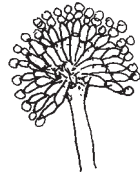
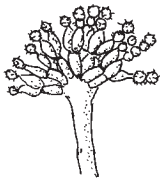
MICROSCOPIC MORPHOLOGY: Septate hyphae; the conidiophores are long (400–800 × 8–18 µm), and when fully mature, the walls are characteristically rough/spiny, especially at the apex, i.e., along the area just below the vesicle. Both uniseriate and biseriate phialides are formed over most of the vesicle; they are loosely radiate and may cluster to form columns of conidia with age. Conidia are 3–6 µm in diameter with smooth or slightly rough walls.

See [Table 26 \(p. 299\)](#) for differential characteristics of the most commonly encountered *Aspergillus* species.



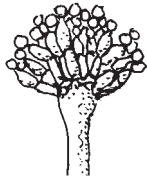
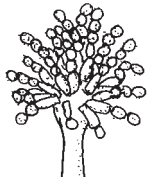
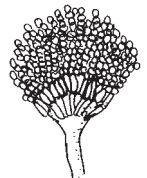
For further information, see
 Amaike and Keller, 2011
 Anaissie et al. (ed.), 2009, pp. 271–296, 561–570
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 14
 Raper and Fennell, 1973, pp. 357–404

TABLE 26 Differentiating characteristics of the most common species of *Aspergillus* (see detailed description of genus and species on pp. 293–308)^a

Species	Pathogenicity	Macroscopic morphology ^b	Microscopic morphology of conidiophores	Microscopic morphology of phialides	Illustration of microscopic morphology
<i>A. fumigatus</i> ^c (p. 295)	Most common cause of invasive disseminated aspergillosis; frequent agent of sinusitis	Velvety or powdery; at first white, then turning dark greenish to gray. Reverse white to tan	Short (<300 μm) Smooth	Uniseriate; usually only on upper two-thirds of vesicle, parallel to axis of conidiophore	
<i>A. niger</i> (p. 297)	Most common in ear infections; frequently in aspergilloma; rarely disseminated	Deep cottony; at first white to yellow, then turning black. Reverse white to yellow	Long (400–3,000 μm) Smooth	Biseriate; cover entire vesicle; form “radiate” head	
<i>A. flavus</i> (p. 298)	Involved in pulmonary, systemic, sinus, ear, and other infections; produces aflatoxins	Velvety; yellow to green or brown. Reverse goldish to red-brown	Medium (400–800 μm) Rough; pitted; spiny	Uniseriate and biseriate; cover entire vesicle; point out in all directions	
<i>A. versicolor</i> (p. 301)	Only occasionally involved in pulmonary or other infections	Velvety; at first white, then yellow, orangey, tan, green, or occasionally pinkish. Reverse white; may be yellow, orange, or red	Medium (200–500 μm) Smooth	Biseriate; loosely radiate; cover most of vesicle (Hülle cells may be present)	
<i>A. calidoustus</i> (p. 302)	Occasional cause of infection in various body sites	Velvety; brownish or olive-gray. Reverse yellowish brown	Short (130–300 μm) Smooth; brown when mature	Biseriate; loosely cover upper half to three-quarters of vesicle (can form irregular, elongate Hülle cells)	

(continued)

TABLE 26 Differentiating characteristics of the most common species of *Aspergillus* (see detailed description of genus and species on pp. 293–308)^a (continued)

Species	Pathogenicity	Macroscopic morphology ^b	Microscopic morphology of conidiophores	Microscopic morphology of phialides	Illustration of microscopic morphology
<i>A. nidulans</i> (p. 305)	Can cause infection at various sites; seen in patients with chronic granulomatous disease	Velvety; usually green, but buff to yellow where cleistothecia form. Reverse tan or purplish red	Short (<250 μm) Smooth; brown	Biseriate; short; columnar; cleistothecia usually present with reddish ascospores; Hülle cells often abundant	
<i>A. glaucus</i> (p. 306)	Rarely involved in nail, ear, and systemic disease	Feltlike; green with yellow areas. Reverse yellow (osmophilic, growth enhanced by 20% sucrose in medium)	Medium (300–700 μm) Smooth	Uniseriate; radiate to very loosely columnar; cover entire vesicle (cleistothecia generally present)	
<i>A. terreus</i> (p. 307)	Involved in infections in wide variety of body sites	Usually velvety; cinnamon-brown. Reverse yellow to brown	Short (<300 μm) Smooth	Biseriate; compactly columnar (round hyaline cells produced on mycelium submerged in agar)	
<i>A. clavatus</i> (p. 308)	An agent of allergic aspergillosis; rarely involved in infections in various body sites	Dense; green. Reverse white or tan	Long (500–2,000 μm) Smooth	Uniseriate; closely crowded on huge clavate vesicle (~200 $\times$ 40 μm)	

^a *A. tanneri* is not included in Table 26 because conidial structures develop only when grown on Czapek agar or malt extract agar. See p. 303.

^b Classically studied on Czapek-Dox agar; color of colonies may be somewhat different on various media.

^c *A. fumigatus* grows well at 45°C or higher.

Aspergillus versicolor

(For general information about the genus *Aspergillus*, see [p. 293](#).)

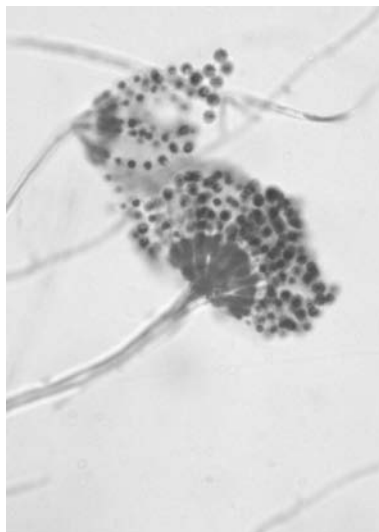
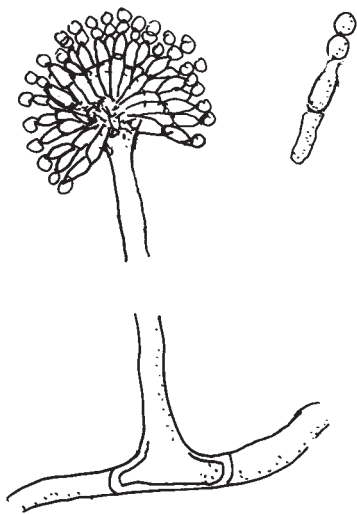
PATHOGENICITY: Occasionally involved in infections of various sites of the body, most commonly in the lung. The organism is widespread in the environment.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface is suedelike, often having radial grooves; color varies, but most commonly green or tan with spots of yellow, orange, or pink. Reverse may be white, yellow, orange, or red. Colony diameter is relatively smaller than most other species of the genus. ([Color Plates 139](#) and [140](#).)

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, medium length ($200\text{--}500 \times 4\text{--}7 \mu\text{m}$); vesicles $9\text{--}16 \mu\text{m}$ in diameter. Biseriate, with metulae approximately the same size as the phialides, loosely covering half to all of the vesicle. Small conidial heads occasionally form, resembling penicilli. Conidia are round, slightly or clearly rough, and $2\text{--}3.5 \mu\text{m}$ in diameter. Hülle cells may occasionally be present.

See [Table 26](#) ([p. 299](#)) for differential characteristics of the most commonly encountered *Aspergillus* species.



For further information, see
Charles et al., 2011
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Siqueira et al., 2016

Aspergillus calidoustus (previously considered *Aspergillus ustus*)*

(For general information about the genus *Aspergillus*, see p. 293.)

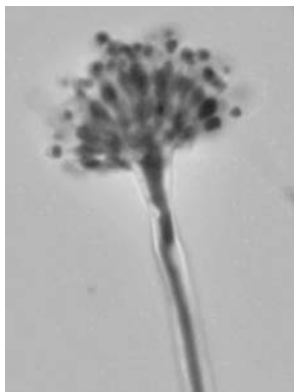
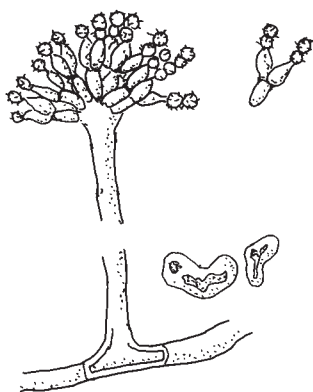
PATHOGENICITY: Has been reported as a very occasional etiologic agent of infection in various sites including heart, lung, ears, burn tissue, and skin. Present in low numbers in soil. Isolates of *A. calidoustus* tend to be resistant to the currently licensed antifungal triazoles but have low MICs to amphotericin B.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface light yellowish brown or dull grayish brown to olive-gray, occasionally with light yellow edges; may produce yellow-brown or purplish droplets on mycelium; texture velvety. Reverse yellowish brown. (Color Plate 141.)

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, brown when mature, short ($130\text{--}300 \times 4\text{--}7 \mu\text{m}$); vesicles $9\text{--}15 \mu\text{m}$ in diameter. Biseriate, with metulae slightly shorter than the phialides, loosely covering upper half to three-quarters of vesicle. Conidia are rough walled and $3\text{--}4 \mu\text{m}$ in diameter. Hülle cells that are irregularly shaped, usually elongate, may be present.

See Table 26 (p. 299) for differential characteristics of the most commonly encountered *Aspergillus* species.



Hülle cells of *A. calidoustus*. Courtesy of Deanna A. Sutton, doctorfungus.org.

For further information, see

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Hageskal et al., 2011

Raper and Fennell, 1973, pp. 543–557 (as *A. ustus*)

Varga et al., 2008

* Until recently, clinical isolates fitting this description were considered to be *Aspergillus ustus*. Molecular, morphological, and physiological data place those isolates in a new distinct species, *A. calidoustus*. *A. calidoustus* differs phenotypically from *A. ustus* by being able to grow well at 37°C and able to produce a secondary indole metabolite that is tested for by a rather complicated method utilizing Ehrlich reagent.

PATHOGENICITY: This newly recognized species causes potentially fatal chronic invasive pulmonary and disseminated aspergillosis in patients with chronic granulomatous disease. *A. tanneri* has also been found to have elevated MICs to amphotericin B, voriconazole, and itraconazole. These elevated MICs correlate with the fatal outcomes in the two initial patients reported with this organism, who had received antifungal therapy.

RATE OF GROWTH: Slow; mature in 14 days. Optimum growth temperature is 30°C; grows slower at 37°C. The preferred media for this organism are malt extract agar (MEA) and Czapek agar. It grows fastest on MEA, slightly slower on Czapek agar. Very slow growth on cornmeal agar; mature in 21 days.

COLONY MORPHOLOGY: The organism may appear initially on Sabouraud medium as white, nonsporulating colonies. When grown on MEA or Czapek agar, the surface is white when young and becomes yellow over time. Reverse remains colorless.

MICROSCOPIC MORPHOLOGY: Uniquely, this species of *Aspergillus* does not produce conidiophores when grown on most routine fungal media such as Sabouraud dextrose agar and potato dextrose agar; only septate hyphae are seen. When grown on MEA or Czapek agar, the conidiophores are smooth, long, slender, and 2–4 µm wide; vesicles are 5–7 × 7–10 µm. It is biserial with metulae shorter than the phialides, each metula bearing two or more phialides covering one-half to two-thirds of the vesicle. The conidia are round, hyaline, smooth, and 2–3 µm in diameter. Conidiophores lacking vesicles, and bearing phialides of reduced size, are often found on trailing hyphae; these structures appear almost penicillate.

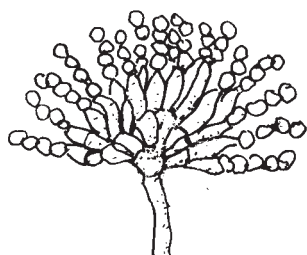
IMPORTANT NOTE: As most clinical laboratories may not have Czapek agar or MEA readily available, a nonsporulating hyaline mould that is thought to be clinically causing refractory pulmonary or disseminated infection should be submitted for molecular identification as early as possible to guide patient care.

A. tanneri is not included in [Table 26](#), as it does not form conidia on routine media.

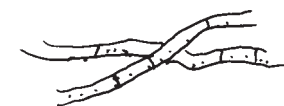
(continued on following page)

* The species name, "*tanneri*," was given in memory of one of the initial patients diagnosed with this organism.

Aspergillus tanneri (sp. nov.) (continued)



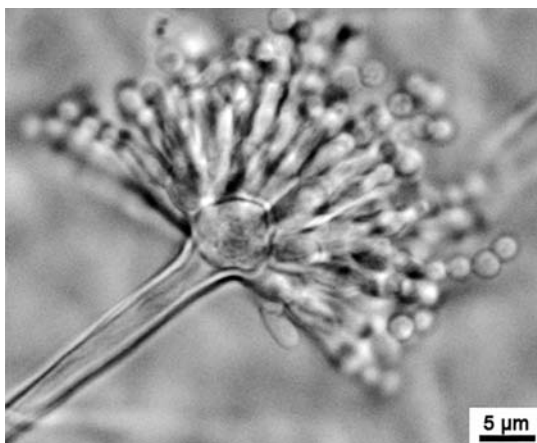
On MEA.



Septate hyphae on SDA or PDA.



Penicillate-like structures.



On MEA.



Penicillate-like structure.

For further information, see
Sugui et al., 2012

Aspergillus nidulans complex

(For general information about the genus *Aspergillus*, see [p. 293](#).)

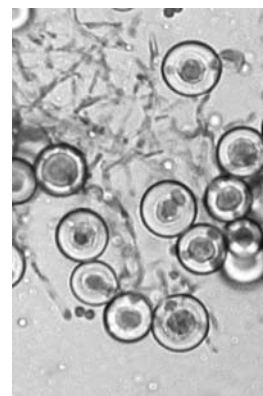
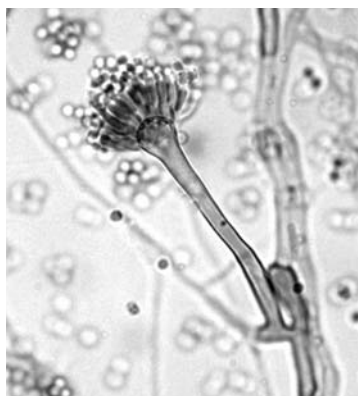
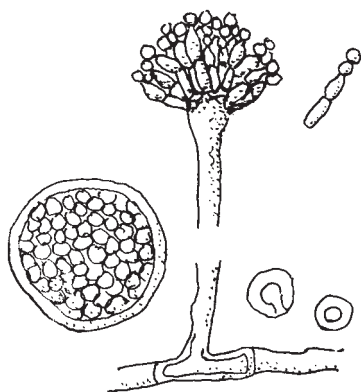
PATHOGENICITY: A soil organism with widespread distribution. Has been reported as the etiologic agent of infection in a variety of sites including lung, brain, bone, eyes, nasal sinus, and skin, as well as disseminated infection. It causes more lethal infections in patients with chronic granulomatous disease than do the other species of *Aspergillus*.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface velvety, green; buff to yellow or purplish brown areas if cleistothecia are present; white border. Reverse buff, brownish orange, or deep reddish purple. ([Color Plates 142, 143, and 144](#).)

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, short (usually $<200\text{ }\mu\text{m}$ long $\times$ $3\text{--}6\text{ }\mu\text{m}$ in diameter), and brown, darkening with age. Vesicles $8\text{--}12\text{ }\mu\text{m}$ in diameter. Phialides biserial with the metula and phialide being equal in length, forming only on the upper half of the vesicle. Conidia are round, smooth or slightly rough, and $3\text{--}4\text{ }\mu\text{m}$ in diameter. Sexual state exhibits round cleistothecia ($100\text{--}250\text{ }\mu\text{m}$ in diameter) producing red ascospores ($3\text{--}4 \times 4\text{--}6\text{ }\mu\text{m}$) upon extended incubation (within 14 days). The cleistothecia are commonly surrounded by numerous round Hülle cells ($10\text{--}25\text{ }\mu\text{m}$ in diameter).

See [Table 26 \(p. 299\)](#) for differential characteristics of the most commonly encountered *Aspergillus* species.



Hülle cells of *A. nidulans*.
Courtesy of Victoria Ruppert.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Henriet et al., 2012
Kidd et al., 2016, p. 21
Raper and Fennell, 1973, pp. 491–542

Aspergillus glaucus

(For general information about the genus *Aspergillus*, see [p. 293](#).)

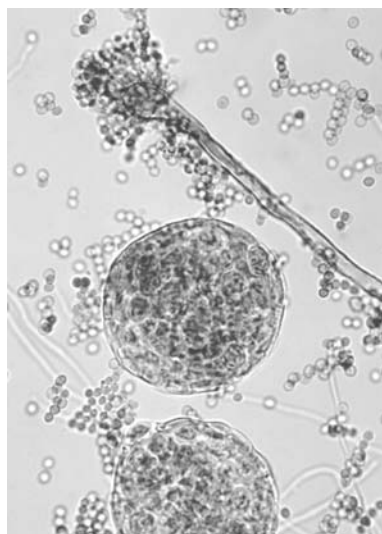
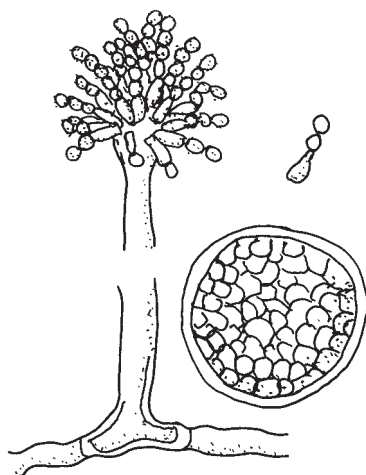
PATHOGENICITY: Worldwide distribution, prefers dry environment; seldom encountered clinically. Occasional reports of infection of nails, ear, brain, and cardiovascular and visceral sites.

RATE OF GROWTH: Moderately slow (mature in 7–21 days) on routine medium. Species is osmophilic, so the growth rate is significantly enhanced by addition of 20% sucrose to the medium.

COLONY MORPHOLOGY: Surface feltlike, dull green with yellow areas of cleistothecia production. Reverse white to yellow.

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, medium length ($300\text{--}700 \times 8\text{--}15 \mu\text{m}$). The phialides are uniseriate and may be loosely columnar or radiate, covering most of vesicle. Conidia rough, $5\text{--}8 \mu\text{m}$ in diameter. Sexual state exhibits round yellow cleistothecia, $60\text{--}150 \mu\text{m}$ in diameter. Ascospores mature slowly (~ 14 days), are $5\text{--}7 \times 3\text{--}5 \mu\text{m}$, with a central furrow/groove.

See [Table 26 \(p. 299\)](#) for differential characteristics of the most commonly encountered *Aspergillus* species.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Klich, 2002, pp. 52–53
 Raper and Fennell, 1973, pp. 147–196

Aspergillus terreus complex

(For general information about the genus *Aspergillus*, see p. 293.)

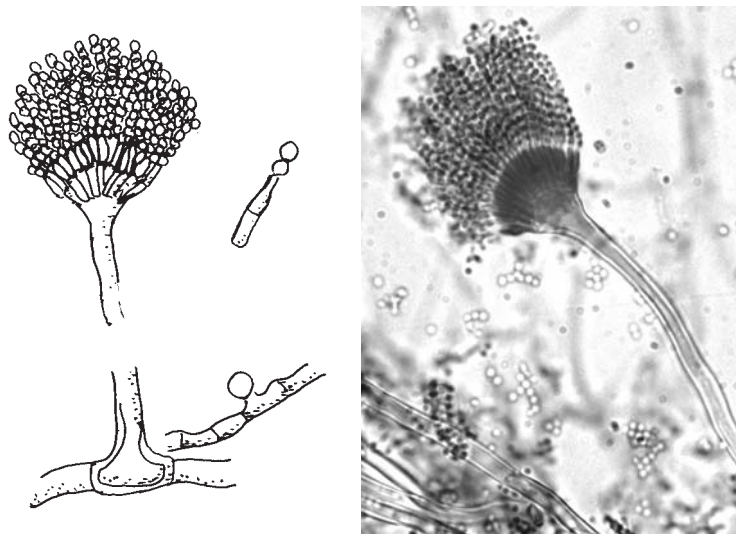
PATHOGENICITY: Widespread in the environment. Known to regularly cause infection in a wide variety of body sites with possible dissemination; also an agent of allergic bronchopulmonary aspergillosis. It is unique among the *Aspergillus* spp. in its capability of *in vivo* adventitious conidiation and its intrinsic resistance to amphotericin B.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface tan to cinnamon (with sugar) brown, texture velvety or powdery. Reverse yellow to tan. (Color Plates 145 and 146.)

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth, relatively short (<300 μm long and 4–7 μm in diameter). Vesicles 10–20 μm in diameter. Phialides biserial, with the metulae and phialide being equal in length, forming only on the upper half of the vesicle, compactly columnar. Conidia are round, smooth, 2–2.5 μm in diameter. Round solitary conidia are commonly produced along sides of hyphae submerged in medium.

See Table 26 (p. 299) for differential characteristics of the most commonly encountered *Aspergillus* species.



For further information, see

Anaissie et al. (ed.), 2009, pp. 271–296, 561–570

de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Kidd et al., 2016, p. 25

Lass-Flörl et al., 2005

Raper and Fennell, 1973, pp. 567–577

Walsh et al., 2003

Aspergillus clavatus

(For general information about the genus *Aspergillus*, see [p. 293](#).)

PATHOGENICITY: A soil organism with widespread distribution. Seldom found in clinical isolates but has been implicated in diseases including allergic aspergillosis, pulmonary infections, endocarditis, infection of the external ear canal (otomycosis), and various toxic syndromes.

RATE OF GROWTH: Rapid; mature in 3 days.

COLONY MORPHOLOGY: Surface green with white border; densely packed with conidia that readily spread on agar to produce additional colonies. Reverse is white to tan. ([Color Plate 147](#).)

MICROSCOPIC MORPHOLOGY: Septate hyphae; conidiophores smooth and large (500–2,000 $\mu\text{m} \times 10\text{--}30 \mu\text{m}$). Phialides are uniseriate and closely packed on surface of extremely large club-shaped vesicle (usually 40–60 μm wide, up to 250 μm long). Conidia are smooth, round to oval (2.5–4 $\times$ 3–6 μm).

See [Table 26 \(p. 299\)](#) for differential characteristics of the most commonly encountered *Aspergillus* species.



For further information, see
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Raper and Fennell, 1973, pp. 137–146

Penicillium spp.

PATHOGENICITY: Commonly considered contaminants, but found in a variety of diseases in which their etiologic significance is uncertain. Among the wide range of multiple *Penicillium* spp., only a few have caused invasive disease; these include *P. chrysogenum* and *P. citrinum*. They have been known to cause corneal, cutaneous, external ear, respiratory, and urinary tract infections, as well as endocarditis after insertion of valve prostheses. Disseminated disease has been reported in severely immunocompromised patients. Many strains produce toxins. Identification of the exact species of a possibly pathogenic isolate of *Penicillium* is best accomplished with molecular methods. Recovery of *Penicillium* from a clinical specimen should still prompt the pursuit of other organisms that may be causing disease.

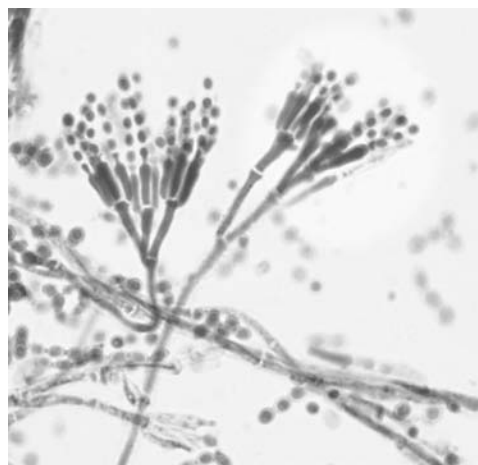
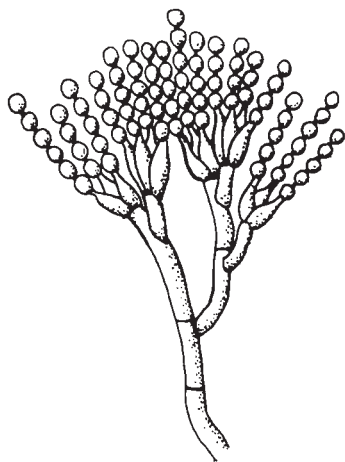
See also *Talaromyces marneffei* (formerly *Penicillium marneffei*) (p. 176).

RATE OF GROWTH: Rapid; mature within 4 days. Usually no or poor growth at 37°C.

COLONY MORPHOLOGY: Surface at first is white, then becomes very powdery and bluish green with a white border. Some less common species differ in color and texture. Reverse is usually white, but may be red or brown. (Color Plate 148.)

If the isolate produces a red reverse and diffuse pigment in the agar, *T. marneffei* (p. 176) must be considered, and the organism should be tested for thermal dimorphism; this is especially relevant if the patient has recently visited Southeast Asia.

MICROSCOPIC MORPHOLOGY: Hyphae are septate (1.5–5 μm in diameter) with branched or unbranched conidiophores that have secondary branches known as metulae. On the metulae, arranged in whorls, are flask-shaped phialides that bear unbranched chains of smooth or rough, almost round conidia (2.5–5 μm in diameter). The entire structure forms the characteristic “penicillus” or “brush” appearance.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 150

Paecilomyces variotii

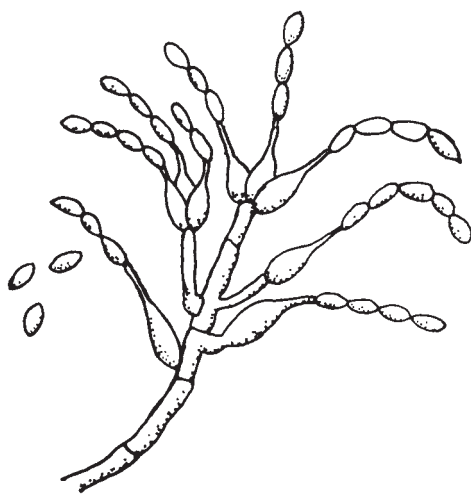
PATHOGENICITY: *Paecilomyces variotii* is associated with sinusitis and pneumonia in immunocompromised patients, as well as post-cataract endophthalmitis, wound infections, cerebrospinal fluid shunt infection, vascular catheter-associated infections, and keratitis.

RATE OF GROWTH: Rapid; mature within 3 days.

COLONY MORPHOLOGY: Surface is flat and powdery or velvety; yellowish brown or sand color, usually with a lighter border. Reverse is off-white, light yellow, or pale brown. (Color Plate 149.) Grows at 37°C; some isolates may also grow between 40 and 50°C.

MICROSCOPIC MORPHOLOGY: Resembles *Penicillium* spp. (p. 309), but the phialides of *P. variotii* are more elongated and taper to a long, slender tube, giving them the shape of drawn-out tenpins; they bend away from the axis of the conidiophore and may appear singly along the hyphae. The conidia (~2–4 × 3–5 µm or more) are elliptical or oblong and occur in long, unbranched chains. Subspherical to pyriform chlamydospores (4–8 µm in diameter) may be present singly or in short chains.

For differentiation of *Paecilomyces variotii* and *Purpureocillium lilacinum*, see Table 27 (p. 314).



For further information, see
 Anaissie et al. (ed.), 2009, pp. 317–321
 Barker et al., 2014
 Chamilos and Kontoyiannis, 2005
 de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
 Kidd et al., 2016, pp. 145–147

Purpureocillium lilacinum (formerly *Paecilomyces lilacinus*)

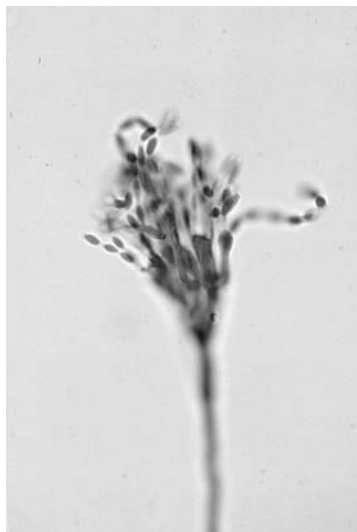
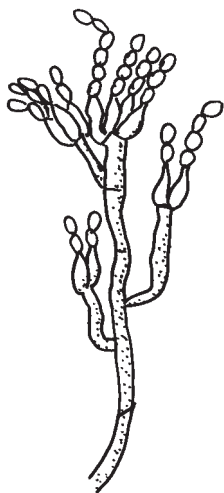
PATHOGENICITY: *Purpureocillium lilacinum* most commonly causes endophthalmitis (post-cataract surgery) and keratitis. It may also cause invasive sinusitis in immunocompetent and diabetic patients. Among immunocompromised patients, it most frequently causes subcutaneous infections and, less often, deep infection, including pneumonia and osteomyelitis.

RATE OF GROWTH: Rapid; mature within 3 days.

COLONY MORPHOLOGY: Surface is flat and powdery or velvety; may be pinkish, mauve, violet, or reddish gray; often has whitish border. Reverse is off-white or pinkish. (Color Plate 150.) Growth does not usually occur above 37°C.

MICROSCOPIC MORPHOLOGY: Conidiophores are long (400–600 μm), rough walled, branch, and form rough-walled metulae that support densely clustered phialides that are flask or short tenpin shaped (oval at their base and tapered to a short, narrow neck). The conidia are smooth or slightly rough walled, oval or fusiform ($\sim 2.0\text{--}2.2 \times 2.5\text{--}3.0 \mu\text{m}$) in swerving chains.

For differentiation of *Purpureocillium lilacinum* and *Paecilomyces variotii*, see Table 27 (p. 314).



For further information, see
Barker et al., 2014
de Hoog et al., Atlas of Clinical Fungi; Version 4.1.4
Kidd et al., 2016, p. 161
Pastor and Guarro, 2006

Scopulariopsis spp.

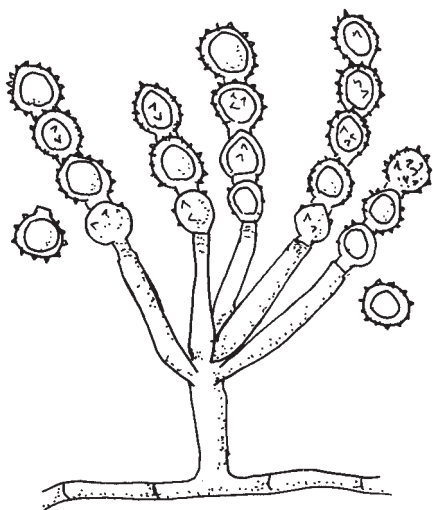
PATHOGENICITY: Known to infect the nails (usually toenail) and are associated with subcutaneous and invasive infection at various sites, including endocarditis, sinusitis, and disseminated infection, primarily in immunocompromised patients. Isolates of *Scopulariopsis brevicaulis* tend to be resistant *in vitro* to antifungal triazoles and have characteristically high MICs to amphotericin B that exceed safely achievable concentrations. *Scopulariopsis* spp. are also commonly encountered as contaminants.

RATE OF GROWTH: Rapid; mature within 5 days.

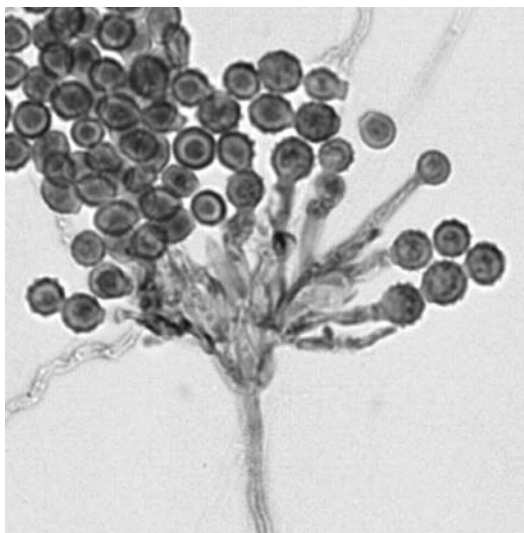
COLONY MORPHOLOGY: Surface is at first white and glabrous and then usually becomes powdery light brown with a light tan periphery. Some less frequently encountered species may be dark grayish, brown, or black. Reverse is usually tan with brownish center; occasionally darker. (Color Plates 151 and 152.)

MICROSCOPIC MORPHOLOGY: Septate hyphae with short, often branched conidiophores bearing annellides that may be cylindrical or tenpin shaped (swollen at the base and narrowing to an extended apex). The annellides form in brushlike groups or singly. The conidia, in chains, are roundish (4–9 μm in diameter), thick walled, rough and spiny when mature, sometimes slightly pointed at the apex, and cut off at the base, forming a short neck. *Scopulariopsis* spp. somewhat resemble *Penicillium* spp. (p. 309) but have shorter and sometimes simpler conidiophores; the conidium-bearing cells are annellides (rather than phialides) and may be more cylindrical; the conidia are larger, rough, and uniquely shaped by being cut off at one end and sometimes slightly pointed at the other.

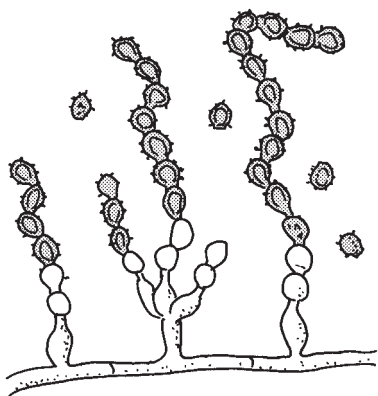
The two species that are most commonly encountered in the clinical laboratory and have been reported to cause invasive disease are *S. brevicaulis* and *S. brumptii*. For differentiation of these species, see Table 28 (p. 314).



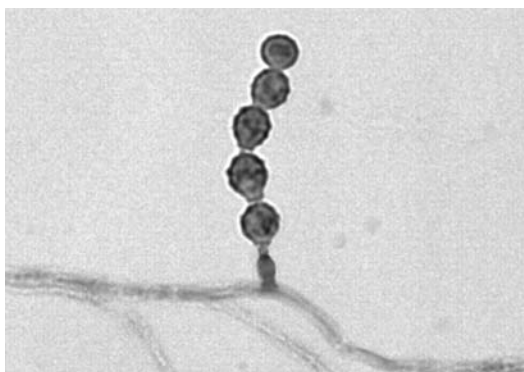
S. brevicaulis.



S. brevicaulis.



S. brumptii.



S. brumptii.

For further information, see
Anaissie et al. (ed.), 2009, pp. 318–322
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Kane et al., 1997, pp. 252–254
Kidd et al., 2016, p. 184
Yu et al., 2016

TABLE 27 Differential characteristics of *Paecilomyces variotii* versus *Purpureocillium lilacinum*

Organism	Colony color	Conidiophores	Phialides	Conidia	Temp
<i>Paecilomyces variotii</i>	Tan to yellowish brown	Smooth walled	More broadly spaced; 2–7 form on each metula	Smooth; 2–4 × 3–5 mm (or longer)	Reported to grow well at 50°C, but many do not grow at >40°C
<i>Purpureocillium lilacinum</i>	Pinkish, violet, or reddish gray	Rough walled	Densely clustered; 2–4 form on each metula	Smooth or rough; 2.0–2.2 × 2.5–3.0 mm	Restricted at 37°C

TABLE 28 Differential characteristics of *Scopulariopsis brevicaulis* versus *Scopulariopsis brumptii*

Organism	Pathogenicity	Colony	Anellides			Conidia	
			Shape	Size (μm)	Arrangement	Diameter (μm)	Color
<i>S. brevicaulis</i>	Frequently involved in nail infections; occasional reports of invasive disease	Light brown	Fairly cylindrical	2.5–3.5 × 9.0–25.0	Most in brushlike groups, some single	5.0–8.0	Hyaline or pale brown
<i>S. brumptii</i>	Occasionally found in pulmonary disease	Dark gray or grayish brown	Like tenpins; swollen at base, narrowing at apex	2.5–3.5 (at base) × 5.0–10.0	Most single, some in groups	3.5–5.5	Dark brown to blackish

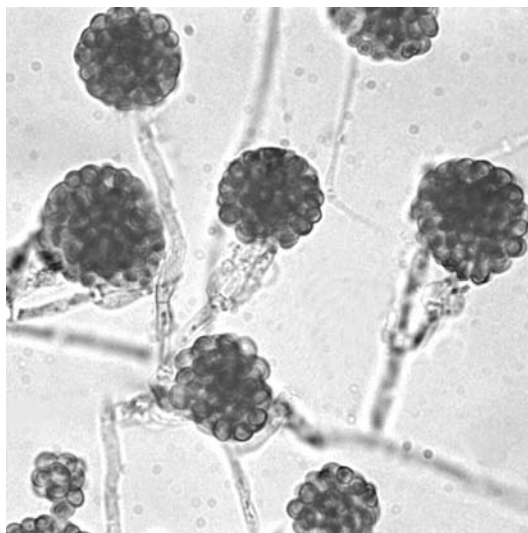
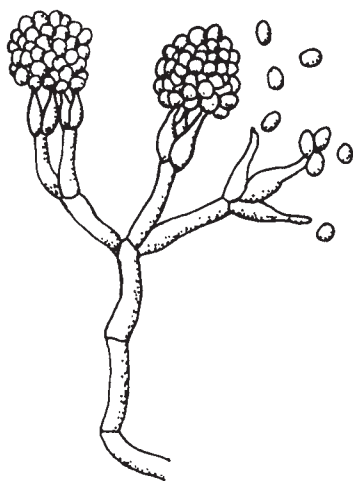
Gliocladium sp.

PATHOGENICITY: Commonly considered a contaminant; not known to cause disease.

RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: Surface is at first white. The center then becomes dark green; some strains may be pinkish. Fluffy growth spreads over plate in 1 week. Reverse is white. (Color Plates 153 and 154.)

MICROSCOPIC MORPHOLOGY: The hyphae, conidiophores, and phialides are similar to those of *Penicillium* spp. (p. 309); however, the conidia of *Gliocladium* do not remain in chains but clump together with the conidia of adjacent phialides to form large clusters or balls.



For further information, see
Barron, 1977, pp. 177-179
Kidd et al., 2016, p. 104

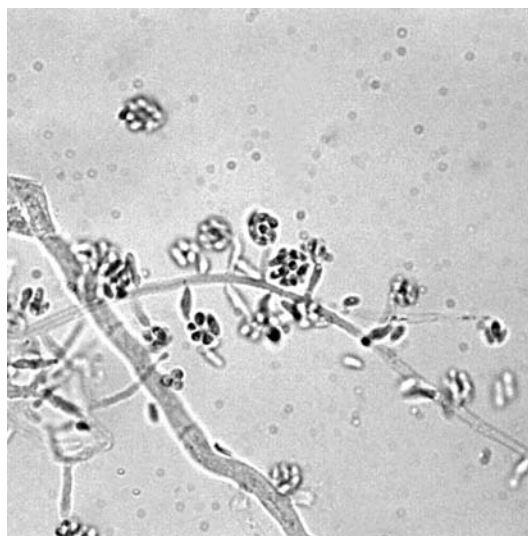
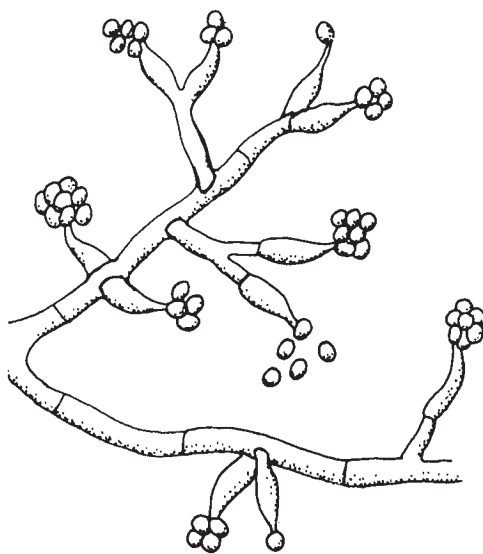
***Trichoderma* sp.**

PATHOGENICITY: Commonly considered a contaminant, but there has been an increasing number of reports of infections, particularly caused by *Trichoderma longibrachiatum*, in immunocompromised patients and cases of peritonitis in patients undergoing peritoneal dialysis. As MICs may be elevated among isolates, *in vitro* susceptibility testing is warranted for those isolates suspected of causing disease.

RATE OF GROWTH: Rapid; mature within 5 days.

COLONY MORPHOLOGY: White fluff covers agar in a few days and then becomes more compact and woolly. Green patches are eventually produced due to formation of conidia (typically at the margin of the colony). Reverse is colorless or light orangey tan to yellow. (Color Plates 155 and 156.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate. Conidiophores are short and often branched at wide angles; phialides are flask shaped and form at wide angles to the conidiophore. Conidia are round (3–4 μm in diameter) or slightly oval (2–3 $\times$ 2.5–5.0 μm), single celled, and clustered together at the end of each phialide. Clusters are easily disrupted unless microscopic preparations are handled with exceptional care.



For further information, see
 Chouaki et al., 2002
 de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
 Kidd et al., 2016, p. 193
 Sandoval-Denis et al., 2014

PATHOGENICITY: Cause adiaspiromycosis, a pulmonary disease seen mainly in rodents and small animals living in soil; only occasionally in humans. This mycosis is characterized by the *in vivo* enlargement, without multiplication, of inhaled conidia. The mycosis is usually self-limited, benign, localized, and relatively asymptomatic but may be more severe; disseminated infection has been reported in patients with AIDS. Lung biopsy is usually required for diagnosis; sputum or bronchoalveolar lavage will not suffice. *E. crescens* is the main etiologic agent in animals and the only agent of the disease in humans. *E. parva* is reported occasionally in immunocompromised patients. *E. pasteuriana* does not cause adiaspiromycosis; it has been reported from only one case of a cutaneous disseminated infection in an HIV-positive patient.

RATE OF GROWTH: Moderate; mature in 7–14 days. For primary isolation, minced lung tissue should be cultured on routine mycology agar at 25°C; both *E. parva* and *E. crescens* grow best at 20–25°C. *E. parva* grows well in the presence of cycloheximide; *E. crescens* is somewhat inhibited.

COLONY MORPHOLOGY: Surface is white, occasionally buff to pale brown in the center; some areas may be glabrous, while others will have tufts of matted mycelia; slight radial grooves may form. Reverse is cream to pale brown.

MICROSCOPIC MORPHOLOGY: Hyphae are septate (1–2.5 µm wide); conidiophores are at right angles to the vegetative hyphae and produce conidia that are round or almost so (2–4.5 µm in diameter) and may become finely roughened with age. The conidiophores may swell at the apex and produce two or three conidia on short, narrow denticles. The conidia also form directly on short stalks along the sides of the hyphae.

When the hyphae and conidia are incubated at their maximum temperatures on enriched medium, the hyphae become distorted and usually disintegrate while the conidia swell to become round, thick-walled adiaconidia (formerly called adiaspores). The adiaconidia of *E. crescens* are produced at 37°C and measure 20–140 µm in diameter. The adiaconidia of *E. parva* are best produced at 40°C and range from 10–25 µm in diameter. Adiaconidia *in vivo* are larger and have thicker walls. *E. pasteuriana*, at 37°C, forms budding cells (2–4 µm long), with no adiaconidia.

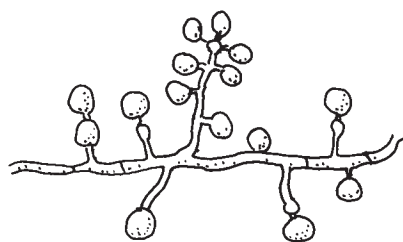
At various temperatures and stages of development, *Emmonsia* may resemble a number of other fungi, including *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Histoplasma capsulatum*, and *Chrysosporium* spp.

Note: Isolates may give false-positive results with the *B. dermatitidis* DNA probe, exoantigen, and direct immunofluorescent-antigen tests.

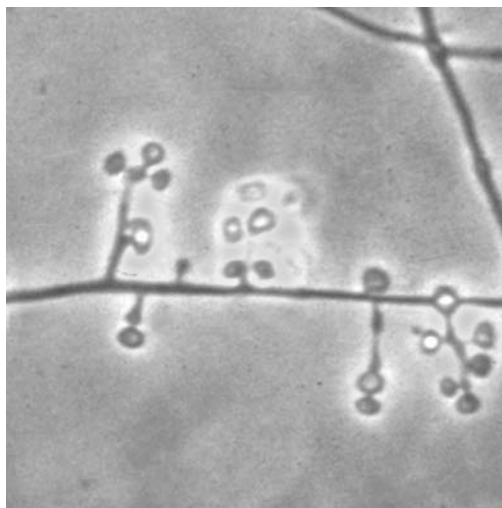
(continued on following page)

* In the older literature, these organisms are referred to as *Emmonsia parva* var. *crescens* and *Emmonsia parva* var. *parva* or *Chrysosporium parvum* and *Chrysosporium parvum* var. *crescens*.

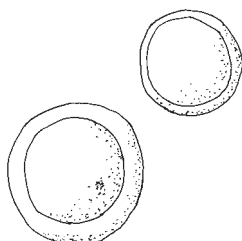
Emmonsia spp. (continued)



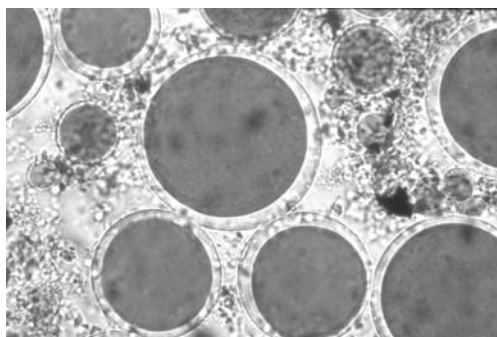
At 25°C.



At 25°C. Courtesy of Lynne Sigler.



At 37°C.



At 37°C. Courtesy of Lynne Sigler.

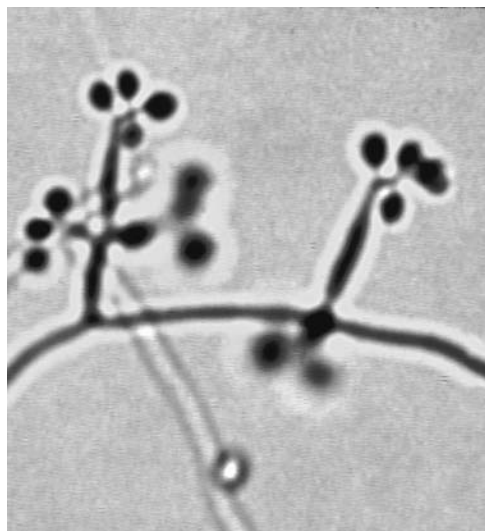
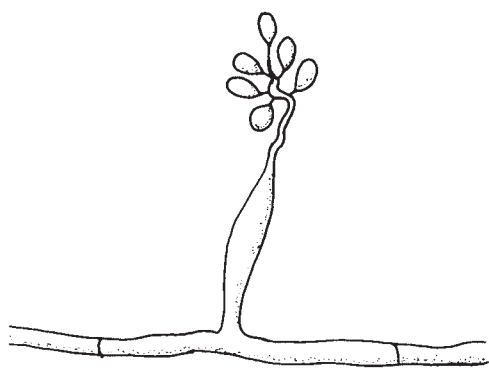
For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Schwartz et al., 2015

PATHOGENICITY: Commonly considered a contaminant. Not often involved in human infection, but there have been reports of several cases of keratitis (corneal infection) and one disseminated infection in a leukemia patient. It is known to be pathogenic in insects, especially silkworms, where it has historical significance for one of the first known causes of fungal disease.

RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: Surface is white to cream, occasionally pinkish; fluffy to powdery. Reverse is white. (Color Plate 157.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate, narrow, and delicate. Conidia-producing structures are rather flask shaped with a narrow zigzag terminal extension bearing a conidium at each bent point (sympodial geniculate growth). Conidia are small (2–4 μm in diameter), one celled, and round to oval, each forming singly on a tiny denticle. It is best to examine young cultures before dense clusters of conidiogenous cells form, making it difficult to observe the characteristic arrangement of conidia.



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kidd et al., 2016, p. 30
Kwon-Chung and Bennett, 1992, pp. 745, 799–800
McGinnis, 1980, pp. 186–187, 192–193
Rippon, 1988, pp. 730–731, 760

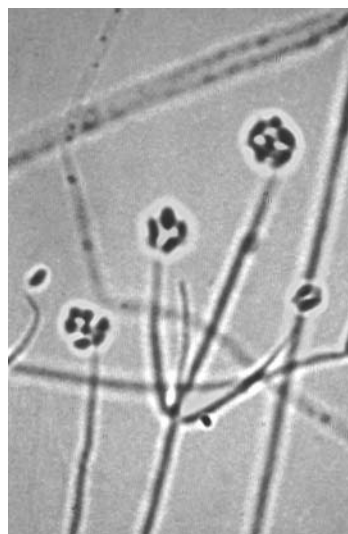
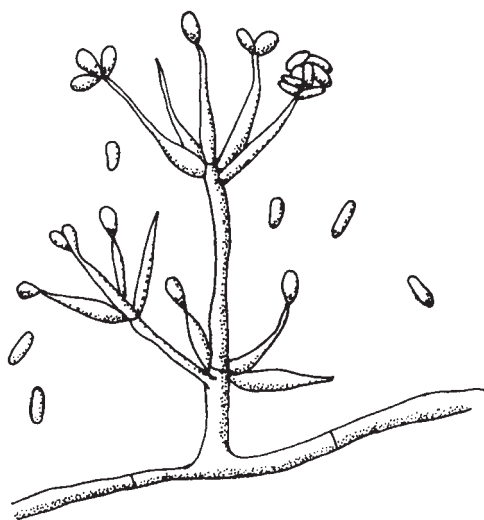
Verticillium* spp.

PATHOGENICITY: *Verticillium* spp. are well known as important plant pathogens. They are also commonly known as contaminants in clinical laboratories. Reported as rare agents of keratitis, endophthalmitis post-cataract surgery, peritonitis, and cutaneous infection post-renal transplantation.

RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: Surface is at first white and then may become pinkish brown, red, green, or yellow; powdery or velvety in texture; spreading. Reverse is white or rust in color. (Color Plate 158.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Conidiophores are simple or branched at several levels and in whorls (i.e., verticillate); phialides are very elongate, having pointed apex, also arranged in whorls. Conidia are oval and single celled, appear singly or in clusters at ends of phialides, and remain in place only if slide preparations are handled with great care.



For further information, see
Kidd et al., 2016, p. 228
Subbarao, 2011

* Several new species of *Verticillium* have been identified.

Acremonium spp. (formerly *Cephalosporium* spp.)*

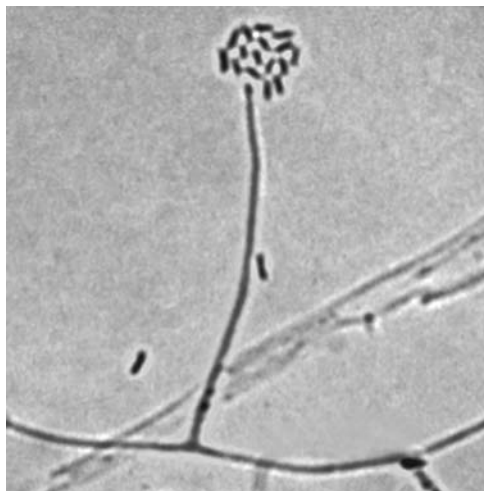
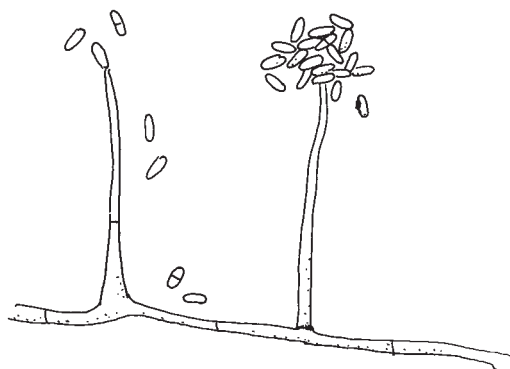
PATHOGENICITY: *Acremonium* spp. are most commonly known as etiologic agents of white grain mycetoma and localized infections of the nails and cornea. Disseminated infections have been reported in immunosuppressed patients. The organisms may also be encountered as contaminants.

RATE OF GROWTH: Moderately rapid; mature within 5–7 days.

COLONY MORPHOLOGY: At first compact, glabrous; later becomes feltlike, powdery, or cottony. May be white, yellowish, light gray, or pale rose in color. Colony does not spread. Reverse is colorless, pale yellow, or pinkish. (Color Plates 159 and 160.)

MICROSCOPIC MORPHOLOGY: Extremely delicate. Hyphae are septate. Phialides are erect, unbranched, tapering, have no conspicuous collarette, and form directly on the fine, narrow hyphae; most (but not all) have a septum at the base delimiting them from the hyphae. Conidia are oblong ($2\text{--}3 \times 4\text{--}8 \mu\text{m}$) and usually one celled but occasionally two celled. The conidia form easily disrupted clusters at the tips of the phialides.

To differentiate *Acremonium* from similar genera, see Table 16 (p. 212).



For further information, see

Anaissie et al. (ed.), 2009, pp. 318–321

de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4

Guarro et al., 1997

Kidd et al., 2016, p. 1

Roilides et al., 1995

Summerbell et al., 2011

* Several studies reveal that the genus *Acremonium* is polyphyletic, with some species separating into two separate clades; e.g., the genus *Sarocladium* has been extended to include members of the clade containing *Acremonium strictum* and *Acremonium kiliense*. Further validation of these observations is necessary before adopting these changes in clinical laboratories.

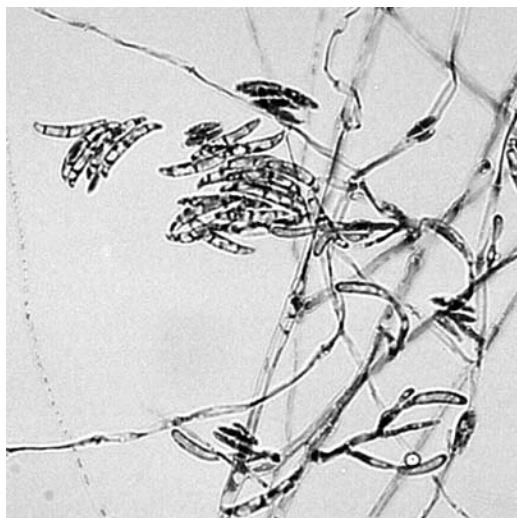
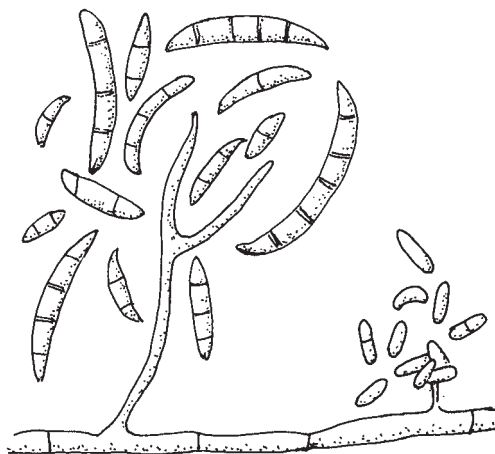
***Fusarium* spp.**

PATHOGENICITY: *Fusarium* spp. are common worldwide causes of mycotic keratitis. Among immunocompetent patients, *Fusarium* spp. also cause mycetoma, sinusitis, septic arthritis, and onychomycosis. They are increasingly the cause of sinopulmonary and disseminated infections in profoundly neutropenic patients; in these cases, the organism can often be cultured from characteristic cutaneous lesions and from blood cultures. Additionally, lethal toxicoses, among which are fumonisins and trichothecenes, have been reported in individuals who ingested food prepared from grain that had been overgrown by toxin-producing species. The susceptibility profiles differ widely among isolates, with variably high MICs being observed for amphotericin B and triazoles. Many *Fusarium* spp. are plant pathogens that can be traumatically inoculated into the cornea or subcutaneous tissues.

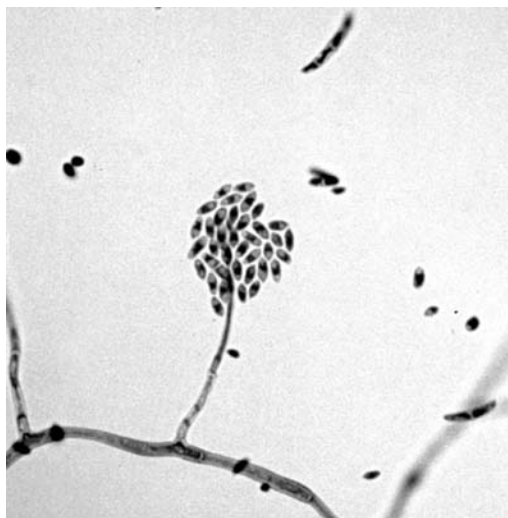
RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: At first white and cottony, but often quickly develops a pink or violet center with a lighter periphery. Some species remain white or become tan or orangey. *F. solani* is unique in becoming blue-green or bluish brown where clusters of conidiogenous cells develop. Reverse is usually light, but may be deeply colored. (Color Plate 161.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. There are two types of conidiation: (i) unbranched or branched conidiophores with phialides that produce large ($2-6 \times 14-80 \mu\text{m}$), sickle- or canoe-shaped macroconidia (with three to five septa); and (ii) long or short simple conidiophores bearing small ($2-4 \times 4-8 \mu\text{m}$), oval, one- or two-celled conidia singly or in clusters resembling those of *Acremonium* spp. (p. 321). Chlamydoconidia may be present in some species. Definitive identification of members of the *Fusarium solani* complex, *Fusarium oxysporum* complex, *Fusarium verticillioides*, and *Fusarium proliferatum* requires molecular characterization.



Macroconidia.



Microconidia.

For further information, see
Anaissie et al. (ed.), 2009, pp. 309–315, 573–574
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
DeLucca and Walsh, 2015
Kidd et al., 2016, pp. 94–100
Nucci and Anaissie, 2007

***Lecythophora* spp.**

PATHOGENICITY: Have occasionally been involved in subcutaneous abscess, keratitis, endophthalmitis, sinusitis, peritonitis, and endocarditis.

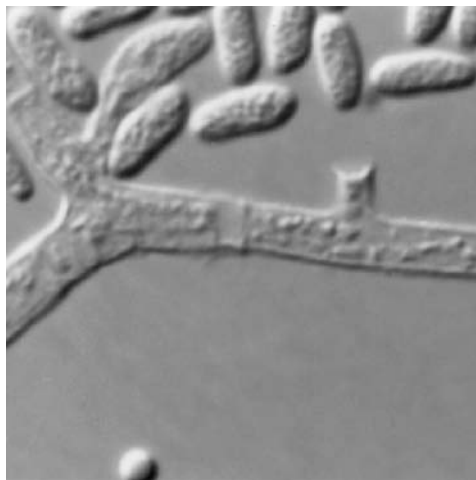
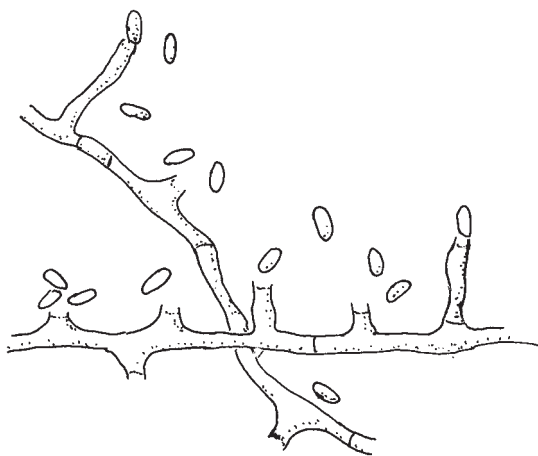
RATE OF GROWTH: Moderate; mature within 7 days.

COLONY MORPHOLOGY: At first flat, smooth, moist to slimy, somewhat yeast-like, pink to salmon or orange; may develop clumps of erect hyphal fuzz. If isolate produces chlamydospores, the center of the colony will become blackish brown. Reverse is pink or tan.

MICROSCOPIC MORPHOLOGY: Hyphae septate, hyaline, or very lightly pigmented. Phialides usually do not have a septum at the base; most are extremely short, volcano-shaped structures along the sides of the hyphae, but they may be larger and flask shaped or nearly cylindrical. Phialides have parallel-sided collarettes that may require oil immersion microscopy to be seen. Conidia are hyaline or almost so, single celled ($1.5\text{--}2.5 \times 3.0\text{--}6.0 \mu\text{m}$), oval to cylindrical, and sometimes slightly curved. May form chlamydoconidia that are brown, $\sim 4.5 \times 7.0 \mu\text{m}$, thick walled, broadly club shaped, and cut off at the base.

Note: The two clinically encountered species of *Lecythophora* differ in that *L. mutabilis* produces brown chlamydoconidia that eventually turn the colony brown, whereas *L. hoffmannii* does not produce chlamydoconidia and the colony therefore remains pink or salmon.

To differentiate *Lecythophora* from similar genera, see Table 16 (p. 212).



Courtesy of Wiley Schell.

For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

Trichothecium roseum

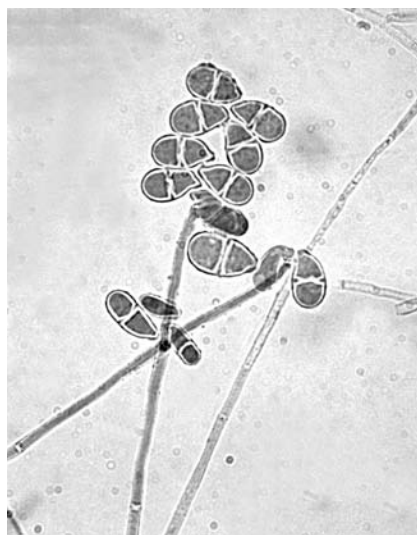
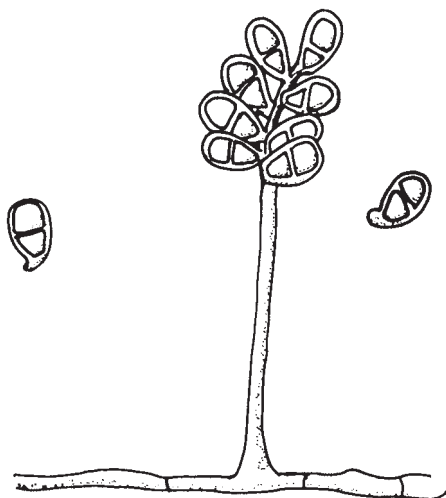
PATHOGENICITY: Commonly considered a contaminant. Not known to cause infection.

RATE OF GROWTH: Rapid; mature within 4 days.

COLONY MORPHOLOGY: At first white and woolly and then becomes pink or peach colored. Reverse is light. (Color Plate 162.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Conidiophores are long, slender, and mostly unbranched. Conidia ($8\text{--}10 \times 12\text{--}18 \mu\text{m}$) are smooth, slightly thick walled, two celled, and pear or club shaped, with a well-marked truncate attachment point frequently off to one side, forming a “foot.” The conidia are produced in alternating directions and remain side by side in an elongated group.

The long conidiophores and the smooth walls, attachment points, and arrangement of the conidia serve to distinguish this organism from *Microsporum nanum* (p. 267).



For further information, see
Kidd et al., 2016, p. 223

***Chrysosporium* spp.**

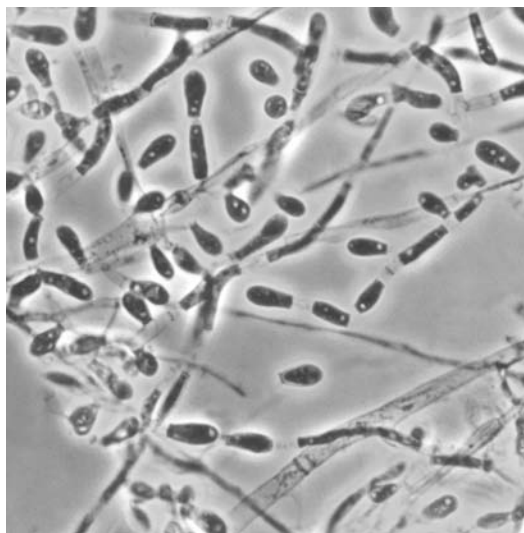
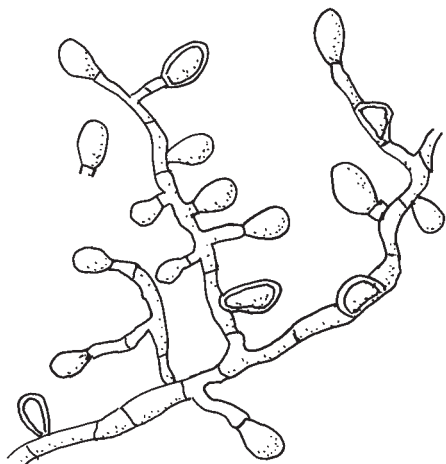
PATHOGENICITY: Commonly considered contaminants. Isolated from specimens of skin and nails, but their significance is often uncertain. There has been a report of systemic infection in a patient with chronic granulomatous disease and a cerebral infection in an HIV-positive patient. More commonly found in skin infections of snakes, chameleons, crocodiles, iguanas, and dogs.

RATE OF GROWTH: Moderately rapid; mature within 6 days.

COLONY MORPHOLOGY: Varies greatly among the many species. May be spreading or compact; surface cottony or powdery; flat or raised; usually white, yellow, or tan but may be pink or slightly orange. Reverse is usually white, yellow, tan, or brown but may be another color. (Color Plates 163 and 164.)

MICROSCOPIC MORPHOLOGY: Septate hyphae. Conidia may form directly on the hyphae or at the ends of simple or branched, stalklike, short or long conidiophores. Conidia are usually one celled ($2-9 \times 3-13 \mu\text{m}$), clavate, with a rounded apex and broad flattened base; the walls may be thin or a bit thick and are most often smooth, occasionally rough. A fringe or remnant of supporting cell wall may stay on the base of the conidium after it matures and detaches. Intercalary conidia (referred to as alternating arthroconidia by some mycologists) are sometimes formed; they are cylindrical or barrel shaped or may bulge on only one side. *Chrysosporium* is an asexual form of *Nannizziopsis vriesii*; thus, large sexual fruiting bodies (ascocarps) may occasionally be seen in culture.

- The morphology of *Chrysosporium* may resemble that of *Emmonsia parva* (p. 317), but adiaconidia do not develop at 37°C; some species will not grow at 37°C.
- Young conidial formation may mimic that of *Blastomyces dermatitidis* (p. 172), but it will not display thermal dimorphism and will be negative with specific nucleic acid probe.
- Morphology may also resemble that of microconidium-producing *Trichophyton* spp., and some species of *Chrysosporium* will grow on dermatophyte test medium (p. 409) and turn it red.
- For differentiation of *Chrysosporium* from *Sporothricum*, see Table 29 (p. 328).



Courtesy of Lynn Sigler.

For further information, see
de Hoog et al., Atlas of Clinical Fungi, Version 4.1.4
Kane et al., 1997, pp. 261–311 (highly informative)
Kidd et al., 2016, p. 52

TABLE 29 Differential characteristics of *Chrysosporium* versus *Sporotrichum*^a

Organism	Colony	Media with cycloheximide ^b	Alternating arthroconidia	Urease ^c	Temp tolerance	Large ^d chlamydospores at 37°C
<i>Chrysosporium</i> (p. 326)	Usually discrete; may spread to cover entire plate	Growth	Remain in chains; may be rare or absent	+	Grows slowly or not at all at 37°C	0
<i>Sporotrichum</i> (p. 329)	Spreads, rapidly covers entire plate	No growth	Usually abundant; often break from hyphae and form clusters	0	Grows well at 37–40°C; some grow at 45°C	+

^a Abbreviations: +, positive; 0, negative.^b Cycloheximide at 0.04%, as in Mycosel agar.^c Christensen's urea with 10 days of incubation.^d Chlamydospores up to 60 mm in diameter when isolate is subcultured.

Sporotrichum pruinosum

PATHOGENICITY: Commonly considered a contaminant. Has been found in sputa from patients with chronic respiratory disorders, but its significance is unclear.

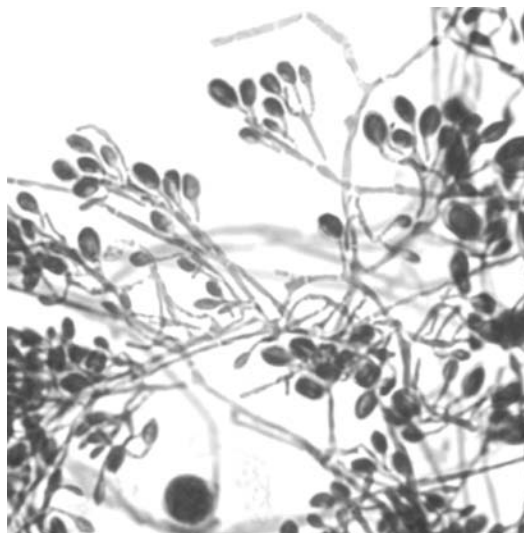
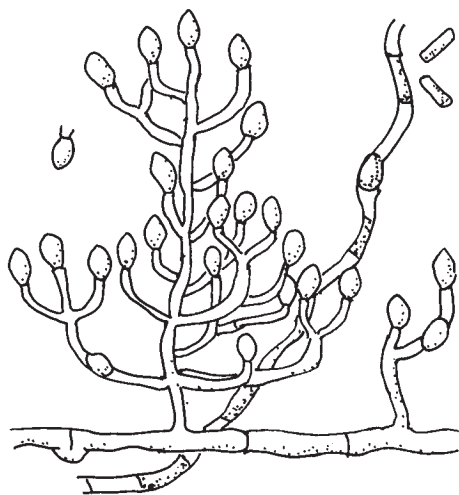
RATE OF GROWTH: Rapid; mature within 3–5 days. Thermotolerant, grows well at 40°C; many strains can grow at 45°C. It is inhibited by cycloheximide.

COLONY MORPHOLOGY: Spreads rapidly to cover entire surface of plate. Colony is initially cottony, then powdery; at first white, may become cream, yellowish, tan, pinkish, or slightly orange. Reverse is colorless or light tan. (Color Plate 165.)

MICROSCOPIC MORPHOLOGY: Hyphae are broad, lightly pigmented, and septate with bridges known as clamp connections at the septa. Conidiophores form at acute angles to the hyphae and then branch at acute angles; each branch ends with a conidium, giving a tree- or candelabrum-like appearance. The conidia are single celled, ovoid (3–6 × 5–10 μm), typically somewhat pointed at the apex and abruptly flattened at the base. The conidia often retain a portion of attached conidiophore after separation. The conidial walls are fairly thick and are usually smooth. Alternating arthroconidia are often abundant; they may be cylindrical or barrel shaped.

Large (up to 65 μm in diameter), round to oval, thick-walled chlamydospores form when incubated at 37°C, and may also develop at 25°C.

To distinguish *Sporotrichum* from *Chrysosporium*, see Table 29 (p. 328).



For further information, see
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4
Kane et al., 1997, pp. 302–303

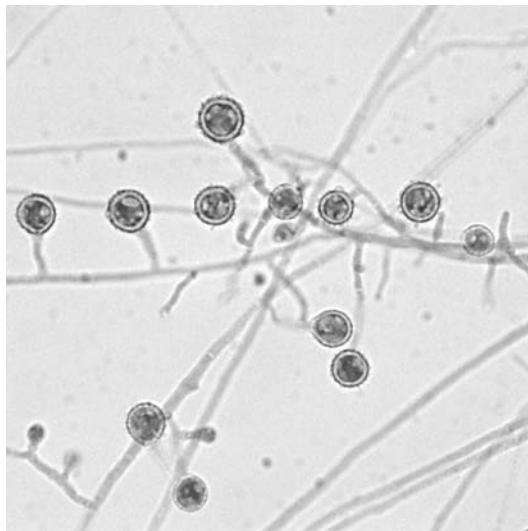
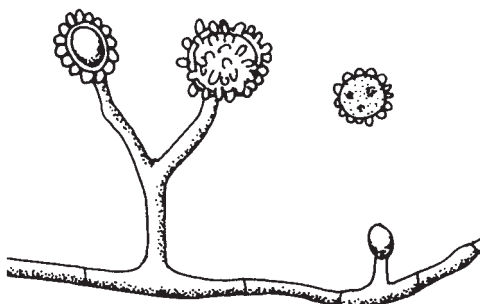
***Sepedonium* sp.**

PATHOGENICITY: Commonly considered a contaminant; not known to cause disease.

RATE OF GROWTH: Moderately rapid; mature within 7 days.

COLONY MORPHOLOGY: At 25–30°C, colonies are at first white and waxy, then become fluffy, and with age often turn yellow. At 37°C, there is little or no growth. Reverse is white. (Color Plate 166.)

MICROSCOPIC MORPHOLOGY: Hyphae are septate with simple or branched conidiophores. Conidia are large (7–17 μm), round, thick walled, and usually rough and knobby. Differs from *Histoplasma capsulatum* (p. 170) in not forming microconidia, not converting to a yeast at 35–37°C, and not reacting with the DNA probe specific for *H. capsulatum*.



For further information, see
Kidd et al., 2016, p. 185

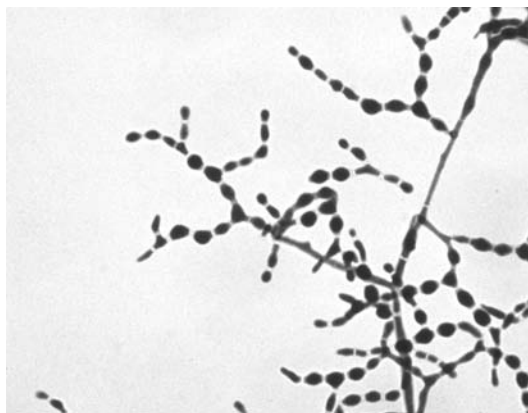
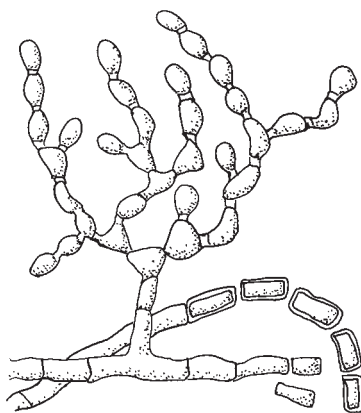
Chrysonilia sitophila (formerly *Monilia sitophila*)

PATHOGENICITY: Commonly considered a contaminant; has rarely been involved in peritonitis, eye infections, and occupational asthma. It is the asexual state of *Neurospora sitophila*. *Neurospora* spp. are routinely used in experimental studies of eukaryotic cell biology.

RATE OF GROWTH: Rapid; mature within 3 days.

COLONY MORPHOLOGY: White at first and then salmon colored. Thin, floccose mycelium rapidly spreads over surface of agar.

MICROSCOPIC MORPHOLOGY: Hyphae are septate; simple conidiophores produce branching chains of oval conidia (5–10 × 10–15 μm). The conidia are arthroconidia and are connected by narrow disjunctors. The older hyphae break up, forming thick-walled rectangular arthroconidia.



Courtesy of James Harris.

For further information, see
Barron, 1977, p. 227
de Hoog et al., *Atlas of Clinical Fungi*, Version 4.1.4

PART III

Basics of Molecular Methods for Fungal Identification

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HealthSystem, Evanston, IL

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Introduction

Molecular techniques for identification of fungi now play a significant and growing role in clinical mycology. While phenotypic methods relying on morphology and, in some instances, biochemical reactions remain the mainstay of fungal identification, molecular methods are increasing in use and offer distinct advantages in their ability to

- Identify an organism that is seen in substantial amounts on direct microscopic examination but does not grow on culture
- Identify a mould that is not producing microscopic reproductive structures (conidia or spores) or produces only unrecognized structures that are noncontributory to routine identification; or identify a yeast that is not represented in the laboratory's phenotypic commercial identification system's database
- Accomplish the above points in a relatively rapid and objective manner as compared to the time and expertise often required for conventional identification, especially when the isolate is not a rapid grower and/or does not have familiar classic morphology
- Definitively identify an organism whose characteristics closely resemble those of other fungi
- Determine the precise genotype of an isolate, an important factor in epidemiologic analyses
- More accurately evaluate the similarities and differences between organisms and adjust taxonomy and nomenclature accordingly

The present downside of molecular identification of fungi is (i) the lack of methods standardization and (ii) the lack of definitive information as to clinical relevance and anticipated antifungal response of organisms that are undergoing changes of long-standing names. The new names, determined by molecular studies, often have no "history" in the medical literature. Therefore, as many clinicians will be unfamiliar with the new nomenclature, it is important to include in the laboratory report information on the relationship

of the new to the old name, e.g., *Lichtheimia* (*Absidia*) *corymbifera* or *Exophiala xenobiotica* (member of the *Exophiala jeanselmei* complex).

This section is not meant to be an instruction on how to perform molecular assays, nor is it meant to cover the entire field of molecular fungal identification. Its goal is to provide basic information enabling laboratorians (i) to be comfortable with molecular dialogue through increased familiarity with the terminology involved and with the principle of various selected example techniques; (ii) to better understand the methods utilized and the tests performed by their reference laboratory; and (iii) perhaps most important, to have greater ease in reading and comprehending additional information regarding this field in its current state, as well as in following the future developments that will surely occur.

Molecular Terminology

Alignment Nucleotide sequences from two or more isolates are compared by lining them up above one another such that linear matches between the nucleotide sequences can be identified. Computer programs are available to align sequences and determine their similarities and differences.

Amplicon Product of nucleotide amplification.

Amplification The creation of numerous copies of a given DNA sequence, most frequently with the use of PCR.

Anneal Binding of two complementary strands of nucleic acid to each other (see also “Hybridization”).

Base The purine or pyrimidine in a nucleotide; represented by A (for adenine), G (for guanine), C (for cytosine), T (for thymine), and U (for uracil).

Base pair (bp) Two complementary nucleotides joined by weak hydrogen bonds; shortest unit of double-stranded DNA (the length of a DNA strand is expressed in number of base pairs).

Basic Local Alignment Search Tool (BLAST) An online computer program that allows comparison of a DNA sequence (from the organism to be identified) to a library of known sequences (of identified organisms) to find the closest matches, or “hits” (see [p. 348](#) for more details).

Clade A group of organisms that are genetically linked by descent from a common ancestor.

Cladogram A type of phylogenetic diagram (tree); the branches of the tree are of equal or arbitrary length. It therefore shows common ancestry, but does not indicate the amount of evolutionary change or divergence between the organisms included in the diagram. (See “Phylogram.”)

Complementary The two strands of DNA are held together by hydrogen bonds between the bases: A to T and G to C; thus, A is complementary to T and G to C. Hence, given the sequence “ATGGAC,” the complementary bases would be

“TACCTG.” The individual strands of DNA run in opposite directions, and to indicate directionality the two ends of the DNA fragment are conventionally termed **5-prime** (5′) and **3-prime** (3′). The representation of the sequence and directionality in the above DNA strand would be:

5′-ATGGAC-3′
3′-TACCTG-5′

(When a DNA nucleotide sequence is presented in text, it is always written from the 5′ to 3′ end.)

Conserved genes/sequences Conserved genes are those having nucleotide sequences that have remained relatively constant and are similar across one or several species, genera, or the entire fungal kingdom; these are genes that have not evolved significantly over time. Compare “Variable genes/sequences.”

Ct value In real-time PCR assays, the Ct value indicates the number of real-time PCR cycles at which the measured fluorescence signal crosses above the level/threshold of background fluorescence. The higher the Ct value, the lower the amount of target DNA present in the sample.

Denaturation The process of breaking the double-stranded DNA into two complementary strands; usually performed by heating to 95°C or by use of chemicals.

Dendrogram A branching diagram showing the genetic relatedness of organisms.

Deoxynucleotide triphosphate (dNTP) A nucleotide used by DNA polymerase to add bases to a growing strand of DNA during amplification. There are four dNTPs corresponding to the four bases: dATP, dCTP, dGTP, and dTTP.

Dideoxynucleotide triphosphate (ddNTP) A variation of the dNTPs that does not have the 3′ OH group. If used in DNA amplification, it terminates chain elongation, as the 3′ OH is crucial for addition of the nucleotides to the growing DNA strand. They are essential in Sanger-type DNA sequencing procedures.

DNA Deoxyribonucleic acid; chains of genetic material made of units, each containing one of any of the four bases of either a purine (adenine [A], guanine [G]) or a pyrimidine (cytosine [C], thymine [T]). The bases are attached to a five-carbon (pentose) sugar, deoxyribose (a base + the sugar = a nucleoside). A phosphate group joins the sugar backbone of the units, making a chain or strand of nucleotides (base + sugar + phosphate). Bases bind to their complement on another chain of DNA, forming double-stranded DNA.

DNA polymerase The enzyme that enables DNA replication; it is responsible for adding nucleotides to an existing DNA template segment.

Downstream Referring to the position on a DNA strand, downstream is the region toward the 3′ end of the strand.

Gel electrophoresis A technique used for determining and separating distinct DNA fragments. An agarose gel matrix is inoculated at one end with material to be analyzed (usually the product of PCR), and an electric current is applied to the gel. DNA fragments migrate various distances, dependent upon their size and electrical charge, forming “bands” that are visualized by staining.

Gene An ordered sequence of DNA on the chromosome that encodes a specific protein or RNA molecule.

Genome The entire genetic content of an organism.

Genotype A group of organisms sharing a specific genetic constitution; it is based on the entire genetic identity of the organisms, including gene forms that do not show as phenotypic characteristics.

Housekeeping gene A gene that is essential to the routine functioning of the cell, is always present, and is expressed (i.e., “turned on”) under all conditions. They are considerably conserved within a species.

Hybridization Binding of complementary strands of nucleic acid. Hybrids can form between DNA-DNA, DNA-RNA, or RNA-RNA; to “anneal” two strands and to “hybridize” them are synonymous.

Master mix A mixture containing the ingredients or factors necessary for PCR to occur, e.g., MgCl_2 , buffer, nucleotides, polymerase, and primers. Extracted DNA is added to the master mix prior to thermocycling.

Multicopy gene A gene that is repeated several times within each cell of a given fungus. These are attractive gene targets for use in molecular identification, as the presence of several copies is likely to increase sensitivity of detection.

Multiplex assay Any technique that can identify several DNA targets simultaneously, optimally in one tube or well (vessel).

Nucleic acid Deoxyribonucleic acid (DNA) or ribonucleic acid (RNA).

Nucleotide sequence The order of the nucleotides in a strand of DNA or RNA; it is conveyed by the first letter of the base in each nucleotide. A sample sequence is “ACCGT.”

Nucleotides The building blocks (base + sugar + phosphate) of DNA and RNA strands.

Oligonucleotides Short stretches of chemically synthesized single-stranded DNA (<50 bp, usually 18–30 bp); commonly used as primers or probes.

Phenotype The observable characteristics of an organism, such as colony and microscopic morphology and biochemical reactions.

Phylogeny The evolutionary history of an organism.

Phylogram A type of phylogenetic diagram (tree) in which the branch lengths are proportional to the amount of inferred evolutionary change. (See “Cladogram.”)

Polymerase chain reaction (PCR) A technique involving repeated thermal cycling and use of enzyme DNA polymerase enzyme to create multiple copies of a selected specific template DNA sequence (see [p. 341](#)).

Primer A synthetically constructed short strand of oligonucleotide (~18–24 bp long) that can bind to, and initiate amplification of, an existing longer strand of DNA. Primers are almost always used in pairs: forward primers (complementary to

the 5' end of the DNA segment to be amplified) and reverse primers (complementary to the 3' end); DNA amplification occurs by adding complementary nucleotides to the 3' end of the hybridized primers. (See "Complementary.")

Probe Short segments of nucleotides (of DNA or RNA) synthesized to be complementary (able to hybridize) to a specific region on the single-stranded DNA or RNA from the organism in question. The probe is commonly labeled with a fluorescent or chemiluminescent marker to enable detection of the specific hybridization reaction and identification of the organism.

Query (noun) The primary mechanism for retrieving information from a database for the identification of the organism under investigation; (verb) to test material (specimen or isolate) for detection or identification of an organism.

Real-time PCR Detection of specific nucleic acid amplification products as they accumulate during PCR in real time using various fluorescence-based chemistries (see [p. 342](#)).

RNA Ribonucleic acid. Similar to DNA except (i) the pentose sugar is ribose instead of deoxyribose; (ii) the base thymine (T) is replaced with uracil (U), and therefore A pairs with U; and (iii) it usually consists of only one strand.

Sequencing Procedure for the determination of the order of nucleotides in a DNA or RNA strand. Discussed in further detail on [pp. 345–351](#).

Single-copy gene Only one copy of the gene is present in each cell; not a preferred target, as amplification may not be optimum (multicopy genes are more likely to provide a higher sensitivity of detection).

Target The particular gene or DNA sequence being amplified and/or detected. The length of a target could vary from a few to several hundred base pairs, depending on the technique being used.

Template A nucleic acid strand, on which the complementary primer anneals and extends; it serves as the blueprint for production of the new strand of DNA.

Thermal cycling Repeated and controlled fluctuation in temperatures, each optimum for a stepwise reaction in PCR.

Threshold value The level of reaction at which a real-time PCR procedure is considered positive (see also "Ct value").

Universal primers Primer pairs that can be used to amplify DNA from a wide range of fungi (also known as "panfungal" primers); they are usually complementary to conserved regions of genes.

Variable genes/sequences Variable genes have nucleotide sequences that differ between species or genera of fungi; these are generally more rapidly evolving genes. Compare "Conserved genes/sequences."

Overview of Classic Molecular Identification Methods

There are three major procedural steps followed in most classic molecular identification techniques.

1. DNA from the isolate must first be extracted in a purified form to serve as the starting material. It is imperative to release the DNA from the cells and rid it of lipids, proteins, and various factors that can inhibit amplification and/or identification, and retrieve as much pure DNA as possible. This may be the most vital and sometimes problematic step for successful test results. A number of manual and automated DNA extraction methods have been developed.
2. Amplification, most often performed by PCR, results in millions of copies of the DNA segment of interest (further described below).
3. Analysis of the DNA is performed following amplification, or simultaneously with amplification, by a variety of methods (e.g., real-time PCR, sequencing, complementary probe hybridization, etc.), yielding final identification of the isolate.

Many, but not all, techniques for molecular identification require of the laboratorian a preliminary impression of the nature of the isolate (i.e., whether it is a yeast versus a mould, hyaline versus dematiaceous, etc.) in order to guide the choice of primers and/or probes to be employed. The more robust methods do not have this limitation.

A sampling of the many methods available today is described on [pp. 341–354](#).

Fungal Targets

The success of molecular identification of a fungal isolate depends on the choice of a reliable target sequence or gene. While the choice is commonly guided by the presumptive identification of the isolate, the technique being employed, and the level to which identification is being sought, optimal gene targets possess several general characteristics.

- Present in multiple copies, in order to provide good PCR sensitivity
- Relatively conserved, to ensure amplification from a broad range of fungi when utilizing panfungal primers; following amplification, variable regions within the amplicon are desirable for species- and genus-specific identification
- An optimum size (~500 bp), short enough to be sequenced with ease in automated sequencers but long enough to provide adequate information for identification
- Presence of its sequence information in databases, for reliable comparison and accurate interpretation of results

The most successful gene target that has been consistently used for molecular identification of fungi is the **ribosomal DNA (rDNA) complex**; it is a multicopy collection of genes that codes for ribosomal RNA. In eukaryotes, this gene complex comprises the 18S (small-subunit rDNA), a 5.8S subunit, and the 28S (large-subunit rDNA) genes. Interspaced between these three gene segments, and separating them from one another, are regions that have historically been called “spacers.” Internal

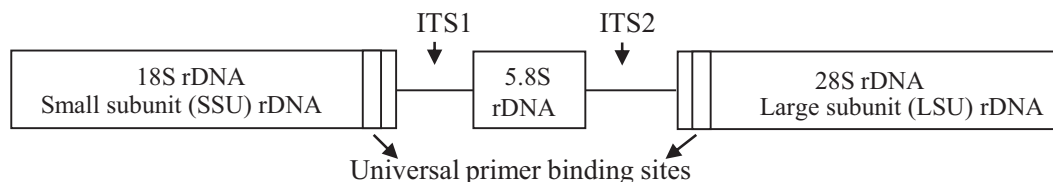


FIGURE 1 Ribosomal DNA (rDNA) gene complex.

transcribed spacer 1 (ITS1) is the region between the 18S and 5.8S genes, and ITS2 is the region between the 5.8S and the 28S genes (Fig. 1).

The ITS1 and ITS2 regions are relatively variable among species, making them good targets for species identification. The other regions of the rDNA are more conserved across the different species of a genus while being variable among genera; therefore, they are targeted for genus-level identification. In rare instances, however, the heterogeneity between species or the similarity within an individual species (of the ITS1 and -2 regions) is not sufficient to provide adequate species identification. Other genes commonly used for fungal identification include β -tubulin, calmodulin, D1/D2 region of 28S rRNA, and elongation factor (EF-1 α).

Selected Current Molecular Methodologies

Amplification and Non-Sequencing-Based Identification Methods

Amplification techniques are utilized to produce abundant copies of a selected DNA segment of interest (target DNA) derived most commonly from a fungal isolate but occasionally (and in the future, more frequently) from a patient's specimen. Successful amplification, providing an extremely heightened concentration of target DNA, obviously increases the sensitivity and success of the tests that are simultaneously or subsequently run on the amplicon, i.e., tests for detection, identification, and, in some instances, quantitation of the organism(s) being sought/queried/investigated. The most commonly used amplification method is PCR.

PCR

PCR is capable of increasing small amounts of DNA from the organism of interest over a millionfold in just a few hours. The reaction utilizes (i) primer pairs that are complementary in sequence to the ends of the target DNA, (ii) thermostable DNA polymerase enzyme (e.g., *Taq* polymerase) that reads the target DNA code and assembles the copy, and (iii) nucleotide triphosphates as the building blocks of the new DNA strand.

There are three major steps in each PCR cycle.

- 1. Denaturation:** Heating the reaction at $\sim 95^{\circ}\text{C}$ for 20–30 s to break the double-stranded DNA of the target organism into two single strands to be used as templates.
- 2. Annealing:** A cooling step at ~ 50 – 65°C for 20–60 s for the primers to hybridize to their complementary sequences on the now single-stranded template DNA.

- 3. Extension:** A heating step at 72°C for 30–60 s to allow the thermostable polymerase to extend the newly forming DNA strand. Nucleotides complementary to the template are added to the 3' end of each annealed primer until a complete complementary strand of target DNA is formed.

At the end of each cycle, the amount of target DNA present is theoretically doubled. The newly formed strands can now serve as templates for the successive cycles. After repeating the cycle many times (typically 30–50 times), the target sequence is amplified over a millionfold. PCR by itself simply amplifies the DNA, and a separate technique is often required after PCR to detect the amplicon. The amplicon is usually visualized at the end of the reaction by gel electrophoresis or is examined by probes labeled with dyes to assist in detection and identification. Other methods incorporate detection, identification, and sometimes quantitation with PCR, eliminating the need to perform additional procedures. (See <http://www.sumanasinc.com/webcontent/animations/content/pcr.html> for an animated presentation on PCR.)

Nested PCR

A method used to increase the sensitivity of a PCR reaction by adding a second round of amplification using a set of primers that is specific to a sequence found within the DNA of the initial PCR amplicon. It is typically used to obtain more amplicon when the first round of amplification yields an insufficient amount. Additionally, since the “nested” primer set uses the initial amplicon as the template to generate a second amplicon, the effects of extraneous inhibitors and nonspecific binding sites are minimized. The result is often an increase in the sensitivity and specificity of the assay.

Real-Time PCR

Real-time PCR (rtPCR, qPCR, or qrt-PCR; RT-PCR, when used properly, indicates reverse transcriptase PCR, not real-time) allows simultaneous detection and quantitation of amplification of one or more DNA targets in a single reaction, and as the name suggests, it does this as the amplification cycles occur. The primers used are similar to those used in conventional PCR, but real-time PCR additionally utilizes fluorescently labeled probes (or other fluorescent detection chemistry) and a thermocycler capable of measuring fluorescence. Typically, the level of fluorescence recorded is proportional to the concentration of the amplicon being produced. No further analysis of the products is required. Real-time PCR results are expressed as Ct values (the cycle number at the threshold level that indicates fluorescence above the background; the lower the Ct value, the greater the amount of target DNA present in the sample). Real-time assays have the attributes of rapid results, high sensitivity, ability to determine the amount of target DNA present, and performance in a closed system, reducing the chance of extraneous DNA contamination. Various fluorescence identification strategies have been developed with multiplex capabilities, enabling recognition of several targets simultaneously. The most commonly used identification strategies in real-time PCR include the following.

Melting Curve Analysis

The temperature at which double-stranded DNA “melts” (or separates) and becomes single strands is known as the melting temperature, or T_m . The T_m depends on the length of the DNA strand and on the nucleotide sequence; the higher the GC

content, the higher the T_m . In the melting curve analysis method, a fluorescent dye, SYBR green, is typically used; its special characteristic is that it intercalates (becomes inserted) only into double-stranded DNA and fluoresces 1,000-fold more intensely when inserted within the two strands. Therefore, as more PCR amplicons are formed, the fluorescence increases due to intercalation of the SYBR green within the double-stranded product. When the temperature is increased, the amplicon melts (i.e., the DNA strands separate), the dye is released, and the fluorescence decreases. Instrumentation is employed that consists of a thermocycler, an optical system, and software that graphically depicts the fluorescent signals and performs the analysis of the T_m for identification of the organism.

Several commercial systems have incorporated real-time PCR and melting curve analysis in an integrated platform. The FilmArray technology implements nested multiplexed PCR in combination with melting curve analysis for simultaneous detection and identification of pathogens. The reagents are freeze-dried in pouches and rehydrated in the instrument. Extraction of nucleic acids, followed by PCR, and subsequent detection of products are performed in “blisters” and “arrays” within the pouch. The entire assay is automated, with limited hands-on time. Data of fluorescent images and corresponding temperature are then analyzed for a melting curve to identify the pathogens.

FRET

Fluorescence resonance energy transfer (FRET) is a technique that uses two probes to examine a PCR amplicon from the DNA of the isolate to be identified. The probes are designed to be complementary to regions close to each other on the DNA of the amplified product, and each of the two probes is labeled with a different fluorescent dye. Upon exposure to a laser, positive emission occurs only if both probes hybridize to the amplified DNA and are positioned close to one another, no further than five bases apart. In such an event, the laser excites one dye to produce light energy that is transferred by FRET to the second dye on the adjacent probe. The wavelength of the light emitted by the second fluorescent dye is detected, recorded, and presented graphically by the real-time instrument. Analysis of the results yields identification of the isolate.

TaqMan 5' Nuclease

A probe is utilized that hybridizes to a portion of the target DNA to be amplified by a primer. The probe is labeled with a fluorescent dye at the 5' end and a quencher at the 3' end. The fluorophore and quencher act in unison and prevent emission of fluorescence by the probe. As the PCR amplification occurs, the primers are extended by the polymerase enzyme; the 5' nuclease activity of the polymerase chews up the hybridized probe (the enzyme does not affect the unbound probes). When the hybridized probe is degraded, the fluorophore is released and is no longer in close proximity to the quencher. The fluorescence emitted is measured and quantified by the real-time instrument and analyzed by a computer program provided with the instrument to identify the isolate. Identification of multiple targets (i.e., multiplexing) is possible by using TaqMan probes labeled with different dyes that can be recorded and graphed separately by the instrument.

Molecular Beacons

These are probes designed to have a fluorescent dye at the 5' end, a quencher dye at the 3' end, and a central secondary "hairpin-loop" structure. An example of a molecular beacon:

Fluorescent dye-5'-**GCGAGCTAGCATCCGATCTTATGTGCTCGC**-3'-Quencher

Since the two ends of the probe (underlined and bolded) are complementary to each other, they hybridize, forming the stem of the hairpin loop, while the sequence of the central portion of the loop is complementary to the target DNA. In the absence of the complementary DNA, the probe appears as in Fig. 2.

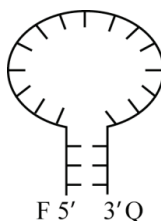


FIGURE 2 Molecular beacon hairpin-loop structure. F, fluorophore; Q, quencher.

As long as the hairpin structure is maintained, the proximity of the quencher to the fluorophore prevents fluorescence. When hybridization to target DNA amplicon occurs, the hairpin-loop structure straightens out, separating the two dyes from each other (Fig. 3). When this happens, fluorescence emission occurs and is recorded and measured by the instrument. As in the TaqMan assay above, multiplexing is possible by the use of several probes coupled to different dyes.

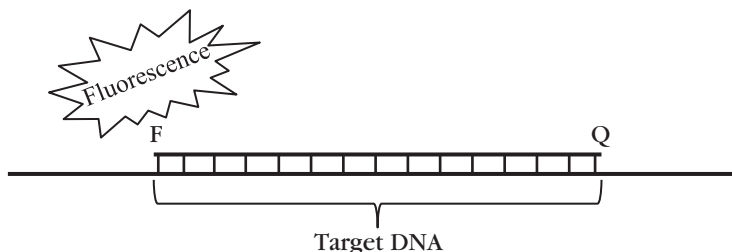


FIGURE 3 Hybridization of molecular beacon probe to target DNA with resultant fluorescence. F, fluorophore; Q, quencher.

Microarray

Various unlabeled DNA probes are "spotted" onto glass slides that have been chemically activated to bind and retain the probes. For identification of an isolate, its target DNA is amplified by PCR using fluorescent dye-labeled primers. The resultant fluorescent amplicon is applied to the microarray slide and allowed to hybridize to probes having complementary sequences. After a washing step that removes unbound

amplicon, the array is exposed to a laser beam that detects fluorescence on the probe spots that are hybridized with the labeled amplicon. The fluorescent signals are usually read, measured, and analyzed by computer for organism identification.

As only a minuscule amount of probe is required, a microarray can accommodate thousands of different probes and is ideal for multiplexing (i.e., testing for multiple organisms in one sample). It is also amenable to high-throughput testing (i.e., testing multiple samples simultaneously) on one microarray slide by dividing the slide into compartments of probes on which different samples are tested. In place of glass slides, the solid surface supporting the probes can be nylon membranes, silicon chips (e.g., Affymetrix), or microscopic beads, as in the Luminex assay, described on p. 352.

Repetitive-Element PCR

Repetitive-element PCR (rep-PCR) uses primers targeting multiple repeated sequences within the fungal genome. Amplification with these rep-PCR primers generates several amplification products that vary in size and quantity depending on the genetic composition of the organism. Analysis of the amplicons yields a unique pattern (fingerprint) for a large variety of organisms and facilitates differential identification to species, subspecies, and strain level.

A commercial system is available that provides separate kits for yeasts or moulds (DiversiLab; bioMérieux, Durham, NC). The PCR products are analyzed on a microfluidic chip analyzer (provided with the system) that separates the products based on size and fluorescent intensity. The pattern generated (within ~4 h) for each isolate is compared to a library of confirmed fungi supplied with the system for identification of the unknown isolate. The system has been most commonly used for bacterial strain identity in epidemiologic studies.

Sequencing-Based Identification Methods

DNA sequencing is a technique that determines the nucleotide sequence of a gene or DNA segment and has proved to be a powerful and useful tool for identification of microorganisms, including fungi. As the need for sequencing grows, new high-throughput sequencing techniques are being developed at a rapid rate.

Sanger Sequencing

At the time of this writing, the method most used for identification of clinically encountered fungi is a modification of the original Sanger sequencing or “chain termination method,” also known as “cycle sequencing” (commonly referred to as “first-generation sequencing technology”). This method determines the nucleotide sequence in the test organism by synthesizing a complementary strand of the DNA target utilizing DNA polymerase enzyme, similar to PCR. However, unlike PCR, the forward and reverse primers are used in separate reactions, so that only one strand is generated with each cycle. Also unlike PCR, two chemically distinct sets of nucleotides, deoxynucleotide triphosphates (dNTPs, which add bases to the elongating strand of DNA) and dideoxynucleotide triphosphates (ddNTPs, which stop/terminate chain elongation), are used. Each of the four types of ddNTP is labeled with a different dye. As the reaction

proceeds, DNA synthesis is terminated randomly each time a ddNTP is incorporated. The cycle reinitiates synthesis from the initial position each time the chain is terminated. As each different ddNTP (ddATP, ddCTP, ddGTP, or ddTTP) is labeled with a different-color dye, all terminated fragments contain a dye at their elongating end. Following a sufficient number of cycles to allow for optimal generation of extended products (which are essentially DNA chains terminated at different lengths), the products are analyzed by capillary electrophoresis-based automated analyzers. The results are displayed as colored peaks (a sequencing chromatogram) indicating each differently colored ddNTP addition to the new complementary strand. Examination and analysis of the color and height of the peaks yield the nucleotide sequence of the target DNA.

For an extensively illustrated description of the basic and modified Sanger sequencing method, see:

- http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Obenrader/sanger_method_page.htm

An animated presentation with written explanation can be found at:

- <http://www.dnalc.org/view/15923-Cycle-sequencing.html>

Next-Generation Sequencing

Also known as massively parallel sequencing and/or deep sequencing, next-generation sequencing (NGS) is a DNA sequencing technology similar in principle to Sanger sequencing in that it provides DNA sequence information by synthesis of the target DNA. However, it can provide a large amount of data in a shorter period of time. For example, using Sanger sequencing, it took a decade to finalize the draft sequence of the human genome, whereas newer NGS technologies have the potential to perform the task in a day. An additional advantage of NGS is the capability of identification of multiple organisms in a single specimen, which is extremely difficult using Sanger sequencing.

For an illustrated description of the technologies in use, see:

- <https://www.ebi.ac.uk/training/online/course/ebi-next-generation-sequencing-practical-course/what-you-will-learn/what-next-generation-dna->

While NGS is the most commonly used technology for research, its use in clinical practice is still limited. The platforms currently in use are Illumina (Solexa) sequencing, Ion Torrent Proton and Personal Genome Machine (PGM) sequencing, and SOLiD (sequencing by oligonucleotide ligation and detection). An extensive critical review of the existing technologies and their advantages can be found in Goodwin et al. (2016).

The high capacity and low cost of these new technologies make them attractive alternative means for sequencing fungi. The NGS platforms have been used to identify isolates and to look for presence of antifungal resistance markers. Their use is well established for outbreak investigation as well, such as the *Candida auris* multistate outbreak investigation (Vallabhaneni et al., 2016).

Pyrosequencing

Known as “sequencing by synthesis,” pyrosequencing differs from the Sanger sequencing method by not utilizing chain termination with dideoxynucleotides. Instead, it involves continuous development of a complementary DNA strand

with a chemiluminescent signal occurring with each consecutive addition of a program-determined nucleotide. Single strands of DNA amplicon to be identified are hybridized to a sequencing primer and incubated with four enzymes (DNA polymerase, ATP sulfurylase, luciferase, and apyrase) and two substrates (adenosine 5'-phosphosulfate and luciferin).

One of the four dNTPs (containing A, C, G, or T) is added to the solution. If its base is complementary to the first base in the template sequence, it is incorporated into the newly forming strand; this causes the release of pyrophosphate, which initiates an enzymatic cascade with other substances in the solution, resulting in the production of ATP and the generation of light that is detected and recorded as a peak on a pyrogram. Each light signal is proportional to the number of nucleotides containing the same base incorporated at that point on the strand. The unincorporated nucleotides and ATP are degraded by the apyrase in the solution before another nucleotide is added. When a dNTP is added that is not complementary to the next base on the template, no incorporation occurs, no light is generated, and the nucleotide is degraded before the next round. Addition of dNTPs continues, one after the other, until the strand is complete. The completed pyrogram corresponds to the order in which the complementary nucleotides were added to the strand, and thus reveals the underlying DNA sequence. Since results are collected as the reaction occurs (real time), no additional analysis is required other than comparison to a reliable database of fungal sequences.

An animated presentation is available at:

- <http://www.sumanasinc.com/webcontent/animations/content/highthroughput2.html> [high-throughput pyrosequencing using microbeads]

Note: A kit available commercially (Biotage, Charlottesville, VA) has been used for identification of yeasts commonly encountered in the clinical laboratory by determining nucleotide sequences of short stretches of the ITS rDNA region (Borman et al., 2010; Montero et al., 2008).

DNA Barcoding

DNA barcoding refers to a procedure for sequence-based identification of an isolate to species level by using short segments of DNA that are highly characteristic of, and unique to, the particular species and able to differentiate it from all others. These segments, or markers, are known as “barcodes.” DNA barcoding essentially involves

- Determining unique signature barcodes for each fungal species proposed to be identified
- Creating a database of curated standard barcodes by sequencing authoritatively identified isolates, confirming the accuracy of their barcodes, and continuously maintaining the database
- Amplifying and sequencing the “barcode” genes and comparing them to the standard barcode database to identify a patient isolate

Studies are ongoing for generation of high-quality barcodes for all medically important fungi. Those that have been defined are internal to various genes and are capable of identifying several fungal groups.

Major challenges in developing this method include locating reliable isolates from which to determine the barcodes for each species, standardizing the methodology, and maintaining reliable barcode databases. A few databases focus on maintaining sequences used for barcode-based identification.

- The Barcode of Life Data Systems (BOLD) (<http://www.boldsystems.org/views/login.php>)
- Molecular Mycology Lab of the University of Sydney (<http://www.mycologylab.org/BioloMICSSequences.aspx>)
- Centraalbureau voor Schimmelmcultures (CBS) Fungal Biodiversity Center (<http://www.westerdijkinstituut.nl/>)
- National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) (<http://www.ncbi.nlm.nih.gov/RefSeq>)

Applications of DNA Sequencing

The nucleotide sequence information of an isolate can be used for the following.

Accurate Molecular Identification

In many circumstances, sequencing has proved to be more precise than phenotypic methods. To reach a fungal identification, the sequence information obtained from the patient's isolate is compared for similarity to a reputable database of known, confirmed fungal nucleotide sequences; this method is known as "comparative sequence analysis." It is the most widely used method and currently considered the gold standard for molecular identification of fungi (as well as other microorganisms). There are several databases that contain libraries of defined sequences from around the world. Some databases are curated, i.e., all the sequences have been confirmed with reliable isolates and maintenance of entries is ongoing; other databases accept submissions without further examination/confirmation. A list of commonly used databases is provided below. Comparison of the sequence of the test isolate to the library sequences in the database is performed by a Web-based analysis tool known as BLAST (Basic Local Alignment Search Tool); it is a feature of all sequence databases. In BLAST search, the sequence of the patient's isolate, or "query sequence," is run against the reference database to generate a list of matches, or "hits." A rank-ordered list of sequences, from the most to the least similar, is generated and displayed along with the percent similarity scores. A diagram of a pairwise alignment of the query sequence with each match is also provided. A score of $\geq 99\%$ indicates that the query sequence is essentially the same as the sequence of the "hit." The lower limit of the score required for species-level identification of fungal isolates is not strictly defined, but it is generally agreed that $< 97\%$ is too low for a match. It is also recognized that no single cutoff score can resolve species variability in certain fungal genera. Obviously, sequence-based identification relies on the number, accuracy, and general quality of the sequences being utilized; depending on the level of quality control, these attributes vary between databases. Errors in fungal sequences available at one of the most popular databases, GenBank (which is not curated), are known to be as high as 14 to 20%.

A commercial system that uses comparative sequence analysis, the MicroSeq D2 rDNA Fungal Identification Kit (Applied Biosystems, Foster City, CA), provides a protocol, reagents required for amplification and sequence determination, and a curated database for identification of fungi (>1,000 species) covered by the system.

Following are the websites for various databases of defined sequences.

- <http://www.westerdijkinstitut.nl/>
- <http://www.ddbj.nig.ac.jp/>
- <http://www.ebi.ac.uk/embl/>
- <http://www.ncbi.nlm.nih.gov/genbank>
- <http://isolate.fusariumdb.org/guide.php>

Phylogenetic Analysis

This is a statistical method employed to ascertain evolutionary relationships between isolates. Through molecular characterization of fungal isolates, it has become evident that even morphologically identical fungi may vary enough genetically to be considered separate species, and in some instances to be placed into different clades. There are numerous computer programs available for phylogenetic analysis; a list can be found at <http://evolution.genetics.washington.edu/phylip.html>.

The programs are based on statistical principles that evaluate the differences in the nucleotide sequences among the isolates being characterized and calculate the probability of the isolates being related to one another by evolution from a common ancestor. A phylogenetic or evolutionary tree, usually in the form of a cladogram or a phylogram, is generated to graphically demonstrate the percent of genetic similarity and the probability of the isolates being related. Although there are a variety of ways to depict a phylogenetic tree (see Hall, 2007), the most common type of tree places the isolates on branches that diverge, and the distance between the branched lines provides an estimate of the relative separation from their common ancestor. (See Fig. 4 for a simplified sample tree.) A sequence similarity of 99–100% clusters isolates on the same branch, essentially indicating that they belong to the same species; strains on very close branches may also be included in that species. A lower level of similarity places an isolate on a more distant branch, often resulting in the designation of an unfamiliar species name or even transfer to a different genus. This is the major basis of the nomenclature and taxonomy changes seen throughout the recent clinical mycology literature.

Organism Typing

Sequence information yielding genetic relatedness can also be used to type individual isolates that are involved in an outbreak or are repeatedly recovered from the same patient. Typing of isolates can be performed by various methods, e.g., rep-PCR (see p. 345) and multilocus sequence typing (MLST). MLST basically compares the nucleotide sequences of multiple points (loci) within housekeeping genes of the isolates. The combination of variations in the sequences of these loci within a species characterizes and classifies the isolates into separate subtypes. The website <http://www.mlst.net/> provides information pertaining to choice of loci and data analysis. Typing can

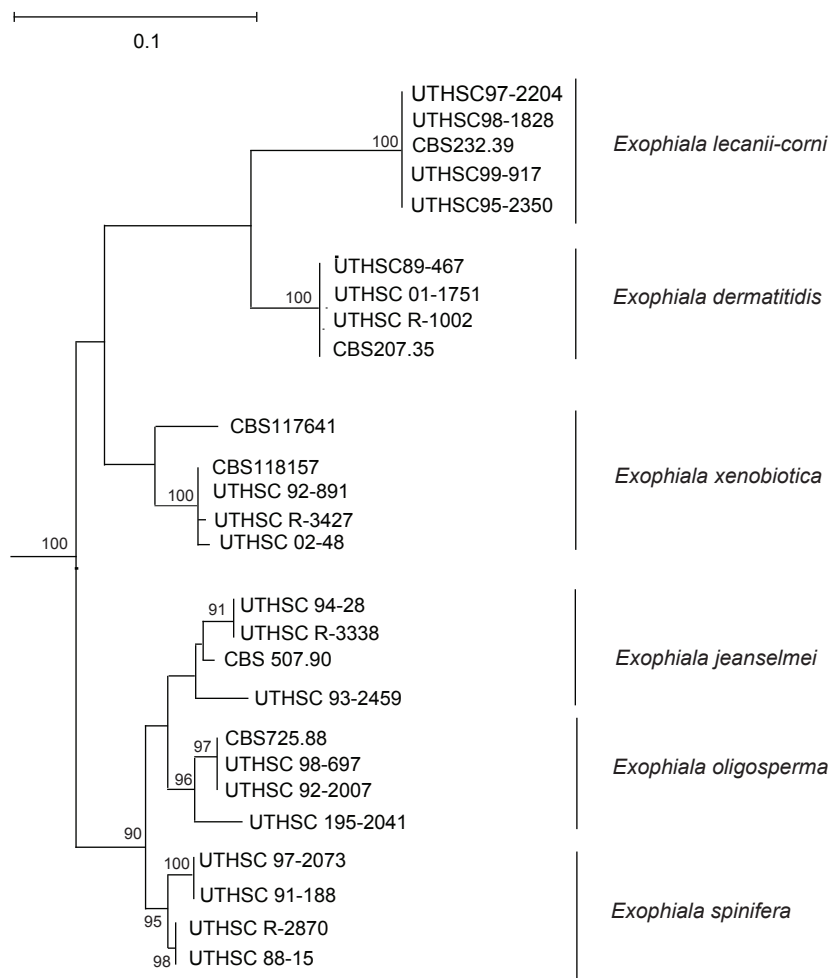


FIGURE 4 Sample phylogenetic tree of select *Exophiala* spp. Strain designations are shown at the end of each branch. (Condensed and adapted from Zeng et al., 2007.) To comprehend the evolutionary branching of the isolates, the tree should be viewed from the left to the right. Each bifurcation (branching) of the tree indicates likely evolutionary divergence. The number (known as the bootstrap value) at the point of bifurcation is a percentage based on numerous repeat sequence analyses and indicates the mathematical strength of the grouping; bootstrap values of >90 are shown at the branches. As the tree branches to the right, more closely related isolates ultimately cluster on the same or neighboring branches and are designated in the phylogram as one species. Nucleotide changes that have taken place, expressed as distance, are shown by the scale bar.

also be carried out by older methods such as pulsed-field gel electrophoresis, restriction fragment length polymorphism, and random amplified polymorphic DNA.

For further information on these and other typing methods, see

de Valk et al., 2008

Gil-Lamaignere et al., 2003

Commercial Platforms and Recently Developed Techniques

AccuProbe Test

AccuProbe (Gen-Probe, San Diego, CA), a long-standing commercially available, FDA-approved system, is based on hybridization of a labeled single-stranded DNA probe to rRNA (natively single stranded) from a growing fungal culture. Due to the large amount of rRNA in fungi, simply lysing the cells provides sufficient target nucleic acid; no further amplification is needed. Only three separate probes are available, i.e., *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides immitis* complex. The test is most commonly used for confirmation of a presumptive identification; the probe to employ is determined by the suggestive phenotypic characteristics of the isolate.

The cells of the patient's fungal isolate are first lysed by sonication and heated briefly at 95°C for 10 min. The rRNA released from the lysed cells is then exposed to the chosen DNA probe at 60°C for 15 min to allow DNA-RNA hybridization. The probes are labeled with the chemiluminescent dye acridinium ester. If the target rRNA is present from the isolate, it binds with the single-stranded DNA probe, and the acridinium ester becomes trapped and protected in the DNA-RNA hybrid. The unbound probe is unprotected and is neutralized by the addition of Selection Reagent at 60°C for 5 min. The test tube is then placed in the system's luminometer, hydrogen peroxide and an alkali are automatically added, and if rRNA-DNA-acridinium ester hybrids are present, an instantaneous flash of chemiluminescence occurs. The flash of light is measured, recorded, and analyzed by the luminometer to determine the qualitative result (positive or negative).

PNA FISH

PNA FISH (peptide nucleic acid–fluorescence *in situ* hybridization) is a synthetic product that closely resembles DNA and can be used as probes for microbial targets. The two compounds differ mainly in their structural backbones. The backbone of a DNA probe is composed of negatively charged sugar phosphates that must overcome a natural destabilizing electrostatic repulsion in order to hybridize with its complementary nucleic acid target, which is also negatively charged. The backbone of a PNA probe is instead composed of noncharged polyamides (peptides) that do not encounter electrostatic repulsion by a complementary nucleic acid, therefore allowing more rapid and secure hybridization to its targets. Additionally, PNA is resistant to degradation by enzymes that can affect DNA probes.

FDA-approved PNA FISH kits are available (AdvanDx, Woburn, MA) for the direct identification of the most commonly encountered *Candida* spp. on smears of blood culture broths that are Gram stain positive for yeasts. rRNA, abundantly produced by the growing yeast cells, serves as species-specific target for the PNA probes; the target sequences are frequently located in regions of the rRNA that

are significantly more accessible to PNA probes than to DNA probes. A variety of fluorescent-labeled individual probes and combination probes are available; the entire procedure is completed in 90 min, and the slides are read with a fluorescent microscope. For further details on the technique, see [p. 376](#).

Luminex xMAP

Luminex xMAP (Luminex, Austin, TX) is a commercially available platform that utilizes PCR amplification of the sample, followed by probe hybridization on an array of beads. The beads, using flow cytometry, then pass within a fluid sheath; the hybridized products are detected by lasers and are presented as numeric values of fluorescence by the provided software.

The system employs 100 distinct types of microspheres (tiny spherical beads; many beads of one type are called a “set”). Each bead type/set contains a different ratio of two dyes, one red and the other infrared; the ratios can be distinguished by laser. Probes complementary to various fungal species (or genera) are designed and synthesized by the user lab (or by an outside source) for a particular study and are attached to beads, i.e., a different probe to each bead type; the number of bead types used depends on the number of probes needed to detect/identify the fungus. Multiple bead types (carrying different probes) can be used in each test well.

Once the bead sets have been produced, target DNA is amplified using PCR primers that are labeled with biotin, resulting in a biotinylated amplicon. The amplicon is then mixed with each of the bead sets bearing probes; if the probe and the amplicon are complementary, annealing occurs. The product is treated with streptavidin-phycoerythrin (SA-PE), and the SA-PE complex binds to the biotinylated amplicon. The beads are then individually passed through a flow cytometer and simultaneously read by a red laser that classifies the beads and a green laser that detects the beads that bear DNA tagged with fluorescent marker (PE). Through the combination of the two sets of information, the identity of the organism involved is revealed. As the tests are run in 96-well microtiter plates, numerous samples can be tested on one plate, providing high throughput; by using various probes on many different bead types in each well, multiple organisms can be detected simultaneously.

T2 Magnetic Resonance

The T2 system (T2 Biosystems, Lexington, MA) is a self-contained, automated, sample-to-answer molecular detection system designed for direct detection of infectious pathogens from clinical samples, without the requirement for an intervening culture step. The T2Candida Panel is an FDA-cleared and CE-marked assay for the direct detection and identification of five common *Candida* species from peripheral whole-blood specimens. A specimen collection tube is loaded directly on the instrument with sequential automated steps of lysis, cellular debris separation, bead-beating to release pathogen nucleic acid, followed by PCR. Amplified products are then hybridized to their specific nucleic acid probes, which are tagged with paramagnetic particles. The detection of the amplified organisms occurs due to a change in arrangement of these paramagnetic particles that provides a specific magnetic resonance signature. Positive results are reported out as one of three categories that include five *Candida* species. The three result categories are based on IDSA (Infectious Diseases Society of America) guidelines for treatment of *Candida* bloodstream infection: *C. albicans* or

C. tropicalis (A/T); *C. krusei* or *C. glabrata* (K/G); and *C. parapsilosis* (P). Hands-on processing time for the specimen is ~5 min, and a result is provided within ~4 h, with a reported analytic sensitivity of 1 CFU/ml. Clinical trials showed sensitivity and specificity compared to culture-based methods of ~91 and 99%. Subsequent work has suggested that in at least some populations, sensitivity may exceed that of culture.

Broad-Panel Molecular Testing and Other Emerging Sample-to-Answer Technologies

In recent years, technical advances in microfluidics and assay design have allowed the production of integrated systems that combine sample preparation (nucleic acid extraction), amplification, and analysis. These so-called “sample-to-answer” platforms have vastly simplified molecular testing and allowed us to think of moderate- or even low-complexity assays using this powerful technology. Together with the increasing development of broad-panel tests, detecting and identifying 10 to 20 pathogens simultaneously, a new paradigm in microbial testing has begun to emerge. These tests have initially been focused on detection of respiratory, and subsequently gastrointestinal, pathogens. As such, most of the tests currently on the market target exclusively bacteria and viruses, with occasional parasitic organisms and some antimicrobial resistance targets also included. At least one FDA-cleared panel, the BioFire (bioMérieux) Blood Culture Identification Panel, also provides detection and identification of five common *Candida* species from positive blood culture media using the FilmArray technology. However, numerous products are in the pipeline and promise increased options for the rapid diagnosis of fungal infections. Panels targeting a much wider variety of fungal pathogens from positive blood cultures, as well as directly from lower respiratory tract samples, are among tests planned for release in the near future. Finally, a recent addition to the marketplace performs direct, automated FISH-based detection and identification and rapid phenotypic susceptibility testing of several common bacteria, together with detection and identification of two common *Candida* species (Accelerate Diagnostics, Tucson, AZ). It can be anticipated that the number of such products available will continue to increase, with detection of an increasing variety of fungal pathogens.

MALDI-TOF

MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) is unique in that it does not depend on analysis of genetic information for identification of an isolate, but is instead based on analysis of a spectrum of organic biomolecules present in the isolate. A small amount of a fungal isolate, or protein extracted from the isolate, is spotted onto a metal plate. The sample is overlaid with a defined organic matrix that, when dried, crystallizes the molecules in the isolate. The plate is then inserted into an instrument for mass spectrophotometry. The sample-matrix mixture is subjected to a pulsed laser beam of appropriate frequency that causes the matrix to be partially ionized and vaporized, carrying intact biomolecules from the isolate into the vapor phase. The vaporized molecules are passed through a vacuum drift tube, where high voltage is applied to accelerate the charged particles toward a detector; the smaller ions reach the detector in a shorter amount of time than do the larger ions. The time required for the charged ions to reach the detector is the “time of flight,” or TOF, which varies according to the mass-to-charge ratio of the

individual molecules. The TOFs exhibited by the various molecules are recorded and yield a unique mass spectrum (pattern) that can be compared to a database for isolate identification.

There are presently two FDA-approved, commercially available MALDI-TOF mass spectrometry systems available in the United States: the Vitek MS from bioMérieux and the MALDI Biotyper CA from Bruker Daltonics (Billerica, MA). Although the initial investment in the instrumentation for these systems is rather costly, the compensation is the subsequent low cost of materials (a few cents) for each organism identification and the rapidity to results (~11 min if just one isolate is tested; 2.5 min per isolate if a batch of 96 isolates is run, with the average time per isolate in published reports being 4 to 6 min).

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Collection and Preparation of Specimens

No matter how experienced the mycologist, isolation and identification of fungi from clinical specimens are not likely to be accomplished unless the specimen is properly collected and sent immediately to the laboratory. Tissue and fluids are the best specimens; swabs should be avoided whenever possible. All specimens should be transported in sterile containers and processed as soon as possible. If a specific fungus is suspected by the physician, the laboratory should be notified, as special media and culture procedures may be needed, and the information will be helpful for the safety of laboratory personnel.

Consult references (p. 469) if more information on specimen handling is required.

BLOOD. As recovery of fungi increases with the volume of blood tested, and since fungemia may be intermittent, at least two blood samples should be separately collected for culture.

Methods and media for fungal blood cultures are available from a variety of manufacturers. The automated, continuous-monitoring blood culture systems (BacT/Alert [bioMérieux, Durham, NC], BACTEC [Becton Dickinson, Sparks, MD], and VersaTREK [Thermo Fisher Scientific, Waltham, MA]) are now used in the majority of clinical microbiology laboratories. Studies have shown their acceptable ability to detect fungemia caused by yeasts; extending incubation to 10 days has been shown in some studies to provide increased recovery of *Candida glabrata* and *Cryptococcus neoformans*.

While the continuous-monitoring broth systems do sometimes yield positive cultures of moulds, lysis-centrifugation (ISOLATOR; Wampole Laboratories, Cranbury, NJ) is highly recommended for the recovery of moulds and is considered essential for the recovery of *Histoplasma capsulatum* in most instances. If only an automated broth culture is performed, *H. capsulatum* can be recovered after the routine 5-day incubation of the bottle by removing a 5-ml aliquot, centrifuging it, streaking the sediment onto a supportive agar medium, and incubating it for up to 4 weeks; 2 weeks' incubation of the subculture is sufficient to detect all of the positives (James Snyder, University of Louisville, personal communication).

If a manual method is to be used, biphasic blood culture medium consisting of broth with an agar slant is significantly better than broth alone. A commercially available example of this method is the SEPTI-CHEK blood culture system (Thermo Fisher Scientific). Conventional broth blood cultures may require 20–30 days before becoming positive and must be subcultured at regular intervals regardless of gross appearance.

For the recovery of most *Malassezia* spp. from conventional broth blood cultures, it is advisable to add palmitic acid or olive oil to make final concentrations of 3 and 5%, respectively. Adding fatty acids to commercial blood culture bottles of the automated systems does not always seem to be necessary. When using lysis-centrifugation (ISOLATOR), sterile olive oil should be applied to the surface of the agar plates before inoculation, or if available, complex media such as Dixon agar or Leeming-Notman agar should also be inoculated.

The membrane filter technique has long been a very successful means of recovering yeasts from blood, but it is impractical for most clinical laboratories. For membrane filtration, 8 ml of blood is collected in sodium polyanethol sulfonate (yellow-stoppered Vacutainer tube; Becton Dickinson) and passed through a

0.45- μ m-pore-size filter. The filter is then placed, inoculated side down, on the center of a plate of supportive medium. The specimen should be kept no longer than 1 h at 35–37°C before being filtered.

Advances in nonculture blood methods for identification of organisms from blood include antigen detection, PCR, quantification of (1→3)- β -D-glucan, and T2 magnetic resonance. Antigen detection in serum includes capsular polysaccharide antigen for *Cryptococcus* spp., galactomannan for *Aspergillus* spp., and mannan antigen for *Candida* spp. Serum for (1→3)- β -D-glucan detects the presence of *Candida* spp., *Aspergillus* spp., and other fungi through a fluorescent signal assay. The T2 assay detects the presence of *Candida* in whole blood using a combination of PCR and magnetic resonance. Whole blood and serum may be used as samples for PCR detection of a wide range of fungi, depending on the assay. More detailed discussion of molecular methods for fungal identification may be found in Part III.

BONE MARROW. Samples of bone marrow should consist of at least 0.5 ml of aspirated marrow. The pediatric ISOLATOR (Wampole Laboratories) is well designed for this small-volume culture. Alternatively, the specimen can be collected in a heparinized syringe and inoculated onto appropriate fungal media at the bedside. Many laboratories use the same bottled-broth methods used for fungal blood cultures (see above), but the blood-to-broth ratio is not optimal; a pediatric blood culture bottle is best in this instance. The specimen should also be smeared and stained with Giemsa or Wright stain if the presence of *H. capsulatum* is a possibility.

CEREBROSPINAL FLUID (CSF). CSF should be centrifuged at $2,000 \times g$ for 10 min. The supernatant fluid should not be decanted unless a portion is needed for cryptococcal antigen testing. With a sterile pipette, the sediment is removed and used to inoculate the medium and prepare smears for microscopic examination. Any remaining sediment is resuspended, and the medium is reinoculated with fairly large amounts of the whole specimen. If <2 ml of specimen is received, it should be inoculated directly onto the media. Filtration of CSF through a 0.45- μ m-pore-size membrane filter, followed by culture of the filter, is sometimes the preferred method.

CUTANEOUS SPECIMENS

A sample of skin, nail, or hair is best placed on clean, strong paper; black paper is often best in order to see tiny particles of scrapings. The paper is folded and taped to form a leakproof packet and transported in an envelope. Commercial transport package systems are available, e.g., Dermapak (<http://www.dermapak.com>; distributor in the United States: <http://www.keyscientific.com>). It is advised to refrain from the use of tubes, as they may accumulate moisture that enhances growth of bacterial contaminants that may be in the specimen, as well as plastic bags or containers, as static electricity can cause specimen to adhere to the plastic.

Skin. Specimens of skin are taken from an area previously cleansed with 70% alcohol. The active, peripheral edge of a lesion is scraped with a scalpel or the end of a microscope slide, and the scales are placed in an appropriate packet as described above.

Nails. When fungal infection is suspected in nails, they should be cleansed with an alcohol wipe and then scraped deeply enough to obtain recently invaded nail tissue.

The most rewarding samples are usually from under the nail, close to the nail bed. If the infection is on the surface of the nail plate, scrapings are taken from that area. Initial scrapings that are likely to be contaminated should be discarded and only the deeper samples sent to the laboratory in a packet as described above.

Scalp and hair. Invaders of the scalp and hair are best isolated by culturing the basal portion of the infected hair. Infected hairs may be selected by placing the patient under a UV light (Wood's lamp; wavelength 365 nm) in a dark room; hairs infected with specific dermatophytes fluoresce under UV light. Hairs that are fluorescent, distorted, or fractured should be chosen for culture.

EXUDATES, PUS, AND DRAINAGE. Specimens of exudates, pus, and drainage are best examined for granules by using a dissecting microscope. If granules are present, the color is noted, and then a portion of the specimen is teased apart gently, crushed between two glass slides, and examined microscopically; the remainder is washed several times in sterile distilled water, crushed with a sterile glass rod or similar appliance, and inoculated onto appropriate media (granules may be bacterial and should be plated accordingly). If no granules are present, the specimen is examined microscopically for hyphae and other fungal elements and directly inoculated onto isolation media.

EYE. Eye cultures for fungi are most successful when the medium is directly inoculated by the ophthalmologist. Corneal scrapings are transferred from a platinum spatula to an agar plate (blood agar plates are the most commonly used in order to simultaneously culture for bacteria) by making a series of C-shaped cuts on the medium. Scraped material should also be smeared on alcohol-cleaned, flamed slides.

FLUIDS. Fluids (e.g., pleural, peritoneal, or joint fluids) must sometimes be collected with heparin to prevent clotting. The fluid is centrifuged at $2,000 \times g$ for 10 min; any clotted material should be minced with a sterile scalpel and combined with the concentrated fluid. At least 0.3 ml of inoculum is placed onto each medium. The use of lysis-centrifugation (ISOLATOR; Wampole Laboratories) is a recommended alternative.

SPUTUM. Sputum should be collected as a first early-morning sample after the patient's teeth are brushed and the mouth is well rinsed; 24-h specimens are not satisfactory, as they easily become overgrown with bacteria and saprobic fungi. Flecks containing pus, blood, or caseous material should be sought and used in culture and smears. Sputum decontaminated for culturing acid-fast bacilli is not acceptable, because the sodium hydroxide in the procedure destroys a large number of fungi; a mucolytic agent without sodium hydroxide may be used with very viscous specimens.

STOOL. Specimens of stool are rarely worth culturing for fungi, as their presence in such a contaminated material is usually without meaning. Growth of a large amount (predominance) of yeast has possible significance, but only in indicating a lack of normal fecal flora. Colonization by yeasts is very common in both healthy

individuals and compromised patients. If fungal infection of the gastrointestinal tract is suspected, biopsy specimens of tissue are usually required for diagnosis. Surveillance cultures of stool have low sensitivity and specificity for detecting invasive fungal disease in immunocompromised patients.

TISSUES. Tissues should be minced with a scalpel or ground with a mortar and pestle or tissue grinder (for recovery of *H. capsulatum*, grinding is essential in order to release the intracellular yeasts and enable their growth on the culture media). If necessary, a small amount of sterile saline or broth may be added to facilitate grinding. If infection with a mucormycete is suspected, the tissue should be minced, not ground or homogenized, as these procedures destroy the hyphae and decrease the viability of the organisms. It is therefore advisable to grind or homogenize one portion of the tissue and mince another portion, and then to inoculate media with a mixture of the two preparations. Direct microscopic examination of tissue is best accomplished with thin paraffin sections stained with Gomori methenamine silver (commonly performed in the anatomic pathology laboratory). The mycology lab should still perform a calcofluor white stain (p. 388) on the minced/ground material. Subcutaneous tissue should be carefully examined for granules; if granules are present, they should be handled as described above (see “Exudates, Pus, and Drainage”).

URINE. Urine should be collected in a sterile container as a first morning “mid-stream” specimen. Upon reaching the laboratory, the urine is centrifuged at $2,000 \times g$ for 10 min, the supernatant is decanted, and ~ 0.5 ml of the sediment is placed on each medium to be used. If quantitation is required, a calibrated loop is used to streak uncentrifuged urine onto a plate of appropriate medium. The interpretation of the colony count is indefinite. The clinical presentation of the patient must be given prime consideration in the determination of the significance of *Candida* spp. in urine. It should be kept in mind that yeast in urine may be a sign of dissemination in severely immunocompromised patients.

For further information on collection and preparation of specimens, see
CLSI, M54-P, 2011
Hazen and Howell, 2010, sections 8.2 and 8.4
McGowan, 2015

Methods for Direct Microscopic Examination of Specimens

Any specimen submitted for fungus culture can be examined microscopically for fungal elements. This examination is made in addition to, not instead of, a culture. As well as providing the physician with early information regarding the possible need for treatment, it may be helpful in determining the significance of the organism that will later be identified on culture. For a guide to interpreting direct microscopic examinations of clinical specimens, see [Part I \(pp. 29–35\)](#).

POTASSIUM HYDROXIDE (KOH) PREPARATION

Most organic substances that might be confused with fungi when viewed microscopically are converted to an almost clear background in the presence of moderately

strong alkaline solutions. The fungi remain unaffected and are therefore more easily demonstrated.

Reagent (Clearing and Staining Agent)

1. Make a 10% KOH solution in Parker Super Quink Ink, permanent blue-black (50 ml of ink + 5 g of KOH pellets). The ink is readily available online.
2. Centrifuge KOH-ink solution at $2,000 \times g$ for 10 min.
3. Pour supernatant into plastic (not glass) sterile tube. Store at room temperature.

Slide Preparation

1. Place a portion of the specimen on a labeled slide.
2. Add 1 drop of KOH-ink solution to the slide; mix.
3. Put a coverslip over the preparation.
4. Heat gently over flame. Do not boil. Portions of nails may demand repeated and prolonged heating for the necessary degree of chemical softening so that the material can be pressed out to afford good visibility; alternatively, submerge the nail in the KOH-ink overnight for complete softening and clearing.
5. The slide must be carefully examined microscopically to detect hyphal segments, spores or conidia, budding yeasts, spherules, or sclerotic bodies. Cotton swabs should not be used in preparing these slides, as the cotton strands may resemble hyphae.

KOH preparations are not permanent; the reagent will eventually destroy the fungi. The addition of a small amount of glycerol to the preparation will preserve it for several days.

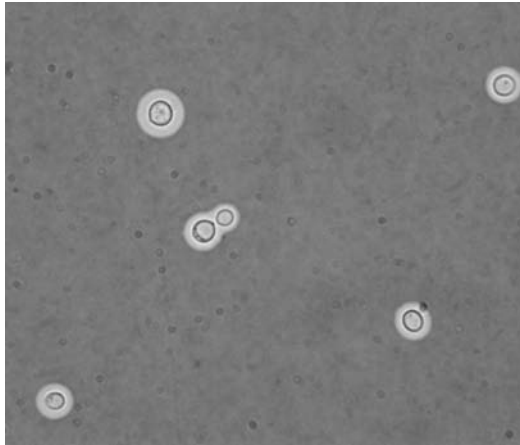
KOH combined with calcofluor white ([p. 388](#)) is a more sensitive method, but a fluorescent microscope with appropriate filter is required.

INDIA INK PREPARATION

1. Place a small drop of India ink on a glass slide.
2. Mix with an equal amount of centrifuged ($2,000 \times g$ for 10 min) spinal fluid sediment or a suspension of isolated yeast. Sputum or pus can be cleared with KOH and heat and then mixed with India ink.
3. Coverslip, and examine the slide for encapsulated yeast cells.

If the India ink is too dark, dilute it by placing a drop of sterile distilled water on the slide next to the edge of the coverslip and allowing the water to slowly diffuse into the ink.

Capsular material is exhibited by the appearance of a clear, well-demarcated halo around a yeast cell. When seen in CSF, it is suggestive of *Cryptococcus neoformans* or *Cryptococcus gattii*, but identification must be confirmed by culture and biochemical testing of the isolate. Other species and genera also produce capsules. The capsules of cryptococci vary from 2–10 μm or more in width; they tend to be small in specimens from immunodeficient patients. Leukocytes may also appear haloed due to leakage of the cytoplasm, but the halo has a fuzzy, irregular appearance at the periphery, and the cell within the halo has a much paler cell wall than does *Cryptococcus*.



Cryptococci often lose their ability to produce capsules when grown on artificial media.

Several antigen detection systems, including the cryptococcal latex antigen test, enzyme immunoassay, and lateral flowthrough device, have been proven to be significantly more sensitive than the India ink preparation and are therefore recommended for the initial diagnosis of cryptococcal disease.

STAINED PREPARATIONS

Calcofluor white, Blankofluor, and Uvitex (p. 388) are different terms for an excellent fluorescent stain that binds to the fungal cell wall for detection of fungi in specimens. It can be combined with KOH (p. 366) if clearing is required. It is a valuable tool and is highly recommended.

Gram stain (p. 392) is most useful in the mycology laboratory for examining specimens for the presence of aerobic actinomycetes. It is commonly used in bacteriology laboratories and may demonstrate mycelial elements and yeast cells, but it does not allow for the best observation of morphologic features. Hyphae, pseudohyphae, and yeast cells of *Candida* spp. in direct exam and in tissue are well stained by Gram stain.

A modified Kinyoun acid-fast stain (p. 387) is used to detect the partially acid-fast filaments of *Nocardia* spp. The routine Kinyoun method is used to stain ascospores.

Wright stain (commonly performed in hematology laboratories) or Giemsa stain (p. 389) is used for detecting intracellular yeast forms of *Histoplasma capsulatum* in blood and bone marrow.

The periodic acid-Schiff (PAS; customarily performed in histology laboratories) and Gomori methenamine silver (GMS) (p. 390) stains are considered the best for demonstrating fungi in tissue.

Primary Isolation

Specimens to be cultured for fungus must be inoculated onto media that in combination will ensure the growth of all fungi that may be clinically significant. A variety

of possible batteries of media can be used, and the ultimate choice often depends on factors such as patient population, fungi endemic in the area, cost, availability, and personal preference. In any case, the battery should include media that have different factors and that complement each other.

There are a few major points to be considered when selecting a battery of fungal media.

- Chloramphenicol and/or gentamicin or other antibiotics are commonly employed to inhibit bacterial contamination. The antibiotics are likely to also prevent the growth of aerobic actinomycetes; therefore, if *Nocardia* or any other filamentous bacteria are suspected, it is necessary to inoculate media lacking the antibiotics commonly used in fungal media (see p. 116).
- Cycloheximide is incorporated in media to inhibit the rapidly growing saprobic fungi that could overgrow slow-growing primary pathogens. It is necessary to simultaneously use media lacking cycloheximide to grow the organisms that are commonly considered saprobes or contaminants but can also act as opportunistic pathogens. In addition, cycloheximide is known to inhibit the growth of some significant pathogens, e.g., *Cryptococcus neoformans/gattii*, *Talaromyces marneffei*, *Aspergillus fumigatus*, *Lomentospora prolificans*, some *Candida* species, and most members of the class Mucor.
- It is also generally recommended that an enriched medium be used to ensure the growth of very fastidious thermally dimorphic fungi. An enriched medium containing an antibacterial agent(s) can serve a double purpose by eliminating contaminant bacteria while supporting fastidious fungal pathogens. Blood enrichment may promote the growth of fastidious dimorphic fungi, but it inhibits conidiation in the mould form.

Media that are commonly used for primary isolation of fungi from specimens are separated into five groups in Table 30 (p. 370). One medium each can be selected from as many groups as necessary, but cost containment dictates the use of as few media as possible while still guaranteeing recovery of possibly significant fungi. In earlier days, one battery of media for all fungal cultures was commonly used. This approach usually increased the number of media utilized. A more efficient system is to establish batteries according to the source of the specimen or the specific organism suspected. It may also be beneficial to use media that have different bases; for example, if using Sabouraud dextrose agar (SDA) without antibiotics, use a different base medium containing antibiotics (e.g., inhibitory mould agar [IMA]).

Note: In a comparative study, IMA was found to be significantly superior to SDA in recovery of fungi on primary isolation (Scognamiglio et al., 2010). Table 31 (p. 371) shows the results of the study. SDA or other antibiotic-free medium is still required for recovery of *Nocardia* and other aerobic actinomycetes.

The following are suggested batteries consisting of a minimum number of media.

- Sterile body sites: one choice from group A and one from group B (or two from group A)
- Respiratory tract: one from group A, one from group B, and one from group C

TABLE 30 Media for primary isolation of fungi

Type of medium ^a	Properties/purpose
A. Without antimicrobial agents SDA (p. 420) PDA or PFA (p. 417) BHI agar (p. 404) SABHI agar (p. 420)	SDA provides classic pigment and morphology, but not necessarily the best primary growth or sporulation; it is poor for the recovery of dermatophytes and other groups of organisms (see Table 31) PDA and PFA enhance production of reproductive structures and colony color BHI enhances the growth of the fastidious dimorphic fungi SABHI has some of the ingredients of both SDA and BHI
B. With antibacterial agent(s) SDA, BHI, or SABHI (usually with chloramphenicol, but may be gentamicin, or penicillin with streptomycin) IMA (p. 413) (usually contains chloramphenicol; some formulations contain gentamicin)	Inhibit bacteria while allowing fungi to grow IMA, BHI, and SABHI are enriched media and provide better recovery of fastidious fungi than does SDA
C. With antibacterial and antifungal agents Mycosel agar (p. 416) (contains chloramphenicol and cycloheximide) SDA, BHI, or SABHI (with any antibacterial agent in group B plus cycloheximide)	Chloramphenicol (or other antibacterial agent) inhibits bacterial growth Cycloheximide inhibits saprobic fungi that could otherwise overgrow pathogens Opportunists and true pathogens may also be inhibited by cycloheximide
D. For dermatophytes DTM (p. 409) RSM (p. 420)	Partially selective and differential for dermatophytes Contain antibiotics to inhibit bacteria and cycloheximide to inhibit many nondermatophytes Contain indicator to demonstrate rise in pH, consistent with growth of dermatophytes
E. Selective and differential for yeasts CHROMagar Candida (p. 406) ChromID Candida (p. 407) Chromogenic Candida Agar (p. 408)	Chloramphenicol inhibits bacteria Differentiation of yeast species is possible due to chromogenic substrates in the agar CHROMagar is able to yield discrete identification of more species of <i>Candida</i> than is ChromID All are useful in detecting mixed cultures of yeasts

^a Abbreviations: SDA, Sabouraud dextrose agar; PDA, potato dextrose agar; PFA, potato flake agar; BHI, brain heart infusion; SABHI, combination of Sabouraud and BHI; IMA, inhibitory mould agar; DTM, dermatophyte test medium; RSM, rapid sporulation medium.

- Skin, hair, and nails: one from group B and one from group C or D
- Throats, urines, and genitals (“TUGs”): one from group E (it has been shown that CHROMagar Candida can be used as the sole primary medium for these specimens; see Murray et al., 2005)

Some special situations require specific media.

- If *Histoplasma capsulatum* or *Blastomyces dermatitidis* is suspected, an enriched medium must be one of the choices (and remember, the yeast phase of these organisms is inhibited by cycloheximide).

TABLE 31 Inhibitory mould agar versus Sabouraud dextrose agar as a primary medium for isolation of fungi^a

Category of fungus	No. of isolates	Growth on both (%)	Growth on IMA only (%)	Growth on SDA only (%)	Statistical significance ^b (P value)
Yeast	578	74.4	20.2	5.4	0.00013
Hyaline hyphomycetes	143	64.3	24.5	11.2	0.00014
Dermatophytes	94	48.9	48.9	2.1	0.001
Dematiaceous	12	58.3	41.7	0	0.001
Mucormycetes	9	33.3	66.7	0	0.001
Thermally dimorphic ^c	4	100.0	0	0	NS
Total	840	69.3	24.9	5.8	0.003

^a Source: Scognamiglio et al., 2010. Abbreviations: IMA, inhibitory mould agar; SDA, Sabouraud dextrose agar; NS, not significant.

^b IMA versus SDA.

^c All four isolates were *Histoplasma capsulatum*.

- If *H. capsulatum*, *B. dermatitidis*, or *Coccidioides* sp. is suspected in a heavily contaminated specimen, yeast extract phosphate medium with ammonia (p. 425) should also be inoculated.
- If an aerobic actinomycete (e.g., *Nocardia*) is suspected, a medium without the antibacterial used in fungal media must be included. Buffered charcoal-yeast extract (BCYE) agar with and without antimicrobials (p. 404) is the medium of choice for *Nocardia* spp.

Whenever possible, and unless otherwise indicated, at least 0.5 ml of specimen should be inoculated onto each agar surface. If tubes are used, the media should be slanted in wide tubes (at least 20 mm in diameter) and optimally left in a horizontal position (on a culture rack designed for acid-fast bacilli) for 24 h after inoculation in order for the inoculum to remain dispersed rather than accumulating at the bottom of the slant. The tubes can then be stood vertically for subsequent incubation. The screw caps must be kept partially loosened to ensure proper atmospheric conditions.

Petri plates provide a larger surface area for isolation of fungi but are more hazardous to handle. If plates are used, they should contain 40 ml of medium and be surrounded by a shrink seal to prevent dehydration; the shrink seal also prevents unintentional opening. Plates should NOT be used if *Coccidioides* is suspected.

The optimal temperature for growth of most clinically encountered fungi is 30°C. If a 30°C incubator is not available, cultures should be incubated at room temperature (~25°C). There is no advantage to simultaneously incubating routine primary cultures at 35–37°C; this should be reserved for when there is reason to suspect the presence of thermally dimorphic organisms or one of the few fungi that prefer the higher temperature. If the incubator is not well humidified, it is essential to place pans of water near the cultures.

Most cultures should be incubated for 4 weeks before being considered negative for fungus. Cultures for yeasts in oral thrush, vaginitis, or urine on chromogenic

medium need to be held at least 48 h; if on routine media, they are held for 5 to 7 days. Cultures suspected of containing thermally dimorphic systemic fungi should be incubated for 8 weeks before being reported as negative.

Macroscopic Examination of Cultures

After initial inoculation and incubation, cultures should be examined for growth every 2–3 days during the first week and at least weekly thereafter. Rapid growers will appear by the first or second time the cultures are checked, whereas slow-growing fungi may not be evident for 2–3 weeks or longer. It is imperative that any yeast, mould, or actinomycete that grows on a primary medium be subcultured immediately to ensure the viability and isolation of the organism. When mature growth develops, the texture and surface color of the colony should be carefully noted. The color of the reverse (underside) of the colony must also be recorded, along with any pigment that diffuses into the medium. It is also helpful to observe whether or not the fungus grows on medium containing cycloheximide.

To ensure the cultivation of all fungi in a specimen (especially the slower-growing pathogens), it is advisable in many cases to hold the cultures for at least a month, even though some fungi may have been isolated. When more than one fungus is seen on the primary culture, a carefully streaked plate is usually necessary for isolation. The lid may be taped closed in several places for safety and prevention of dehydration, but care must be taken not to create anaerobic conditions. Shrink seals are perfect for this situation. As previously mentioned, to prevent dehydration of the plates, it is best if they contain 40 ml of agar, and a pan of water should be placed in the incubator. If *Coccidioides* is suspected, plates should NOT be used.

Microscopic Examination of Growth

It is best to examine a fungus microscopically when the culture first begins to grow and form conidia or spores and again a few days later. In many instances the manner of conidiation or sporulation, which is so important to identification, is obscured in old cultures. Potato flake or potato dextrose agar often promotes conidiation or sporulation better than does SDA. Manipulation of mould growth must always be carried out in a Class II biological safety cabinet.

There are several methods for microscopically examining a fungus culture.

Tease mount. Place a drop of lactophenol cotton blue (LPCB) ([p. 393](#)) on a clean glass slide. With a sterile bent dissecting needle or sterile loop, remove a small portion of the colony from the agar surface and place it in the drop of LPCB. With two dissecting needles or sterile applicator sticks (after they are broken at an angle to provide sharp pointed ends), *gently* tease apart the mycelial mass of the colony on the slide, cover with a coverslip, and observe under the microscope with low-power (100×) and high-dry (430×) magnifications. Unfortunately, this method does not always preserve the original position and structure of the conidia, spores, and other characterizing elements, but it is a very rapid method and is always worth a try. All moulds should be examined with a wet mount before setting up a slide culture.

Cellophane tape mount. Another rapid method of studying the microscopic morphology of a mould is with the aid of clear cellophane tape. Loop back on itself

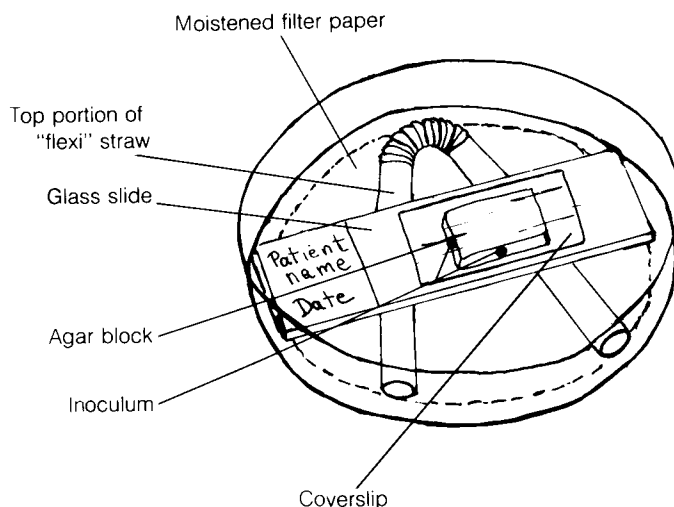
a 1.5-in. (~4-cm) strip of clear tape, sticky side out, and hold the tip of the loop securely with a forceps. Press the lower, sticky side very firmly to the surface of the fungal colony, and then pull the tape gently away; aerial hyphae will adhere to the tape. Then, with the tape strip opened up, place it on a small drop of LPCB on a glass slide so that the entire sticky side adheres to the slide, and examine it under the microscope. This method is usually successful in retaining the original positions of the characteristic fungal structures but has the drawback of requiring the organism to be grown on plated medium.

An alternative method has recently been described. It utilizes frosted tape on an applicator stick. The frosted tape is generally more readily available, more pliable, and easier to tear than is clear tape; the use of an applicator stick better permits sampling of fungi in tubed cultures (see Harris, 2000).

Slide culture. The best method for preserving and observing the actual structure of a fungus is the slide culture. It is not a rapid technique, but it is unsurpassed as a routine means of studying the fine points of the microscopic morphology of fungi. (Always do a tease mount before a slide culture; organisms suspected of being *Histoplasma*, *Blastomyces*, or *Coccidioides* spp. or *Cladophialophora bantiana* should NOT be set up on slide culture.)

The procedure is carried out as follows.

1. Cover the inside bottom of a 100-mm-diameter sterile petri plate with a piece of filter paper.
2. Place a bent glass rod, two pieces of plastic tubing (about 6 cm long, 5 cm apart), or the bending end of a flexible drinking straw in the petri plate.
3. Place a clean, labeled, flamed glass microscope slide on the glass rod, plastic tubing, or bent straw.
4. From a plate of potato dextrose agar (or other agar when desired) poured 4 mm deep, cut a 1-by-1-cm block with a sterile scalpel. Transfer the block to the center of the glass slide.



5. With a heavy nichrome wire needle (22 gauge), plastic inoculating needle, or sterile applicator stick, inoculate the fungus onto the centers of the four sides of the agar block.
6. Place a flamed coverslip over the block, and apply slight pressure to ensure adherence.
7. Place ~1.5 ml of sterile water on the filter paper on the bottom of the petri plate, replace the cover, and incubate the plate at room temperature, preferably in the dark (this can be accomplished by stacking plates in a wire basket and incubating in a designated cabinet with the door closed, but not airtight).
8. Examine periodically for growth, and add water if the plate begins to dry out. The fungus will ordinarily grow on the surface of the slide and also on the undersurface of the coverslip. The closed petri plate can be placed on the microscope stage, and the slide culture can be examined with the low-power (10×) objective.
9. When reproductive structures are well developed, use forceps to carefully remove the coverslip and place it on a drop of LPCB on a second slide.
10. With a heavy needle or applicator stick, gently flip the agar block off the original slide into a container of antifungal disinfectant. Put a drop of LPCB on the slide, and place a new coverslip over it.
11. Both microscopic preparations from steps 9 and 10 can be sealed around the edges with nail polish or mounting fluid and kept for further study or as teaching aids. These slide preparations have been found to last longer if they are stored in a flat position rather than standing on their sides. Huber's modified LPCB with polyvinyl alcohol (p. 393) is now widely used and is definitely the best method available for preserving slide preparations for long periods; it is the only acceptable method if the slide is to be shipped to another laboratory.

Procedure for Identification of Yeasts

The extent to which a yeast needs to be identified depends primarily on the body site from which it is isolated and the clinical relevancy of full identification. The particular needs of the patient population and economic issues in the laboratory also play major roles. The following is a scenario that meets minimum requirements while being cost-effective. More detailed discussion of molecular methods for identification of yeasts may be found in Part III.

Traditionally *Candida albicans*, at least, is differentiated from the other species of *Candida*, but even that level of identification is not usually necessary for isolates from the lower respiratory tract. *Candida* spp. are seldom of clinical significance in the lower respiratory tract, and diagnosis of *Candida* pneumonia is based on histologic examination, not culture. *Cryptococcus neoformans* and *Cryptococcus gattii* are typically the only yeasts of concern in such specimens (aside from the yeast forms of the thermally dimorphic fungi). *C. neoformans* and *C. gattii* can usually be rapidly ruled out by colony and microscopic morphology, with further testing only occasionally needed (see below), and the result reported as "yeast, not *Cryptococcus*."

When appropriate, screening any specimen for only *C. albicans* (not differentiated from *Candida dubliniensis*) on nonchromogenic agar can be rapidly

accomplished by the germ tube test (p. 378), by commercial chromogenic enzyme tests, or by simply examining the colonies (on blood agar or chocolate agar; especially useful method in the bacteriology laboratory) for mycelial projections, commonly called “feet” (Color Plate 44).

If throat, urine, and genital (“TUG”) specimens and blood cultures smear-positive for yeasts have been inoculated directly onto CHROMagar Candida, isolates of *C. albicans*, *Candida tropicalis*, and *Candida krusei* will be identifiable within 48 h, unless differentiation of *C. albicans* versus *C. dubliniensis* is required. (Table 6, p. 132). Images of *Candida* spp. grown on CHROMagar may be viewed at the CHROMagar site (<http://www.chromagar.com/clinical-microbiology-chromagar-candida-focus-on-candida-species-22.html#.WXYq7tMrJ-U>). Isolates resembling *Candida glabrata* on CHROMagar Candida can be identified in 1 h with the rapid assimilation of trehalose (RAT) test (p. 418) (Murray et al., 2005).

The decision on how far to go with species identification of isolates from various body sites lies with each laboratory, the primary factors being the patients’ needs and the clinical relevancy of the results.

With the above in mind, proceed.

If the colony is mucoid. Determination should immediately be made as to whether the isolate may be *C. neoformans/gattii*; this can be accomplished by

- a simple wet prep in sterile water or saline; *C. neoformans* and *C. gattii* appear as round, dark-walled cells of various sizes
- addition of India ink (p. 367) to the wet prep; will exhibit capsules (may be small), if present
- a urease slant (p. 424), inoculated especially heavily at the top of the slant (that area will turn positive more rapidly than the rest of the agar)

If any of the tests listed above suggest *Cryptococcus*, the isolate should be

- inoculated to cornmeal-Tween 80 (or analogous agar that shows microscopic morphology and is glucose free)
- from the cornmeal agar, tested for phenoloxidase with the caffeic acid disk test (p. 379); the caffeic acid test can be performed directly from the primary isolation medium, but a false negative may result if the medium contains dextrose

The first isolate of *Cryptococcus* from a patient should be confirmed with one of the commercial yeast identification kits. If the isolate is shown to be *C. neoformans* or *C. gattii*, it is reasonable to do only the caffeic acid disk test on subsequent isolates. Differentiation of *C. neoformans* and *C. gattii*, if needed, is attainable with canavanine glycine bromothymol blue (CGB) agar (p. 405).

If the colony is not mucoid

1. Perform a germ tube test (p. 378). Only *C. albicans* (and *C. dubliniensis*) will produce germ tubes in serum within 3 h, and they can be differentiated (if desired) by the characteristics in Table 6 (p. 132).
2. If the germ tube test is negative and the colony is small and relatively slow growing, and the wet prep shows small, oval cells, perform the RAT test (p. 418) for the identification of *C. glabrata*.

3. If the isolate is not *C. albicans* or *C. glabrata* and further identification is desired, use a pure culture to perform biochemical tests or proteomic analysis for yeast identification; this is most commonly done with the use of a commercially prepared system, such as one of the following.

Biochemical analysis:

API 20C AUX (bioMérieux, Hazelwood, MO)

API ID32 C (bioMérieux, Marcy l'Etoile, France; not available in United States)

Rapid Yeast ID (Beckman Coulter, Brea, CA)

RapID Yeast Plus (Thermo Fisher, Scientific Remel Products, Lenexa, KS)

Sherlock Microbial ID System (MIDI, Newark, DE)

Uni-Yeast Tek (Thermo Fisher, Scientific Remel Products, Lenexa, KS)

Vitek Yeast Biochemical Card (bioMérieux, Hazelwood, MO)

Vitek 2 ID-YST (bioMérieux, Hazelwood, MO)

Proteomic analysis:

BD Bruker MALDI Biotyper (Bruker Daltonics, Billerica, MA)

Vitek MS (bioMérieux, Marcy l'Etoile, France)

4. Using the Dalmau method (p. 409), inoculate a plate of cornmeal-Tween 80 agar (p. 408) or another yeast morphology agar; this should accompany all biochemical identification systems.

If an acceptable identification is not obtained. After the purity of the isolate is rechecked, subsequent tests may be required for

- fermentation (p. 411)
- assimilation of potassium nitrate or additional carbohydrates (p. 399–403)
- appearance of isolate in Sabouraud broth (p. 422)
- inhibition of isolate by cycloheximide (p. 416)
- urease activity, if not included in the commercial system (p. 424)
- phenoloxidase (caffeic acid disk test [p. 379])

Consult Tables 4–12 (pp. 128, 130, 132, 137, 142, 151, 152, and 164) for identification of genus and species.

Whenever complete identification is not performed, it is advisable to hold the isolate for at least 7 days after reporting and to add a note to the report that is similar to “If further identification is required, please call Microbiology, extension xxxx.”

Direct Identification of Yeasts from Blood Culture (by PNA FISH)

Peptide nucleic acid–fluorescent *in situ* hybridization (PNA FISH) tests (AdvanDx, Woburn, MA) are useful for the direct identification of *Candida* spp. from positive blood cultures that display yeast cells on Gram stain. The system utilizes PNA fluorescent-labeled probes that target the species-specific ribosomal RNA (rRNA) of individual *Candida* spp. (see p. 351 for further explanation of molecular reaction). The products are in kit form and are FDA approved; the procedure is easy, detailed in the package instructions, and completed in 1.5 h. The major steps:

1. A drop of positive blood culture is mixed with a drop of fixative in the well on the slide and allowed to dry; fixation is completed by heat or methanol.

2. A drop of fluorescent-labeled PNA probe is added to the well, coverslipped, and incubated for 30 min at 55°C. (If the isolate's rRNA is complementary to the PNA probe, hybridization will occur.)
3. The slide is immersed in heated wash solution (55°C), the coverslip is removed, and the slide is incubated in the solution for 30 min (PNA probe not hybridized to the yeast cells is removed from the slide).
4. Mounting fluid and coverslip are placed on the slide and examined with a fluorescent microscope within 2 h (the microscope must be equipped with the appropriate filter for PNA FISH available from AdvanDx).

If the species for which the probe is testing is present on the slide, the yeast cells will show fluorescence. When using the probe specific for *C. albicans*:

Green fluorescent cells = *C. albicans*

No fluorescence = species present in the positive blood culture is other than *C. albicans*

There is a dual-probe reagent available that tests simultaneously for *C. albicans* and *C. glabrata*:

Green fluorescent cells = *C. albicans*

Red fluorescent cells = *C. glabrata*

No fluorescence = species other than *C. albicans* and *C. glabrata*

The Yeast Traffic Light kit, using PNA FISH, tests simultaneously for five species of *Candida* in the single drop of positive blood culture broth. The method separates species, or pairs of species, in accordance with their anticipated response to fluconazole:

Green fluorescent cells = *C. albicans* or *C. parapsilosis* (both of these species show good susceptibility to azole therapy; additionally, *C. parapsilosis* has exhibited limited response to echinocandins; hence, the "light is green" for fluconazole use)

Yellow fluorescent cells = *C. tropicalis* (susceptible to fluconazole but higher dose is required for effective treatment)

Red fluorescent cells = *C. glabrata* or *C. krusei* (many isolates of *C. glabrata* may require a high dose of fluconazole for effectiveness, while others may be completely resistant; *C. krusei* is considered innately resistant to fluconazole; an echinocandin is usually more appropriate)

Simultaneous plating of the blood culture broth onto CHROMagar Candida medium (p. 406) can provide differentiation of *C. albicans* from *C. parapsilosis* and *C. krusei* from *C. glabrata*, as well as isolation of yeasts other than those targeted by the above tests. Although the laboratory may perform antifungal susceptibility tests on some of the isolates, the PNA FISH tests yield rapid results on which to base immediate early therapy for the most commonly encountered *Candida* spp.

Isolation of Yeast When Mixed with Bacteria

Yeasts may grow mixed with bacteria on primary culture. It is absolutely essential that only pure cultures be used in assimilation, fermentation, and other biochemical tests for identification.

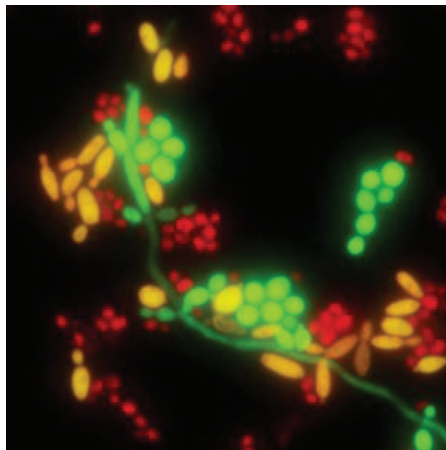


Figure (<http://www.opgen.com/pathogenid/pna-fish-products/candida-pna-fish/>)

Careful streaking of the organisms onto plated medium with or without antibiotics often yields isolated colonies. Likelihood of isolation is increased with the use of a chromogenic yeast agar (pp. 406, 407), inhibitory mould agar (p. 413), or similar media containing antibacterial(s). Cycloheximide (the antifungal in Mycosel agar) inhibits some yeasts.

Persistent bacterial contamination may be eliminated with the following acidification method.

1. Place 10 ml of Sabouraud dextrose broth in each of four tubes.
2. To tube no. 1, add 1 drop of 1 N HCl.
3. To tube no. 2, add 2 drops of 1 N HCl.
4. To tube no. 3, add 3 drops of 1 N HCl.
5. To tube no. 4, add 4 drops of 1 N HCl.
6. Make a suspension of the yeast-bacterium mixture in sterile water. Add a drop of the suspension to each of the four Sabouraud dextrose broth-HCl tubes. Incubate at 25–30°C for 24 h.
7. Subculture a loopful of broth from each tube to plated media and incubate for 48 h.

In most instances, there will be an acid concentration at which the bacteria are inhibited and the yeast is allowed to grow.

Germ Tube Test for the Presumptive Identification of *Candida albicans**

1. Make a very light suspension of a yeastlike organism in 0.5–1.0 ml of sterile serum in a 12-by-75-mm test tube. The optimum inoculum is 10^5 – 10^6 cells per

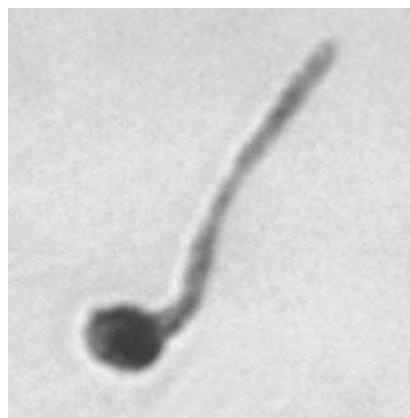
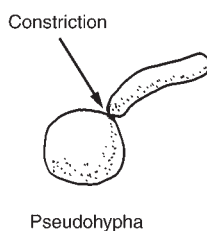
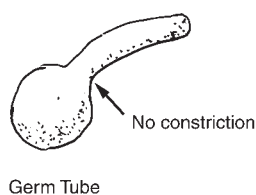
* The germ tube test is also positive with *Candida dubliniensis*, but this organism is seldom encountered; if suspected, further testing must be performed (see Table 6 (p. 132)).

ml; an increased concentration of inoculum often causes a significant decrease in germ tube production and false-negative results. Very few yeast cells should be seen per microscopic field when results are read. Pooled human sera is an excellent medium for production of germ tubes, but it must be negative for hepatitis and HIV and free of antifungal agents. Fetal bovine serum, rabbit serum, and other animal sera are also commonly utilized. A Pasteur pipette tip can be used to inoculate the serum and can be left in the tube during incubation.

2. Incubate at 35–37°C for no longer than 3 h.
3. Place 1 drop of the yeast-serum mixture on a slide with a coverslip. Examine microscopically for germ tube production.

A known strain of *C. albicans* should be tested with each new batch of serum.

Germ tubes are the beginnings of true hyphae and appear as filaments that are NOT constricted at their points of origin on the parent cell. If the filaments are constricted and septate at their points of origin, they are pseudohyphae, not germ tubes.



Positive germ tube test.

Rapid Enzyme Tests for the Presumptive Identification of *Candida albicans*

Several systems are commercially available for the rapid (5–30 min) presumptive identification of *C. albicans* in culture. Each system utilizes two substrates, one for the detection of β -galactosamidase and the other for the detection of L-proline aminopeptidase. Of the clinically encountered yeasts, only *C. albicans* produces both of these enzymes. Evaluations of the systems show them to be rapid, acceptable alternatives to the germ tube test. Manufacturers' instructions must be followed.

Caffeic Acid Disk Test

Caffeic acid is one of several good substrates for the detection of phenoloxidase enzyme activity. When phenoloxidase is present, the caffeic acid is broken down to melanin, resulting in a dark brown to black color. As *Cryptococcus neoformans* and *Cryptococcus gattii* are the only clinically encountered yeasts that produce

phenoloxidase, the test is extremely useful for identification and/or confirmation of these organisms (species separation can be accomplished with canavanine glycine bromothymol blue (CGB) agar [p. 405]). Although birdseed agar may also be used to detect phenoloxidase activity, the advantages of the disk test are speed and improved reliability.

1. Culture the isolated yeast on cornmeal-Tween 80 agar. Because phenoloxidase is inhibited by glucose, it is essential that the growth medium be glucose free; cornmeal-Tween 80 agar is usually the most readily available medium to meet this requirement.
2. Place a caffeic acid disk (available from Remel [Lenexa, KS] and from Hardy Diagnostics [Santa Maria, CA]) on a glass slide. Moisten the disk with 30–40 μl of water.
3. Inoculate the disk with several colonies of the isolated yeast.
4. Incubate the disk at 22–35°C in a moist chamber.
5. Read for development of brown to black pigment. Most positive reactions are seen within 30 min, but the test should be held at least 4 h before being considered negative. On rare occasions, an organism may require several generations of growth on cornmeal agar before the enzyme can be detected. (Color Plate 54.)

Olive Oil Disks for Culturing *Malassezia* spp.

(K. McGowan, Children's Hospital of Philadelphia, personal communication)

Malassezia spp. other than *M. pachydermatis* (p. 158) require long-chain fatty acids for growth. This need is usually met in the laboratory by overlaying an agar plate surface with sterile olive oil. As an alternative method, olive oil-saturated paper disks can be used.

1. Place large ($\frac{1}{2}$ -in. [~ 13 -mm]-diameter) blank disks (catalog no. 231122; BBL, Sparks, MD) in a screw-cap sterile flask or jar (a urine specimen container is suitable).
2. Pour in enough olive oil to reach a depth of 10–20 mm.
3. Place disks (no more than can be easily covered by the oil) in the container and allow to soak in the olive oil; add oil and disks to container as needed. They can be kept for many months without deterioration.
4. When screening for *Malassezia* spp., streak specimen or aliquot of blood culture broth (after ~ 48 h of incubation) onto mycology agar without cycloheximide.
5. Remove a saturated disk from the olive oil, using a swab or forceps (allow excess oil to drip down the side of the container).
6. Place the disk on the area of initial inoculation of the plate.
7. Incubate at 35–37°C.

Malassezia spp., if present, often grow well in the area surrounding the disk. Special media for recovery of *Malassezia* spp., i.e., Dixon agar (p. 410) or Leeming-Notman agar (p. 414), may be required.

Conversion of Thermally Dimorphic Fungi in Culture

A mycelial colony that is morphologically suggestive of a thermally dimorphic fungus at room temperature is classically grown in its yeastlike form at 35–37°C to confirm its identification. This or another confirmation method is essential, as there are several monomorphic moulds that resemble the filamentous phase of thermally dimorphic fungi. In addition, the mould forms of some dimorphic fungi may not be definitive microscopically, and the yeast phase can serve to identify them.

These organisms **MUST** be handled in a biological safety cabinet. To test for the ability of a mycelial form to convert to a yeast phase, proceed as follows.

1. Inoculate mycelial growth onto a fresh, moist slant of brain heart infusion agar in a screw-cap tube. A small amount of brain heart infusion broth added to the tube ensures sufficient moisture. After the fragment of mould inoculum has been emulsified in the moisture, pull it up and place it on the agar slant.
2. Incubate the slant at 35–37°C, preferably in CO₂. Keep the screw cap closed tightly to retain the moisture, but loosen it daily to allow the colony to “breathe.”
3. Periodically examine the slant, and make a wet preparation of any yeastlike area.
4. If only a mycelial form grows, transfer it to another moistened brain heart infusion slant and incubate it again at 35–37°C. It may be necessary to make several serial transfers to attain complete conversion to the yeast phase.

In some instances, *in vitro* conversion is exceedingly difficult or slow, and exoantigen tests or DNA probes may be preferred or required to confirm identification. The manufacturer’s instructions (Gen-Probe, San Diego, CA) should be followed for performing DNA probe tests. For complete instructions on performing the exoantigen test, see Hazen and Howell (2010), pp. 8.9.19–8.9.21.

Sporulation Inducement Method for *Apophysomyces* and *Saksenaea* (Padhye and Ajello, 1988)

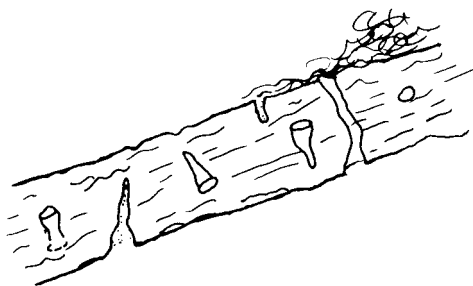
This procedure is recommended for use in identifying all nonsporulating mucormycetes isolated from clinical specimens. The growth on Sabouraud dextrose agar (SDA) at 25°C should consist of broad, nonseptate to sparsely septate, branched, hyaline hyphae without sporangial formation. The manipulations must be carried out in a biological safety cabinet.

1. Grow the isolate on an SDA plate at 25°C for 1 week.
2. Aseptically cut out a 1-cm² agar block permeated with the hyphal growth.
3. Transfer the block to a petri plate containing 20 ml of sterile distilled water and 0.2 ml of filter-sterilized 10% yeast extract. Shrink-seal the plates to prevent spillage.
4. Incubate the blocks in the water solution at 35–37°C (lower temperature will yield fewer or no sporangia). After 5 days of incubation, a thin film of growth should appear over the surface of the water.
5. Make wet preparations (with lactophenol cotton blue) of portions of the film on days 5, 10, and 15 of incubation. Examine microscopically for sporangia. See pp. 193 and 195 for descriptions of *Apophysomyces elegans* and *Saksenaea vasiformis*, respectively.

***In Vitro* Hair Perforation Test (for Differentiation of *Trichophyton mentagrophytes* and *Trichophyton rubrum*)**

1. Place 1-cm-long fragments of healthy human hair in a petri plate or tube. Sterilize by autoclaving at 15 lb/in² for 15 min. The clearest and fastest results are obtained with light-colored hair from a child (<12 years old). Adult hair will give correct results if the hair has not been exposed to hair spray or a combination of bleach and permanent wave (Salkin et al., 1985b).
2. In a sterile 50-ml screw-cap tube, place 8–10 of the sterile hair fragments; add 20–25 ml of sterile distilled water and 0.1 ml of 10% filter-sterilized yeast extract. (Plastic conical tubes used for concentrating specimens for acid-fast bacilli work well; tubes have proven safer to handle than petri plates when doing this test.)
3. Place several fragments of the fungal culture in the tube.
4. Incubate at room temperature for 4 weeks or until a positive reaction is seen (average, 8–10 days). Examine at weekly intervals by placing one or two hairs from the culture onto a slide with a drop of lactophenol cotton blue and a coverslip. Look for wedge-shaped perforations caused by hyphae that penetrate the hairs perpendicularly. (Color Plate 129.)

The hair is perforated by *T. mentagrophytes* but not by *T. rubrum* (see Table 23 [p. 271]).



Positive *in vitro* hair perforation test.

Germ Tube Test for Differentiation of Some Dematiaceous Fungi

Germ tube formation plays an important role in identification of the genera of dematiaceous fungi that form macroconidia with transverse septa, i.e., *Bipolaris*, *Drechslera*, and *Exserohilum* spp.

1. Place a drop of water on a microscope slide.
2. Inoculate the drop of water with a small amount of the actively growing fungus. Examine the slide microscopically to confirm that conidia are present.
3. Place a coverslip over the suspension.
4. Incubate in a moist chamber at room temperature for 8 to 24 h.
5. Examine the slide microscopically to determine the origin and orientation of the germ tubes. See Table 22 (p. 246) for interpretation of results.

Temperature Tolerance Testing

When determining at what temperatures an organism can grow, it is important that the inoculations are equivalent. This is best attained by making an evenly dispersed suspension of the organism in sterile saline and placing the same amount on each slant or plate that will be incubated at various temperatures. One culture must be at optimum temperature (usually 30°C) as a control.

Maintenance of Stock Fungal Cultures

No single method is best for all types of fungi. Regardless of the method employed, a young, actively growing culture is essential for the first step.

OIL OVERLAY TECHNIQUE

Obtain a small but actively growing fungal culture on a short slant of Sabouraud dextrose agar or potato dextrose agar (PDA). Pour over it a good grade of sterile heavy mineral oil (Saybolt viscosity, 330 at 100°F; autoclaved at 15 lb/in² for 45 min in half-filled 250-ml flasks). It is absolutely necessary that the oil cover not only the fungal colony but also the whole agar surface; otherwise, the exposed agar will act as a wick and in time cause the medium to dry out and the colony to die.

The stock cultures are stored upright at room temperature, and most will remain viable for several years. Unfortunately, some fungi must be transferred very often to sustain viability.

To transfer a fungus from an oiled culture, remove a small portion of the colony with a long, disposable inoculating needle or a sterile applicator stick. Drain off the oil along the inside of the tube, and place the inoculum on a fresh agar slant.

WATER CULTURE TECHNIQUE

Rub a sterile moistened swab over the surface of an actively conidiating or sporulating fungus colony; wash the swab off into a screw-cap tube containing ~4 ml of sterile distilled water. Tighten the cap, and store at room temperature. Add sterile distilled water periodically if any evaporation occurs.

To prepare a subculture, shake the water culture to resuspend the fungus. With a sterile Pasteur pipette aseptically transfer 2–3 drops of the suspension to a slant or plate of a supportive fungal medium. Incubate the agar at 25–30°C.

FREEZING TECHNIQUE

Culture the fungus on a slant of PDA in a screw-cap glass test tube. Incubate at 25–30°C until the organism reaches young maturity and is actively producing conidia or spores. Close caps tightly, and place tubes in a freezer. Best results are obtained at –70°C.

To subculture, remove the tube from the freezer and chip a small portion of the colony from the agar slant. Place the chipped section on a fresh agar slant, and immediately return the frozen slant to the freezer. If the culture is allowed to thaw, it should not be refrozen; the fungus must be transferred to a fresh slant, incubated to maturity, and then frozen.

Freezing can also be accomplished by suspending a young reproductive isolate in a cryovial containing 10–15% glycerol and freezing at –70°C. The organisms to most likely fail to survive are *Candida dubliniensis* and the dermatophytes (see Espinel-Ingroff et al., 2004).

Controlling Mites

Mites are tiny arachnids (0.05–0.15 mm long, barely visible to the naked eye) that graze on fungi if given the opportunity. Their most common means of entry into the mycology laboratory is on specimens of hair, skin, or nails or in cultures received from other laboratories that have a mite problem. These tiny creatures can cause enormous problems as they walk from one culture to another, contaminating the cultures with the bacteria and fungi they carry on their bodies.

The very best system of mite control is to prevent their initial entry into the laboratory. If there is a risk of mites, culture plates and tubes should be sealed with parafilm or shrink seals. If mites are detected and have spread, it is advisable to autoclave and discard all of the cultures that are not absolutely essential to save and to thoroughly clean the laboratory with an effective disinfectant. An attempt to save indispensable cultures can be made by transferring them to slants of media containing 0.1% hexachlorocyclohexane or by exposing the mite-infested fungi to naphthalene (mothball) crystals. Exposure can be attained by placing the cultures in a plastic bag along with naphthalene (or other chemicals poisonous to mites); if the fungi are in tubes, the caps must be loosened. Alternatively, a gauze plug containing the crystals can be put in the neck of each tube. By placing petri plates of naphthalene in incubators and refrigerators for at least a week, those areas can be cleared of mites.

Do not conduct yeast assimilation tests in the vicinity of the chemicals; the vapors may be used as a carbon source by the yeasts, yielding false-positive results.

Another method for salvaging cultures relies on the fact that mites are rapidly killed by freezing, while their eggs can survive for 48–72 h at -20°C . Therefore, a culture might be saved by placing it at -20°C for more than 72 h and then subculturing an area that appears to be unaffected by the mites. A dissecting microscope is useful in this instance to select the best colonies.

For further information on mites, see
McGinnis, 1980, pp. 454–456, 559
Pitt and Hocking, 2009, pp. 50–51

Staining Methods

Acid-Fast Modified Kinyoun Stain for *Nocardia* spp. [387](#)

Acid-Fast Stain for Ascospores [388](#)

Ascospore Stain [388](#)

Calcofluor White Stain [388](#)

Giemsa Stain [389](#)

Gomori Methenamine Silver (GMS) Stain [390](#)

Gram Stain (Hucker Modification) [392](#)

Lactophenol Cotton Blue [393](#)

Lactophenol Cotton Blue with Polyvinyl Alcohol (PVA)
(Huber's PVA Mounting Medium, Modified) [393](#)

Rehydration of Paraffin-Embedded Tissue
(Deparaffination) [394](#)

Positive and negative controls must be run on new lots of stain and periodically thereafter. If the stain is not frequently used, it is advisable to run controls each time the staining procedure is performed on an unknown organism.

Acid-Fast Modified Kinyoun Stain for *Nocardia* spp.

The filaments of *Nocardia* spp. usually appear at least partially acid-fast with this staining procedure. Acid-fast organisms stain pink to red.

Procedure

1. Make a smear and fix over flame.
2. Flood slide with Kinyoun carbol-fuchsin for 5 min.
3. Pour off excess stain.
4. Flood slide with 50% alcohol and immediately wash with water.
5. Decolorize with 1% aqueous sulfuric acid (see alternative decolorizer below).
6. Wash with tap water.
7. Counterstain with methylene blue or brilliant green for 1 min.
8. Rinse with water, dry, and examine under oil immersion objective.

Negative and positive controls should be included each time this method is used.

Reagents

Kinyoun carbol-fuchsin (same as for staining mycobacteria)

Basic fuchsin	4 g
Phenol	8 ml
Alcohol, 95%	20 ml
Distilled water	100 ml

Dissolve the dye in the alcohol, and then add the water and phenol.

1% aqueous H₂SO₄ (see alternative decolorizer below)

Concentrated H ₂ SO ₄	1 ml
Distilled water	99 ml

Be sure to add the acid to the water, not vice versa.

Counterstain

Methylene blue	2.5 g
Ethanol, 95%	100.0 ml

or

Brilliant green	0.5 g
Distilled water	100.0 ml

Alternative decolorizing agent

The following decolorizing agent, commonly used in the fluorochrome method for acid-fast bacilli, may be successfully used to substitute for steps 4 and 5 of the above procedure.

Concentrated HCl	0.5 ml
Ethanol, 70%	99.5 ml

Acid-Fast Stain for Ascospores

When staining for ascospores, the classic Kinyoun method is used (as for mycobacteria), i.e., decolorize with solution of:

Concentrated HCl 3 ml
Ethanol, 95% 97 ml

Add acid to alcohol; mix well.

The ascospores will stain red; other cells will take the counterstain.

Ascospore Stain

1. Culture the fungus on a medium that promotes ascospore formation (see p. 398).
2. Make thin smear and fix in flame.
3. Stain with 5% aqueous malachite green (filter before using) containing Tergitol 7 (Sigma Chemical Co., St. Louis, MO) for 3 min.
4. Wash with tap water.
5. Decolorize with 95% ethyl alcohol for 30 s.
6. Wash with tap water.
7. Counterstain with 5% aqueous safranin for 30 s.
8. Wash with water, allow to dry, and examine under oil immersion objective.

Ascospores stain green, and vegetative cells stain red.

The acid-fast stain (above) is also useful for observing ascospores. It should be noted that ascospores may be observed in simple aqueous wet mounts without any stain.

Calcofluor White Stain

Calcofluor white (CFW), which also is known in Europe as Blankofluor and Uvitex, has become a valuable and routine reagent in the clinical mycology laboratory. It binds to β -(1 $\rightarrow$ 3) and β -(1 $\rightarrow$ 4) polysaccharides, such as cellulose and chitin (present in fungal cell walls), and fluoresces when exposed to long-wave UV light. It is exceedingly useful for direct microscopic examination of specimens, as the fungal elements are seen much more easily than with the older traditional plain potassium hydroxide (KOH) preparations. Additionally, it is exceptional for exhibiting certain morphologic structures of fungi that have been isolated on culture.

Preparation of Stain Solution

CFW M2R (Fluorescent Brightener 28)* 100 mg
Evans blue..... 50 mg
Distilled water..... 100 ml

* The powder form is available from Sigma-Aldrich (St. Louis, MO). Several other companies produce prepared solutions of various formulations and names. It is imperative to follow manufacturers' instructions for use; excitation wavelengths may vary and require filters other than those described below.

Mix well. Store at room temperature in a dark bottle.

Procedure

1. Smear specimen onto glass slide. The specimen may be stained while wet or allowed to dry on the slide and rehydrated with the CFW solution. If the slide is to be subsequently reprocessed with another stain, it must first be allowed to dry and then heat fixed before applying the CFW.
2. Add 1 drop of CFW solution. One drop of 10% KOH may also be added if clearing is required.
3. Apply coverslip. Allow slide to sit at room temperature at least 5 min (it often takes a few minutes for the CFW to penetrate the organism).
4. Examine slide with a UV (fluorescent) microscope equipped with an excitation filter that transmits wavelengths between 300 and 412 nm (the optimum wavelength for excitation of CFW M2R is 365 nm) and a barrier filter (530 nm) that removes UV and blue light while transmitting longer wavelengths.

The required light source is a mercury vapor lamp. Quartz halogen bulbs are usually not suitable, as the energy output is too low.

Fungal elements will stand out as bright apple green on a red background; the barrier filters that allow transmission of shorter wavelengths and yield white elements on a blue background are no longer recommended (for eye safety).

Quality control must be performed on a routine basis to ensure the quality of the reagent, procedure, and microscope.

Positive control is a suspension of a yeast or mould, e.g., *Candida* sp. or *Aspergillus* sp.

Negative control is a solution without fungi.

If the slide is to be saved or restained, remove the coverslip, rinse the slide briefly with distilled water, and air dry. The slide can later be restained with CFW or other stains, such as Gomori methenamine silver, Gram, etc.

For further information, see

Harrington and Hageage, 2003

Giemsa Stain

The Giemsa stain is used for the detection of intracellular *Histoplasma capsulatum* in bone marrow or blood smears. The blood smears are made from the buffy coat of centrifuged citrated blood or from the bottom of the tube, where heavily infected cells are often found. *H. capsulatum* is seen as small, oval yeast cells that stain light to dark blue with a hyaline halo due to the unstained cell wall (see [p. 170](#)). Giemsa stain is commercially available.

Procedure

1. Place slide in 100% methanol for 1 min (to fix smear).
2. Drain off methanol.
3. Flood slide with Giemsa stain (freshly diluted 1:10 with distilled water) for 5 min.
4. Wash with water; air dry.

Gomori Methenamine Silver (GMS) Stain

Of the special stains for fungi, methenamine silver nitrate is considered by many to be the most useful for screening clinical specimens. It provides better contrast and often stains fungal elements that may not be revealed by other procedures. Fungi are sharply delineated in black against a pale green background. The inner parts of hyphae are charcoal gray. Certain bacteria (including *Nocardia* spp.), as well as some tissue elements, also take the stain, so it must be remembered that all that is gray or black is not necessarily a fungus.

Histology laboratories routinely perform some method of GMS staining. The following are simpler and faster methods that may be used on smeared material or deparaffinized tissue.

METHENAMINE SILVER STAIN (METHOD OF MAHAN AND SALE (1978))* **Solutions Required (All Are Aqueous Solutions)**

10% chromic acid (chromium trioxide)
5% silver nitrate
3% methenamine (hexamethylenetetramine)
5% borax (sodium borate)
1% gold chloride
1% sodium metabisulfite
5% sodium thiosulfate

Preparation of Stains

Methenamine silver nitrate solution

3% methenamine 40 ml
5% silver nitrate 2 ml
5% borax 3 ml
Distilled water.....35 ml

This solution must be freshly prepared before use; it can be used only once. The other solutions may be reused for up to 1 month provided fungal contamination does not occur.

Light green stock solution

Light green SF-yellowish 0.2 g
Distilled water.....100.0 ml
Glacial acetic acid.....0.2 ml

Solution is stable for 1 year.

Working solution

Stock light green solution 10 ml
Distilled water.....40 ml

Solution is stable for 1 month.

* Prepared reagents for an equally rapid method that employs a microwave oven are commercially available from Sigma Diagnostics (St. Louis, MO).

Procedure

Before starting, heat Coplin jar of methenamine silver nitrate solution in oven or water bath until the solution becomes a deep golden brown ($\sim 95^{\circ}\text{C}$). Then, proceed as follows.

1. If slide to be stained is paraffin fixed, rehydrate (see procedure, [p. 394](#)); other slides must be fixed by heating or submerging in alcohol. Positive-control slides must be included each time the staining procedure is performed.
2. Rinse slides with water.
3. Place slides in reagents in Coplin jars as follows.
 - a. Chromic acid (discard after use) 10 min
 - b. Running tap water 5 s
 - c. 1% sodium metabisulfite 1 min
 - d. Hot tap water 1 min
 - e. Methenamine silver nitrate solution, $\sim 95^{\circ}\text{C}$ 5–10 min

Periodically remove the control slide, wash with water, and observe microscopically to determine when optimal staining has been achieved. The color should never be so intense as to obscure the morphologic detail of a fungus. Prolonged staining time may be required when old and nonviable fungal elements or filaments of actinomycetes are suspected.

When control shows optimal staining, rinse all slides in hot tap water and then in gradually cooler water.

- f. Distilled water Rinse
 - g. 1% gold chloride 10 s
 - h. Distilled water Rinse
 - i. 5% sodium thiosulfate 3 min
 - j. Running tap water 30 s
 - k. Light green working solution 30 s
4. Rinse twice with each increasing concentration of ethanol: 70%, 95%, and absolute alcohol.
 5. Dip slides twice in xylene.
 6. Place drop of mounting medium on slide, and cover with coverslip.

MORE RAPID METHENAMINE SILVER STAIN (Method of Shimono and Hartman (1986))

The solutions and procedure for the Shimono-Hartman method are identical to those for the method of Mahan and Sale (see above), except that the methenamine solution is not preheated and the slides are stained while on a heating platform.

1. Begin staining procedure as for Mahan and Sale's method above, steps 3.a–d.
2. At this point, place slides on a 70°C heating platform or hot plate. The slides can be placed directly on the heated surface or in a large (150-mm-diameter) glass petri plate to handle any spillage.
3. Layer the methenamine silver solution onto the slides.

4. When the control tissue becomes golden brown (~1 min if slide is directly on heating plate, 4–5 min if slide is in a petri plate), remove slides from heat.
5. Continue from step g to completion as in Mahan and Sale's method.

The advantages of this system are that the time required to heat the methenamine solution in volume is eliminated along with the general manipulations and handling of the hot solution. Additionally, a smaller volume of methenamine solution is usually required, resulting in cost saving.

Gram Stain (Hucker Modification)

Fungi are Gram positive but often stain poorly.

Procedure

1. Fix the smear by passing it over a flame.
2. Place crystal violet solution on the slide for 20 s.
3. Wash gently with tap water.
4. Apply Gram iodine solution to the slide for 20 s.
5. Wash gently with tap water.
6. Decolorize quickly in solution of equal parts acetone and 95% ethanol.
7. Wash gently with tap water.
8. Counterstain with safranin for 10 s.
9. Wash with tap water; air dry or blot dry.

Reagents

Crystal violet solution

- a. Crystal violet, 85% dye content 2 g
Ethyl alcohol, 95%.....10 ml
Dissolve the dye in alcohol.
Add distilled water 100 ml
- b. Ammonium oxalate 4 g
Distilled water.....400 ml

Dissolve the ammonium oxalate in the water.

Mix the crystal violet-alcohol solution (a) with the ammonium oxalate solution (b).

Gram iodine solution

- Iodine 1 g
Potassium iodide.....2 g

Dissolve the iodine and potassium iodide completely in 5 ml of distilled water.

- Add distilled water.....240 ml
- Sodium bicarbonate,
5% aqueous solution 60 ml
(i.e., 3 g of NaHCO_3 + 57 ml of distilled water)

Mix well; store in amber glass bottle.

Counterstain

Safranin O 1 g
Ethyl alcohol, 95% 40 ml

Dissolve the dye in the alcohol.

Add distilled water.....400 ml

Mix well.

Note: A slide that has been Gram stained can be decolorized by flooding it with acetone for 30–60 s and rinsing it well with water. Special fungus-staining procedures can then be performed on the slide.

Lactophenol Cotton Blue

Lactophenol cotton blue is used as both a mounting fluid and a stain. Lactic acid acts as a clearing agent and aids in preserving the fungal structures, phenol acts as a killing agent, glycerol prevents drying, and cotton blue gives color to the structures.

Lactic acid 20 ml
Phenol crystals 20 g
(or phenol, concentrated..... 20 ml)
Glycerol (or glycerine) 40 ml
Distilled water 20 ml
Cotton blue 0.05 g
(or 1% aqueous solution 2 ml)

1. Dissolve the phenol in the lactic acid, glycerol, and water by gently heating (if crystals are used).
2. Add cotton blue (Poirrier's blue and aniline blue are analogous to cotton blue).
3. Mix well. For use, see [p. 372](#).

Lactophenol Cotton Blue with Polyvinyl Alcohol (PVA) (Huber's PVA Mounting Medium, Modified*)

This modification of Huber's plastic mount (Huber and Caplin, 1947) is excellent for making permanent mounts of fungal wet preparations or slide cultures. Upon drying for at least 24 h on a flat surface, these mounts are permanent and will not be dissolved in ether, xylene, or alcohols, and the fixed fungal structures remain picture-perfect for years.

Reagents

PVA; molecular weight, 70,000–100,000 (Sigma-Aldrich Co., catalog no. P-1763)
Phenol, purified grade (Sigma no. P-5566 or Fisher Scientific no. 89-949-179)
Lactic acid, ACS reagent (Sigma no. L-1893)
Aniline blue, certified (Aldrich Chemical Co., catalog code 252746). This is analogous to cotton blue.

* Appreciation is extended to Lawrence M. Bobon of Wilmington, DE, for his invaluable contributions in the updating and reinstatement of Huber's method.

Preparation

1. Add 7.5 g of PVA powder to 50 ml of cold deionized water in a beaker.
2. Transfer beaker to a heated stirring plate; add a magnetic rod for mixing.
3. Place a thermometer in the beaker to monitor temperature.
4. Add 22 g of lactic acid (BEFORE adding phenol).
5. Add 22 g of phenol crystals (or 22 ml of melted phenol).
6. Add 0.05 g of aniline blue.
7. Heat and stir the solution until the temperature reaches 90°C. Do not boil or go over 100°C. Remove from hot plate.
8. Dispense into small dropper bottles (washed used ones from blood bank panel cells work well). Tighten dropper bottle caps, and store at room temperature.

Procedure

Place 1 drop of PVA mounting fluid on a slide with a sample of fungal growth. Apply coverslip. Allow to dry on a flat surface.

Huber's modified PVA mounting medium may be used as a replacement for lactophenol cotton blue. Care must be taken to avoid coverslip runover or droppings on the benchtop, since they are difficult to remove. While the solution is in liquid form, it can be cleaned from surfaces with water; after it dries, a razor blade is required to remove the hardened material.

Slides can be examined microscopically at low or high dry power as soon as the slides are prepared. After adequate drying time (2–4 days), the slides can be examined under oil immersion, cleansed in xylene, decontaminated by dipping in disinfectant, or shipped (without risk of leakage) to a reference laboratory.

The solution is available in prepared form as MycoPerm Blue from Scientific Device Laboratory Inc. (Des Plaines, IL).

Rehydration of Paraffin-Embedded Tissue (Deparaffination)

Treat slides as follows.

Xylene (in Coplin jar)..... 12 min

Repeat, using two more jars of xylene.

Absolute ethanol..... Rinse twice

95% ethanol Rinse twice

70% ethanol Rinse twice

Distilled water..... Rinse twice

Proceed with staining procedure.

Media

- Acetamide Agar 397
- Arylsulfatase Broth 398
- Ascospore Media 398
- Assimilation Media (for Yeasts) 399
- Birdseed Agar (Niger Seed Agar;
Staib Agar) 403
- Brain Heart Infusion (BHI) Agar 404
- Buffered Charcoal-Yeast Extract
(BCYE) Agar 404
- Canavanine Glycine Bromothymol Blue
(CGB) Agar 405
- Casein Agar 406
- CHROMagar Candida Medium 406
- ChromID Candida Medium 407
- Chromogenic Candida Agar 408
- Citrate Agar 408
- Cornmeal Agar 408
- Dermatophyte Test Medium (DTM) 409
- Dixon Agar (Modified) 410
- Esculin Agar 410
- Fermentation Broth for Yeasts 411
- Gelatin Medium 412
- Inhibitory Mould Agar (IMA) 413
- Leeming-Notman Agar (Modified) 414
- Loeffler Medium 414
- Lysozyme Medium 415
- Middlebrook Agar Opacity Test for
Nocardia farcinica 415
- Mycosel Agar 416
- Nitrate Broth (for Nitrate
Reduction Test) 416
- Polished Rice, or Rice Grain,
Medium 417
- Potato Dextrose Agar and Potato
Flake Agar 417
- Rapid Assimilation of Trehalose
(RAT) Broth 418
- Rapid Sporulation Medium (RSM) 420
- SABHI Agar 420
- Sabouraud Dextrose Agar (SDA) 420
- Sabouraud Dextrose Agar with
15% NaCl 421
- Sabouraud Dextrose Broth 422
- Starch Hydrolysis Agar 422
- Trichophyton* Agars 422
- Tyrosine, Xanthine, or Hypoxanthine
Agar 423
- Urea Agar 424
- Water Agar 424
- Yeast Extract-Phosphate Agar
with Ammonia 425

Many of the media prepared for mycology are used relatively rarely in the typical clinical microbiology laboratory. Freshness during long storage may be maintained by dispensing agar medium into screw-cap tubes, autoclaving it, allowing it to cool as butts, and storing it (with caps tightly closed) in the refrigerator. The media must be labeled with the name of the medium, the date of preparation, and the date of expiration. When needed, the appropriate number of butts are melted in a boiling-water bath and allowed to cool as slants or cooled slightly and poured into petri plates.

Most of the media can be purchased in dehydrated form. Commercially prepared tubes or plates are also available for most, but not all, of the formulations.

Whenever a new batch of medium is placed in use, positive- and negative-control organisms must be tested. If the medium is not often used, it is advisable to run controls each time an unknown organism is tested.

Acetamide Agar

(For testing the ability of *Nocardia* spp. to utilize acetamide as the sole source of carbon and nitrogen. The medium is commercially available in dehydrated or prepared form.)

Acetamide	10.0 g
Sodium chloride.....	5.0 g
Dipotassium phosphate.....	1.39 g
Monopotassium phosphate.....	0.73 g
Magnesium sulfate.....	0.5 g
Phenol red.....	0.012 g
Agar	15.0 g
Distilled water.....	1,000.0 ml

1. Dissolve by boiling for 1 min; dispense in screw-cap tubes.
2. Autoclave (15 lb/in²) for 15 min.
3. Allow tubes to cool in slanted position or as butts to be melted and slanted as needed.
4. Store tubes in the dark at 2–25°C.

Test Procedure

1. Inoculate the agar slant with the organism to be tested.
2. Incubate at 35°C for up to 14 days.

Interpretation

Positive: Color of the medium changes from yellow-orange to purplish red.

Negative: Color of medium remains yellow-orange.

For phenotypic identification of *Nocardia* spp., see [Table 3 \(p. 118\)](#).

Arylsulfatase Broth

(Used in the battery of tests for phenotypic identification of commonly encountered *Nocardia* spp.)

The medium is essentially Middlebrook 7H9 broth supplemented with the substrate tripotassium phenolphthalein disulfate. If the organism being tested produces the enzyme arylsulfatase, the substrate will be degraded and free phenolphthalein will be released. The presence of free phenolphthalein is indicated by a pink to red reaction when an alkaline reagent (sodium carbonate) is added to the broth.

Medium

If using commercially prepared broth, the one available with the highest concentration of tripotassium phenolphthalein disulfate (0.003 M) should be used. The best sensitivity has been shown with 0.008 M, but this requires preparation by the laboratory. The concentration of the product being used must therefore be considered when comparing results to those in the literature.

Reagent (1 M Sodium Carbonate Solution)

1. Dissolve 5.3 g anhydrous Na_2CO_3 in 50 ml of distilled water.
2. Store in the dark or in brown glass screw-cap bottle. (Discard if solution develops cloudiness, indicating contamination.)

Test Procedure

1. Inoculate broth with a loopful of actively growing isolated organism.
2. Mix well to disperse organism throughout broth.
3. Incubate at 35–37°C in an aerobic (non- CO_2) incubator for 14 days.
4. After incubation, add 6 drops of 1 M Na_2CO_3 to the broth and observe for color change immediately, as the color reaction is not stable.

Interpretation

Positive: A color change of the medium to any shade of pink or red.

Negative: No color change.

Ascospore Media

ACETATE ASCOSPORE AGAR

Potassium acetate	5.00 g
Yeast extract	1.25 g
Dextrose	0.50 g
Agar	15.00 g
Distilled water.....	500.00 ml

1. Dissolve by boiling for 1 min; dispense in screw-cap tubes.
2. Autoclave (15 lb/in²) for 15 min.
3. Allow tubes to cool in slanted position or as butts to be melted and slanted as needed.

GORODKOWA MEDIUM

Dextrose	1.25 g
NaCl	2.60 g
Beef extract	5.00 g
Agar	5.00 g
Distilled water	500.00 ml

1. Dispense into screw-cap tubes.
2. Autoclave (15 lb/in²) for 15 min.
3. Allow to harden as slants or as butts to be melted and slanted as needed.

V-8 MEDIUM FOR ASCOSPORES

V-8 vegetable juice	500 ml
Dry yeast	10 g
Agar	20 g
Distilled water	500 ml

1. Dissolve agar in water by boiling.
2. Mix vegetable juice and dry yeast, adjust to pH 6.8 with 20% KOH, add to agar-water mixture, and mix well.
3. Dispense into screw-cap tube.
4. Autoclave (15 lb/in²) for 15 min.
5. Allow to cool as slants or as butts to be melted and slanted as needed.

Assimilation Media (for Yeasts)

Assimilation is the utilization of a carbon (or nitrogen) source by a microorganism in the presence of oxygen. A positive reaction is indicated by the presence of growth or a pH shift in the medium.

WICKERHAM BROTH METHOD Carbon Assimilation Medium

Yeast nitrogen base	6.70 g
Appropriate carbohydrate	5.00 g
Distilled water	100.00 ml

1. If necessary, heat to dissolve.
2. Sterilize by Seitz or membrane filter.
3. Add 0.5 ml of the solution to 4.5 ml of sterile distilled water in screw-cap tubes.*

These tubes may now be stored in the refrigerator, ready for use, for 1 month.

Note: Care must be taken to ensure that carbon compounds are pure and not mixed with other carbohydrates. It is advisable to check each new lot of a carbon compound

* The carbon and nitrate assimilation media can be distributed in 150- μ l aliquots into 96-well microplates. In that instance, prepare a suspension of the yeast adjusted to an optical density of 0.21–0.29. Inoculate each well with 50 μ l of the suspension (resulting in a final inoculum of 2×10^5 to 2×10^6 CFU/ml). Incubate at 25–30°C.

with control yeasts that can and cannot assimilate it before using the material in assimilation studies. The sugars most commonly employed are listed in [Tables 5, 10, and 11](#) (pp. 130, 151, and 152).

Nitrate Assimilation Medium

Yeast carbon base.....	11.70 g
Potassium nitrate (KNO ₃).....	0.78 g
Distilled water.....	100.00 ml

1. Warm gently to dissolve.
2. Sterilize by Seitz or membrane filtration.
3. Add 0.5 ml of medium to 4.5 ml of sterile distilled water in screw-cap tubes.*

These tubes may be stored in the refrigerator for 1 month.

Tubes of yeast nitrogen base without sugar and yeast carbon base without KNO₃ should be prepared and used as controls to check “carryover” of nutrients that may have been stored within the yeast cell when grown on the previous medium.

Test Procedure

1. Make a suspension of the yeast in sterile distilled water. This suspension should not exceed the turbidity of McFarland no. 1 standard (prepared by mixing 0.1 ml of 1% barium chloride with 9.9 ml of 1% sulfuric acid).
2. Add 0.1–0.2 ml of the yeast suspension to each tube of medium. Include a tube of yeast nitrogen base without any carbon source and a tube of yeast carbon base without KNO₃ as controls for carryover.
3. Incubate tubes at the yeast’s optimal temperature. If the organism grows at 35–37°C, positive reactions are usually more rapid at this temperature. Shaking the culture tubes will also enhance growth.
4. Examine cultures over a period of 7–14 days for dense turbidity caused by growth.
5. The negative-control tubes without a carbon or nitrogen source should show no growth. If growth is present, the test is invalid because of carryover. In such cases, a small amount of growth from each tube should be transferred to another tube of the same medium and the test should be repeated.

For identification of yeasts, see [Tables 5, 10, and 11](#) (pp. 130, 151, and 152, respectively).

AUXANOGRAPHIC PLATE METHOD (HALEY AND STANDARD MODIFICATION) For Carbon Assimilation Tests

Yeast nitrogen base	0.67 g
Noble or washed agar	20.00 g
Distilled water.....	1,000.00 ml

1. Dispense in 20-ml quantities into 18-by-150-mm screw-cap tubes.
2. Autoclave (15 lb/in²) for 15 min.
3. Allow to harden as butts. Store in refrigerator.

Test Procedure

1. Melt a tube of nitrogen base medium in a boiling-water bath; allow to cool to 47–48°C.
2. With a sterile cotton-tipped applicator, make a heavy suspension of a 24- to 72-h yeast culture in 4 ml of sterile distilled water. The density should equal that of a McFarland no. 4 or 5 standard.
3. Pour the yeast suspension into the tube of molten yeast nitrogen base agar. Mix very thoroughly by inverting tube several times.
4. Pour the yeast-agar mixture into a sterile 15-by-150-mm petri plate. Allow to solidify at room temperature.
5. Place carbohydrate disks (available from BBL-Difco Microbiology Systems [Sparks, MD]), evenly spaced, on the plate.
6. Incubate at 30°C for 18–24 h; examine for growth around each disk. Any amount of growth around a disk indicates that the yeast assimilates that sugar. The plates may be reincubated for an additional 24 h, but reincubation is usually not necessary.

For Nitrate Assimilation Tests

Medium

Yeast carbon base.....	12 g
Noble or washed agar.....	20 g
Distilled water.....	1,000 ml

Tube in 20-ml aliquots and autoclave at 15 lb/in² for 15 min. Store in refrigerator.

Peptone solution for growth control

Peptone	10 g
Distilled water.....	100 ml

Sterilize by filtration; store in refrigerator.

Test Procedure

1. Melt a tube of yeast carbon base medium in a boiling-water bath; allow to cool to 47–48°C.
2. Make an aqueous suspension of the yeast to a density equal to a McFarland no. 1 standard.
3. Add 0.1 ml of the yeast suspension to the tube of medium. Mix thoroughly.
4. Pour the yeast-agar mixture into a sterile 15-by-100-mm petri plate. Allow to solidify at room temperature.
5. Place ~1 mg of KNO₃ crystals on agar surface away from the center of the plate.
6. Place ~0.1 ml of peptone solution (growth control) on agar surface opposite the KNO₃ site.
7. Incubate at 30°C for 48–96 h. Growth must occur in the “peptone area” for test to be valid. If growth is seen in the peptone area, examine for growth in the KNO₃ area (growth indicates assimilation of KNO₃).

AGAR WITH INDICATOR METHOD

For Carbon Assimilation Tests

This modification of the Wickerham medium was devised by Adams and Cooper (1974). It is easier to read than the conventional methods, yet it is equally reliable. The medium is in the form of agar slants with an indicator added. It is less troublesome than other formulations, as it can be sterilized by autoclaving after the carbohydrates have been added. The quality of each batch of medium should be tested with standard reference strains of yeasts.

Basal medium

Bromcresol purple (1.6%)	0.2 ml
0.1 N NaOH	1.0 ml
Noble agar.....	2.0 g
Deionized water	90.0 ml

Heat to dissolve.

Stock carbohydrate solution

Carbohydrate.....	1.00 g
(if using raffinose.....	2.00 g)
Yeast nitrogen base	0.67 g
Deionized water	10.00 ml

Mix to dissolve; gently heat if necessary.

Preparation

1. Add each stock carbohydrate solution to a separate portion of the melted agar base; leave one portion free of carbohydrate for use as negative controls.
2. Mix well.
3. Adjust to pH 7.0.
4. Dispense in 5-ml amounts in 16-by-125-mm screw-cap tubes.
5. Sterilize by autoclaving at exactly 10 lb/in² for 10 min.
6. Allow to solidify in a slanted position.
7. Store in refrigerator at 4°C.

Inoculation and Incubation

1. Suspend a 2-mm loopful of pure culture in 9 ml of sterile water.
2. Inoculate each assimilation slant with 0.1 ml of the suspension.
3. Incubate at 25–30°C, examining at 7 and 14 days for abundant growth and acid production (yellow).

Assimilations are considered negative when there is no significant difference between the growth of the organism on the carbohydrate medium and that on the control medium without carbohydrate.

MODIFIED POTASSIUM NITRATE ASSIMILATION TEST (Pincus et al., 1988)

Potassium nitrate.....	1.4 g
Yeast carbon base.....	1.6 g
Bromothymol blue.....	0.12 g
Noble agar.....	16.0 g
Distilled water.....	1,000 ml

1. Adjust final pH to 5.9–6.0.
2. Dispense into screw-cap tubes.
3. Autoclave at 15 lb/in² for 15 min.
4. Cool tubes in slanted position.

Test Procedure

1. Inoculate the surface of the slant with yeast isolate.
2. Incubate at 25–30°C.
3. Examine daily; most positive results are seen within 24 h.

Interpretation

Positive: Slant becomes blue-green or blue.

Negative: Slant remains greenish yellow.

Birdseed Agar (Niger Seed Agar; Staib Agar)

<i>Guizotia abyssinica</i> seed.....	50 g
(Commonly known as niger seed; it is available at most stores that sell bird feed.)	
Distilled water.....	100 ml

1. Pulverize in blender.
2. Add 900 ml of distilled water.
3. Boil for 30 min.
4. Cool and filter through four layers of gauze, and then add enough distilled water to make volume 1,000 ml.
5. Add:

KH ₂ PO ₄	1 g
Creatinine	1 g
Agar	15 g

(Glucose, 1 g, is added in the formulation for identification of *Candida dubliniensis* [p. 129], but it can inhibit pigment production by *Cryptococcus neoformans* and *Cryptococcus gattii*.)

(Chloramphenicol, 1 g, may be added in the formulation for pigment production and isolation of *C. neoformans* and *C. gattii* [pp. 148 and 150]).

6. Mix well. Autoclave (15 lb/in²) for 15 min.
7. Pour into tubes and cool in slanted position or as butts to be melted and slanted or plated as needed.

Test Procedure

A. For pigment production by *C. neoformans* and *C. gattii*

Inoculate with suspected *Cryptococcus* sp. (fresh isolate) and incubate at 25–30°C for no more than 7 days.

Only *C. neoformans* and *C. gattii* produce phenoloxidase, which breaks down the substrate, resulting in the production of melanin and the development of dark brown to black colonies. Colonies of other yeasts are cream to beige. (Color Plate 53.)

Chemically defined tests such as the caffeic acid disk test also detect phenoloxidase and have the advantage of rapidity and sensitivity; see p. 379.

B. For differentiation of *C. dubliniensis* versus *Candida albicans*

1. Inoculate a plate of the medium with a 48-h-old colony; streak for isolation.
2. Incubate at 30°C for 48–72 h. Examine macroscopic morphology of colonies (see Table 6 [p. 132]).

Brain Heart Infusion (BHI) Agar

BHI agar is classically recommended for the cultivation of fastidious pathogenic fungi, such as *Histoplasma capsulatum* and *Blastomyces dermatitidis*. The ingredients are listed here for ease of comparison with other enriched fungal media that are commonly used.

BHI	8.0 g
Peptic digest of animal tissue	5.0 g
Pancreatic digest of casein	16.0 g
Sodium chloride.....	5.0 g
Dextrose	2.0 g
Disodium phosphate	2.5 g
Agar	13.5 g
Distilled water.....	1,000 ml

Antibiotics are often added to inhibit bacteria, and sheep blood is added to further enrich the medium and enhance the growth of fastidious pathogenic fungi.

Buffered Charcoal-Yeast Extract (BCYE) Agar

This medium is best known for cultivation of *Legionella* spp., but it has also proved useful in the recovery of *Nocardia* spp.

Excellent recovery occurs on nonselective and selective BCYE agars. The formulation containing vancomycin (BCYE-PAV) is preferred over the formulation with cefamandole (PAC) for the primary isolation of *Nocardia* spp. from specimens likely to contain other organisms. The media are commercially available. The contents of BCYE media (BBL, Sparks, MD) are

Yeast extract.....	10.0 g
L-Cysteine HCl.....	0.4 g
Ferric pyrophosphate	0.25 g
ACES [N-(2-acetamido)- 2-aminoethanesulfonic acid] buffer ...	10.0 g
Charcoal, activated	2.0 g

α -Ketoglutarate	1.0 g
Agar	15.0 g
Purified water.....	1,000.0 ml

BCYE Selective Agar with PAV has added:

Polymyxin B.....	40,000 units
Anisomycin	80.0 mg
Vancomycin.....	0.5 mg

Canavanine Glycine Bromothymol Blue (CGB) Agar (Kwon-Chung et al., 1982; Klein et al., 2009)

CGB agar is used for the differentiation of *Cryptococcus gattii* versus *Cryptococcus neoformans*.

Solution A

Glycine	10 g
KH ₂ PO ₄	1 g
MgSO ₄	1 g
Thiamine HCl.....	1 mg
L-Canavanine sulfate	30 mg
Distilled water.....	100 ml

1. Dissolve ingredients; adjust to pH 5.6.
2. Filter sterilize the solution using a 0.45 (or 0.22)- μ m filter.
3. Store in refrigerator if not used same day.

Solution B

Bromothymol blue.....	0.4 g
0.01 N NaOH	64 ml
Distilled water.....	36 ml

1. Dissolve the bromothymol blue in the NaOH.
2. Add the water.

Medium Preparation

Distilled water.....	880 ml
Solution B.....	20 ml
Agar	20 g

Mix in flask and autoclave at 25 lb/in² for 15 min. Cool to ~50°C.

Add

Solution A.....100 ml

Mix well and dispense into plates or into tubes for slants.

CGB agar can be stored in the refrigerator for at least 3 months without losing its efficacy.

Inoculate agar with a small amount of the isolate. Incubate at 25–30°C for 48 h.

Interpretation

C. gattii yields a positive reaction. The medium turns from greenish yellow to cobalt blue; this is due to the organism's ability to grow in the presence of L-canavanine and utilize glycine as its sole source of carbon and nitrogen, causing an increase in pH.

C. neoformans yields a negative (or very weak) reaction. The medium remains greenish yellow, as the organism is inhibited by canavanine and does not utilize glycine. If the inoculum is too heavy, a weak reaction may occur; this should be interpreted as negative.

Casein Agar

(For differentiation of aerobic actinomycetes and characterization of some dematiaceous fungi.)

Solution A

Skim milk (dehydrated or instant nonfat dry milk).....	10 g
Distilled water.....	90 ml

Add milk with constant stirring to avoid lumping.

Solution B

Agar	3 g
Distilled water.....	97 ml

1. Autoclave each solution separately at 15 lb/in² for 10 min.
2. Cool both solutions to 45–50°C, and then combine solutions and mix well.
3. Pour into petri plates (or into screw-cap tubes and allow to solidify as butts to be melted and poured as needed).

Inoculate an area approximately the size of a dime (15 mm) heavily with a pure culture. Include positive- and negative-control cultures. Three or four organisms can be tested on one 100-mm-diameter plate if they are evenly spaced. Incubate at 30 or 37°C for 2 weeks. Examine every few days for clearing (hydrolysis) of casein around or directly beneath the colony.

For results with *Nocardia* spp., see [Table 3](#) (p. 118).

For results with “black yeasts,” see [Table 20](#) (p. 227).

CHROMagar Candida Medium

CHROMagar Candida (CAC) medium (Becton Dickinson, Sparks, MD; CHROMagar, Paris, France) is for the isolation of all, and the definitive differentiation of a few, of the most commonly encountered yeasts in the clinical laboratory. ([Color Plate 45](#).) It has proven acceptable for use as the sole primary fungal medium for specimens in which yeasts are the primary concern, i.e., throats, urines, and genitals (“TUGs”) and blood cultures smear-positive for yeast. It is extremely effective in detecting mixed yeast populations in clinical specimens. Colonies grown on CAC (Becton Dickinson) yield valid results when applied to the rapid assimilation of trehalose test (Scientific Device Laboratory Inc., Des Plaines, IL) for *Candida glabrata* (Murray et al., 2005). Isolates from CAC (Becton

Dickinson) have also been shown to yield excellent results when inoculated directly into the Vitek 2 ID-YST card (bioMérieux, Hazelwood, MO) (Aubertine et al., 2006).

Chromogenic substrates produce unique and specific colors for the identification of

Candida albicans (light to medium green; *Candida dubliniensis* often forms darker green colonies on primary isolation, but this property is often lost on subcultures)

Candida tropicalis (dark blue to metallic blue-purple)

Candida krusei (light rose with a whitish border; rough)

Studies have shown that further identification tests for these three species are not necessary.

Other yeasts (cream color or light to dark mauve; *C. glabrata* forms pink colonies but cannot be reliably distinguished on this basis from other yeasts)

Candida rugosa (unique blue-green with whitish border; slightly rough)

Filamentous fungi (colors that may differ from those exhibited on routine mycology agar, but their microscopic morphology does not change)

Notes:

- Plates produce the best color development in the colonies when incubated at 37°C (they should NOT be incubated below 30°C).
- Incubate in atmospheric air, not in CO₂, for 36–48 h.
- It is advisable to minimize exposure of the plates to light both before and during incubation.
- All yeasts and filamentous fungi will grow, while bacteria are inhibited by chloramphenicol. The moulds may not have their typical colony color, but they will retain their characteristic microscopic morphology (Morhaime et al., 2004).

ChromID Candida Medium

(Formerly Candida ID2.)

ChromID Candida (bioMérieux, Durham, NC) is a chromogenic medium for the culture and isolation of yeasts, the identification of *Candida albicans*, and the separation of other yeast species into two groups. Most filamentous fungi will also grow. Bacteria are inhibited. It can be useful in detecting mixed yeast populations in clinical specimens. The plates are incubated in ambient air for 48 h at 35–37°C; some yeasts and yeastlike species require 30°C for optimal growth.

The colony colors indicate the following.

Blue = *C. albicans* or *Candida dubliniensis* (some strains of *Trichosporon* can be blue, but the colony morphology is very different from that of the *Candida* spp.; *C. dubliniensis* is often turquoise)

Pink = *Candida tropicalis*, *Candida kefyr*, *Candida lusitanae*, or *Candida guilliermondii*

White, dry, downy = *Candida krusei*

White or cream, smoother = other yeasts and some filamentous moulds (*Cryptococcus* spp. may be white, pink, or blue)

Note: Plates must always be stored and incubated in the dark.

Chromogenic Candida Agar

Chromogenic Candida Agar (Brilliance Candida Agar [Oxoid, Basingstoke, UK]) consists of a proprietary chromogenic substrate for detection and identification of the following *Candida* spp. by colony morphology and color: *C. albicans*, *C. tropicalis*, and *C. krusei*.

C. albicans/*C. dubliniensis* (light to medium green)

C. tropicalis (dark blue)

C. krusei (pink-brown with a whitish border)

C. glabrata and other yeasts (beige, yellow, brown)

Citrate Agar

(Used in the battery of tests for the phenotypic identification of *Nocardia* spp.)

Simmons citrate agar is commercially available in dehydrated form or prepared as slants. It is used to test for the ability of the isolate to utilize sodium citrate as its sole source of carbon.

Test Procedure

1. Inoculate slant lightly with a pure culture of the isolate.
2. Incubate for 10–14 days at 35–37°C in an aerobic atmosphere.
3. Observe slants periodically for color change to blue.

Interpretation

Positive: Growth with the slant turning an intense blue color.

Negative: No growth to trace growth with no change in color (medium remains dark green).

For application to identification of *Nocardia* spp., see [Table 3 \(p. 118\)](#).

Cornmeal Agar

Cornmeal.....	40 g
Agar	20 g
Tween 80 (polysorbate 80).....	10 ml
Distilled water.....	1,000 ml

1. Mix cornmeal well with 500 ml of water; heat to 65°C for 1 h.
2. Filter through gauze and then paper until clear; restore to original volume.
3. Adjust to pH 6.6–6.8; add agar dissolved in 500 ml of water.
4. Add Tween 80; autoclave (15 lb/in²) for 15 min.
5. Dispense into petri plates or into screw-cap tubes to form butts to be melted and poured as needed.

Cornmeal with Tween 80 is used in distinguishing the different genera of yeasts and the various species of *Candida* and can also be useful in slide cultures, as it stimulates conidiation in many fungi.

If 10 g of dextrose is added to the medium in place of the Tween 80, the medium can be used to differentiate *Trichophyton mentagrophytes* from *Trichophyton rubrum* on the basis of pigment production (see [Table 23](#) [p. 271]).

For studying the morphology of yeasts, the **Dalmau method** is recommended. It is performed by using one-fourth or one-third of a cornmeal-Tween 80 agar plate for each organism. ([Color Plate 65.](#))

1. Make one streak of a young, actively growing yeast down the center of the area (do not cut the agar); make three or four streaks across the first to dilute the inoculum.
2. Cover with a 22-by-22-mm coverslip.
3. Incubate at room temperature, in the dark, for 3 days.
4. Examine by placing the plate, without its lid, on the microscope stage and using the low-power ($\times 100$) and high-dry ($\times 430$) objectives. The most characteristic morphology (especially the terminal chlamydospores of *Candida albicans*) is often found near the edge of the coverslip.

C. albicans should be included as a control for production of chlamydospores and blastoconidia.

Dermatophyte Test Medium (DTM)

Specimens from hair, skin, or nails may be inoculated directly onto DTM and incubated at room temperature with the cap of the culture tube loose. Dermatophytes change the color of the medium from yellow to red within 14 days. Care must be taken in specimen collection and interpretation of results, as many contaminants and other fungi increase the number of false-positive changes in color. DTM does not interfere with macroscopic morphology and microscopic characteristics of the dermatophytes, but it cannot be used to study pigment production because of the intense red color of the indicator. ([Color Plate 128.](#))

Phytone	10.0 g
Dextrose	10.0 g
Agar	20.0 g
Phenol red solution	40.0 ml
0.8 M HCl	6.0 ml
Cycloheximide (Upjohn)	0.5 g
Gentamicin sulfate (Schering)	0.1 g
Chlortetracycline HCl (Lederle)	0.1 g
Distilled water	1,000.0 ml

1. Dissolve the phytone, dextrose, and agar by boiling them in the water.
2. While stirring, add 40 ml of phenol red solution (0.5 g of phenol red dissolved in 15 ml of 0.1 N NaOH made up to 100 ml with distilled water).
3. While stirring, add the 0.8 M HCl.
4. Dissolve cycloheximide in 2 ml of acetone, and add to hot medium while stirring.
5. Dissolve gentamicin sulfate in 2 ml of distilled water, and add to medium while stirring.

6. Autoclave at 12 lb/in² for 10 min, and cool to ~47°C.
7. Dissolve chlortetracycline in 25 ml of sterile distilled water in sterile container, and add to medium while stirring.
8. Dispense into sterile 1-oz (~30-ml) screw-cap bottles or screw-cap tubes; slant and cool. The final pH of the medium is 5.5 ± 0.1, and the medium should be yellow in color.
9. Store in refrigerator at 4°C.

Dixon Agar (Modified) (Ellis et al., 2007, p. 183)

(For culturing *Malassezia* spp. Characteristic colony morphology of the individual species is better demonstrated on this medium than on Leeming-Notman medium [p. 414], but Leeming-Notman may provide better recovery of some species.)

Malt extract (Oxoid L39)	9.0 g
Ox bile, desiccated (Oxoid L50)	5.0 g
Bacto tryptone	1.5 g
Oleic acid	0.5 g
Tween 40	2.5 ml
Glycerol	0.5 ml
Bacto agar	3.0 g
Distilled water	250.0 ml

1. Soak ingredients in a small amount of the water for 15 min.
2. Bring remaining water to boil, add to the soaked ingredients, and bring to boiling again; stir constantly.
3. Dispense into tubes.
4. Autoclave at 121°C for 10 min.
5. Allow tubes to cool in slanted position or pour into petri plates. If the medium is to be used relatively rarely, allow to solidify as butts to be melted and poured into petri plates as needed.

Esculin Agar

(Used in the identification of *Nocardia* spp. and in the differentiation of *Candida norvegensis* from similar *Candida* spp.)

When esculin (a glycoside) is hydrolyzed, it yields esculetin; esculetin, in the presence of an iron salt, forms a brown-black complex that diffuses into the medium. The medium, containing esculin, ferric citrate, and supportive ingredients for growth, is commercially available (this formulation does not contain bile salts).

Test Procedure

1. Streak several actively growing isolated colonies onto the slanted medium.
2. Incubate tubes aerobically at 35–37°C (with caps loosened) for 24–72 h for *Candida* spp. and for up to 10 days for *Nocardia* spp.
3. Observe for a black diffusing color in the medium surrounding the colonies.

Interpretation

Positive hydrolysis: Black color diffused in medium.

Negative: No black color in the medium.

For *Nocardia* spp., see [Table 3](#) (p. 118); for select *Candida* spp., see [Table 8](#) (p. 137).

Fermentation Broth for Yeasts

Broth

Bromothymol blue.....	0.04 g
Powdered yeast extract	4.50 g
Peptone	7.50 g
Distilled water.....	1,000.00 ml

Stock Carbohydrate Solutions

Carbohydrate.....	6 g
Distilled water.....	100 ml

1. Filter sterilize through a 0.22- μ m-pore-size filter.
2. Dissolve bromothymol blue in 3 ml of 95% ethanol. Add to other ingredients.
3. Dispense 2-ml aliquots into narrow screw-cap tubes.
4. Place Durham tube (mouth down) into each tube of broth.
5. Autoclave at 15 lb/in² for 15 min. Allow to cool.
6. Add 1 ml of each carbohydrate solution to separate tubes of broth. (After the carbohydrates have been added, the broths may be stored for 1 month in the refrigerator.)

Test Procedure

1. Inoculate each of the carbohydrate broths with a pure culture of the organism grown on a sugar-free medium. Be sure that the Durham tube is completely filled with broth before incubating.
2. Incubate at room temperature for 10–14 days, examining at 48- to 72-h intervals for production of gas (observed in Durham tube).

Gas production is the only reliable evidence of carbohydrate fermentation; acid production (color change in indicator) may simply indicate that the carbohydrate has been assimilated. All fermented carbohydrates will also be assimilated, but many carbohydrates that are assimilated are not necessarily fermented. For identification of yeasts, see [Table 5](#) (p. 130) and [Table 11](#) (p. 152).

Yeast fermentation broth is commercially available from Remel (Lenexa, KS) (with bromothymol blue) and from Becton Dickinson/BBL (Sparks, MD) (without bromothymol blue).

Gelatin Medium

FRAZIER PLATE METHOD

The Frazier plate method is the most rapid and reliable test for the ability of microorganisms to decompose gelatin (i.e., exhibit proteolytic activity); it is therefore highly recommended over the old conventional butt method.

Agar

Standard Methods Agar (BBL)
or
Plate Count Agar (Difco).....24 g
Distilled water.....1,000 ml

1. Heat to dissolve, mixing well.
2. Remove from heat; cool slightly.
3. Add
Gelatin.....4 g
4. Mix thoroughly until gelatin dissolves.
5. Autoclave at 15 lb/in² for 12 min.
6. Dispense into small petri plates or into screw-cap tubes and allow to solidify as butts to be melted and poured as needed.

Developer (Acid Mercuric Chloride)

Mercuric chloride (HgCl₂) 12 g
Distilled water.....80 ml
Concentrated HCl 16 ml

Prepare in order listed; HgCl₂ will dissolve only after HCl is added. Mix well.
(An aqueous saturated solution of ammonium sulfate may also be used as a developer.)

Test Procedure

1. Inoculate the agar surface in one central spot ~15 mm in diameter.
2. Incubate at room temperature until growth is mature.
3. Flood surface of plate with developer.

Mercuric chloride (or ammonium sulfate) precipitates gelatin, causing the medium to become cloudy where gelatin is intact.

Gelatin decomposition (a positive test result) is demonstrated by a clear zone around the colony.

See [Table 2 \(p. 115\)](#) and [Table 17 \(p. 218\)](#).

BUTT LIQUEFACTION METHOD

Brain heart infusion.....	25 g
Gelatin.....	120 g
Distilled water.....	1,000 ml

1. Heat to dissolve ingredients; adjust to pH 7.4–7.6.
2. Dispense 5- to 8-ml aliquots in 16-by-125-mm screw-cap tubes.
3. Autoclave at 15 lb/in² for 15 min. Allow to harden as butts.

Test Procedure

1. Inoculate the surface and just below the surface of the medium with the organism to be tested.
2. Incubate tubes at room temperature for 4 weeks.
3. Examine at weekly intervals for proteolytic activity by placing in refrigerator for 1 h and observing for liquefaction. If the medium solidifies in the refrigerator, proteolysis has not occurred and the tube should be returned to room temperature and retested the following week.

See [Table 2 \(p. 115\)](#) and [Table 17 \(p. 218\)](#).

Inhibitory Mould Agar (IMA)

IMA is an enriched medium that contains chloramphenicol (some formulations also contain gentamicin) but no cycloheximide; bacteria are inhibited, while fungi grow well. When specimens are contaminated with bacteria, IMA is especially useful in recovering fungi that are inhibited by cycloheximide and would not grow on Mycosel agar, e.g., *Cryptococcus neoformans/gattii*, *Lomentospora prolificans*, the mucormycetes, many species of *Candida* and *Aspergillus*, and most saprobic or opportunistic fungi. IMA has been shown to be superior to Sabouraud dextrose agar as a primary medium (Scognamiglio et al., 2010); see [Table 31 \(p. 371\)](#). IMA is commercially available in dehydrated and prepared form (BBL Microbiology Systems, Cockeysville, MD, and many other suppliers). The ingredients are listed below to allow comparison with other enriched fungal media.

Pancreatic digest of casein.....	3.0 g
Peptic digest of animal tissue	2.0 g
Yeast extract.....	5.0 g
Dextrose	5.0 g
Starch.....	2.0 g
Dextrin	1.0 g
Chloramphenicol	0.125 g
Sodium phosphate.....	2.0 g
Magnesium sulfate	0.8 g

Ferrous sulfate	0.04 g
Sodium chloride.....	0.04 g
Manganese sulfate	0.16 g
Agar	15.0 g
Distilled water.....	1,000.0 ml

Leeming-Notman Agar (Modified)

(Leeming and Notman, 1987; Kaneco et al., 2007)

(For isolation of *Malassezia* spp. Provides better recovery of some *Malassezia* spp. than does Dixon agar [p. 410]. It is also a good medium for maintenance of *Malassezia* cultures.)

Bacteriological peptone.....	10.0 g
Glucose	10.0 g
Yeast extract.....	2.0 g
Ox bile, desiccated	8.0 g
Glycerol.....	10.0 ml
Glycerol monostearate	0.5 g
Olive oil.....	20.0 ml
Agar	15.0 g
Deionized water	1,000.0 ml

1. Combine the above ingredients and let stand for ~10 min.
2. Adjust pH to 6.2 with 1 M HCl.
Tween 60 5.0 ml
3. Warm Tween 60 to 60–70°C before adding to mixture.
4. Boil mixture briefly to dissolve components.
5. Autoclave 15 lb/in² for 20 min.
6. Cool to ~50°C. (Add antimicrobial solutions if desired: chloramphenicol 50 µg/ml; cycloheximide 200 µg/ml.)
7. Pour into petri plates. Store in refrigerator.

Loeffler Medium

(For testing proteolytic activity of *Cladosporium* species and similar organisms [the gelatin test, p. 412, also is a test for proteolytic activity].)

The medium may be obtained in dehydrated form or bought commercially prepared from a number of sources.

Test Procedure

1. Place a fragment of the fungus on the agar slant, taking care not to place the inoculum on the bottom part of the slant that is usually covered with liquid.
2. Incubate all tubes at room temperature for 4 weeks.
3. Examine tubes at weekly intervals for evidence of proteolytic activity, i.e., disintegration of medium.

Usually, saprophytic species of *Cladosporium* are proteolytic, whereas pathogenic species, such as *Cladophialophora carrionii*, *Cladophialophora bantiana*, and *Cladophialophora boppii*, are not (see Table 17 [p. 218]).

Lysozyme Medium

Basal Glycerol Broth

Peptone	1.0 g
Beef extract	0.6 g
Glycerol	14.0 ml
Distilled water	200.0 ml

1. Combine ingredients; mix well to dissolve.
2. Pour 95 ml of broth into a separate container.
3. Dispense the remainder into screw-cap tubes in 5-ml quantities. These will be used as control tubes.
4. Autoclave the tubes and the separate portion at 15 lb/in² for 15 min.

Lysozyme Broth

Lysozyme	50 mg
0.01 N HCl	50 ml

1. Sterilize by filtration.
2. Aseptically add 5 ml of lysozyme solution to the 95 ml of basal glycerol broth.
3. Mix well.
4. Dispense 5-ml aliquots into sterile screw-cap tubes.
5. Store all tubes in the refrigerator.

Test Procedure

1. Inoculate a tube of control broth (glycerol broth without lysozyme) and a tube of lysozyme broth with the organism to be tested.
2. Incubate at 25–30°C until control tube shows good growth. The organism must grow in the control tube for the test to be valid. Growth in the lysozyme broth indicates resistance to the enzyme.

A known *Streptomyces* sp. should be used as a susceptible control organism; i.e., growth must occur in the control tube but not in the lysozyme tube to prove the lysozyme is active.

Middlebrook Agar Opacity Test for *Nocardia farcinica* (Flores and Desmond, 1993)

Of the clinically encountered *Nocardia* spp., *N. farcinica* has the distinct characteristic of causing opacity (dense milky whiteness) in the medium around the colonies when grown on Middlebrook 7H10 or 7H11 agar. The media are commercially

available from a variety of suppliers. The opacity occurs on plated or slanted medium within 10 days, but may be seen a few days earlier on plates and is best observed by viewing from the bottom of the plate.

Mycosel Agar

Mycosel agar is a selective medium that is frequently part of the battery that is used for primary isolation of fungi. It contains chloramphenicol to inhibit the growth of bacteria and cycloheximide to inhibit fungi that are commonly considered saprobes. However, cycloheximide also inhibits the growth of some known pathogens, e.g., *Cryptococcus neoformans/gattii*, *Aspergillus fumigatus*, *Lomentospora prolificans*, *Talaromyces marneffe* (formerly *Penicillium marneffe*), some species of *Candida*, and most mucormycetes, as well as many other opportunistic fungi.

The medium is also used to test an organism's ability to grow in the presence of cycloheximide; the interpretive information may state the level of cycloheximide against which the reference organisms were tested. Mycosel agar contains 0.04% cycloheximide.

Mycosel is produced in prepared and dehydrated form by Becton Dickinson (Sparks, MD) and by other suppliers; the formulations may vary slightly.

Papaic digest of soybean meal	10.0 g
Dextrose	10.0 g
Agar	15.5 g
Cycloheximide	400.0 mg
Chloramphenicol	50.0 mg
Distilled water.....	1,000.0 ml

Nitrate Broth (for Nitrate Reduction Test)

(Used in the battery of tests for phenotypic identification of commonly encountered *Nocardia* spp.)

The medium and reagents are commercially available. Manufacturers' instructions must be followed, as there may be slight variations in brands.

Reagents

- Reagent A: 0.8% sulfanilic acid in 5 N acetic acid
- Reagent B: 0.6% *N,N*-dimethyl-1-naphthylamine in 5 N acetic acid
- Zinc dust

Test Procedure

1. Emulsify a large loopful of growth from a pure culture into the broth.
2. Incubate, with caps loosened, at 35–37°C aerobically for 3 days.
3. Following incubation, add reagent A and then reagent B (amount in accordance with manufacturer's instructions) to broth. The appearance of a red color in the

broth within 2 min indicates that the nitrate has been reduced to nitrite (i.e., a positive nitrate reduction reaction).

4. If the broth remains colorless, add a pinch (~20 mg) of zinc dust (addition of too much zinc dust may hide the reaction or cause a too rapidly disappearing reaction). No red color developing within 5–10 min indicates that nitrate was reduced beyond nitrite, and the test is positive for nitrate reduction. The development of a red color after the addition of zinc indicates that nitrate is still present, i.e., the test is negative for nitrate reduction.

For application of results to *Nocardia* spp., see [Table 3](#) (p. 118).

Polished Rice, or Rice Grain, Medium

The medium is used on the rare occasion when an isolate of *Microsporium audouinii* exhibits abortive forms of macroconidia similar to those of a poorly sporulating *Microsporium canis*.

“Polished” rice is the regular common white rice available in grocery stores. The outer husk has been removed, and the layers of bran are milled until the grain is white.

Each test consists of

Polished rice (without added vitamins)	3.2 g
Distilled water.....	10.0 ml

1. Place ingredients in a wide screw-cap tube or vial. Prepare enough tubes/vials for the test organism(s) and two controls (a known *M. audouinii* and a known *M. canis*).
2. Autoclave at 15 lb/in² for 15 min.
3. Allow to cool.

Test Procedure

1. Inoculate the surface of the rice grains with the actively growing isolate. Incubate for up to 14 days at 30°C.
2. Also inoculate a routine supportive medium as a growth control.

M. audouinii grows very poorly or not at all; it often produces only a brown discoloration. *M. canis* shows good growth of white mycelium with production of a lemon yellow pigment (other dermatophytes are also able to grow well on the rice).

Potato Dextrose Agar and Potato Flake Agar

Potato dextrose agar and potato flake agar are available commercially prepared or in dehydrated form and should be prepared according to manufacturers’ instructions. They support the growth of most clinically encountered fungi, and some laboratories use one of them as a primary medium. They can sometimes stimulate the production of conidia and pigment when other media fail to do so and are therefore often recommended for slide cultures or for inducing an isolate to exhibit a characteristic pigment.

Rapid Assimilation of Trehalose (RAT) Broth

(Stockman and Roberts, 1985, and personal communication)

The RAT test is used for the rapid identification of *Candida glabrata*. It is ONLY for yeasts that

- form colonies that are relatively slow growing and small
- are microscopically small and oval and have terminal budding
- form neither germ tubes nor pseudohyphae in the germ tube test
- are suggestive of *C. glabrata* on CHROMagar Candida agar

The RAT test is based on the utilization of trehalose in the presence of a protein inhibitor, cycloheximide. This broth contains more nitrogen and carbohydrate than the traditional Wickerham assimilation broth and incorporates an indicator, bromcresol green, that has a low pH range (3.8–5.4). *C. glabrata* utilizes the trehalose and produces acid more quickly than do other yeast isolates. (Color Plate 51.)

PREPARATION OF REAGENTS

Yeast Nitrogen Base

A. Stock solution

1. Add 6.7 g of yeast nitrogen base to 100 ml of distilled water.
2. Dissolve by gently heating.
3. Filter sterilize.

B. Working solution

Dilute by combining 20 ml of the stock yeast nitrogen base solution with 80 ml of sterile distilled water.

40% Trehalose

1. Add 4 g of trehalose to 10 ml of distilled water.
2. Dissolve by gently heating at 56°C.
3. Filter sterilize.

Bromcresol Green

1. Add 0.1 g of bromcresol green to 7.1 ml of 0.01 N NaOH. (Prepare 0.01 N NaOH by adding 0.4 g of NaOH to 1,000 ml [1 liter] of distilled water. Mix to dissolve.)
2. Dilute by adding 117.9 ml of distilled water.
3. Filter sterilize.

Cycloheximide

1. Add 0.1 g of cycloheximide to 10 ml of distilled water.
2. Gently heat to dissolve.
3. Filter sterilize.
4. Aliquot in 0.5-ml amounts. Store at –30°C (stable for 6 months).

PREPARATION OF RAT BROTH

Yeast nitrogen base (working solution)	80 ml
40% trehalose	10 ml
Bromcresol green	10 ml
Cycloheximide	0.4 ml

1. Combine ingredients and mix well.
2. Adjust pH to 5.4–5.5.
3. Dispense into sterile screw-cap tubes.

TEST PROCEDURE

1. Dispense 3 drops of RAT broth into as many microtiter wells as are needed for tests and controls.
2. Using a microtiter grid form, label each space as to culture number or control.
3. Emulsify a **HEAVY** inoculum of the yeast into the broth in the appropriate well.
4. Incubate plate for 1 h at 37°C in ambient air; do not cover with tape.

(When the test is finished, the used wells can be covered with tape and held until the remainder of the wells have been used.)

INTERPRETATION

Positive: Broth changes from a blue to yellow; *Candida glabrata*.

Negative: Broth remains blue or green; isolate is NOT *C. glabrata* (further testing is required for complete identification).

QUALITY CONTROL

Positive control: *Candida glabrata*

Negative control: *Candida albicans*

Notes:

- This method is approved by the Clinical Laboratory Standards Institute (CLSI, M35-A2, 2008).
- Do not incubate longer than 1 h at 37°C. Depending on the source medium, false positives can occur if overincubated; CHROMagar Candida is a better source medium than Sabouraud dextrose agar (see Murray et al., 2005).
- Some reports indicate that incubation at 42°C for 3 h may increase the specificity of the test; this is not necessary if the yeasts to be tested are properly selected as described above. Only those meeting the criteria should be tested by this method.
- Beware: bacteria and *Prototheca* spp. can give positive results.

A RAT slide test (based on the above method of Stockman and Roberts), incubated at 37°C for 1 h, is available from Scientific Device Laboratory Inc. (Des Plaines, IL).

Another commercially available RAT test (Thermo Scientific Inc., Scientific Remel Inc., Lenexa, KS) has a slightly different formulation; it is performed in small tubes and incubated at 42°C for 3 h.

Rapid Sporulation Medium (RSM)

RSM is designed for the isolation and identification of dermatophytes; it contains chloramphenicol and chlortetracycline for the inhibition of bacteria and cycloheximide for the inhibition of saprobic fungi. Bromothymol blue in the formulation changes from yellow to blue-green in the presence of alkaline metabolites that are characteristically produced by dermatophytes; notably, the pH color change does not obscure the natural pigment of the isolate. It is very similar to dermatophyte test medium (p. 409) but differs in promoting increased production of conidia and enabling better visualization of the isolates' color. RSM is manufactured and distributed by Hardy Diagnostics (Santa Maria, CA).

SABHI Agar

SABHI agar combines two commonly used media. It has the glucose content of Sabouraud dextrose agar and the calf brain and beef heart infusions (albeit half as much) found in brain heart infusion agar. Antibiotic and/or sheep blood can be added to the formulation. It is favored as a medium for primary isolation of fungi in a number of clinical mycology laboratories.

Glucose	21.00 g
Neopeptone	5.00 g
Proteose peptone.....	5.00 g
Calf brains, infusion.....	100.00 g
Beef heart, infusion	125.00 g
Sodium chloride.....	2.50 g
Disodium phosphate	1.25 g
Agar	15.00 g
Distilled water.....	1,000 ml
(Optional: Chloramphenicol	50 mg
Sheep blood.....	100 ml)

1. Mix reagents (without antibiotic or blood) and bring to boil.
2. Autoclave at 15 lb/in² for 15 min.
3. If antibiotic and/or blood is to be added, cool medium to 50–52°C.
4. Add chloramphenicol (in 10 ml of sterile distilled water).
5. Add the sheep blood.
6. Dispense into plates, or tubes for slants, and cool.

Sabouraud Dextrose Agar (SDA)

SDA is much used in mycology laboratories but has limitations as a medium for primary isolation of fungi directly from specimens (see Scognamiglio et al., 2010).

EMMONS MODIFICATION

The Emmons modification differs from the original formula in that it has an approximately neutral pH and contains only 2% dextrose.

Most mycologists no longer consider it necessary or desirable to use 4% sugar, as in the original formula, and a pH near neutrality has been found to be better for some fungi. The very acid original formula once recommended for suppression of bacterial contaminants can now be replaced by media containing antibiotics.

Dextrose	20 g
Peptone	10 g
Agar	17 g
Distilled water.....	1,000 ml

Final pH, 6.9.

ORIGINAL FORMULA

Some workers still prefer the original formula.

Dextrose	40 g
Peptone	10 g
Agar	15 g
Distilled water.....	1,000 ml

Final pH, 5.6.

1. To prepare either of the formulas, dissolve the ingredients by boiling, and dispense in tubes if slants are to be made. Leave in bulk if plates are to be poured.
2. Autoclave at 15 lb/in² for 10 min.
3. Allow tubes to cool in slanted position or pour plates.
4. Store in refrigerator.

Both formulations of SDA are commercially available in prepared or dehydrated form.

Sabouraud Dextrose Agar with 15% NaCl

Testing for tolerance to 15% sodium chloride (NaCl) can be valuable for identifying some dematiaceous (black) fungi.

SDA.....	500 ml
NaCl.....	75 g

1. Heat to dissolve.
2. Dispense in tubes; autoclave at 15 lb/in² for 10 min.
3. Allow to solidify as butts to be melted and slanted as needed.

Test Procedure

1. Place a pinpoint inoculum of the organism to be tested on a slant of the agar.
2. Incubate at room temperature.

The organism is considered to be strongly inhibited if its colony diameter is <2 mm at 21 days. If the colony surpasses 2 mm, the organism is considered tolerant of 15% NaCl.

Sabouraud Dextrose Broth

Dextrose	20 g
Peptone	10 g
Distilled water.....	1,000 ml

Final pH, 5.7.

1. Dissolve ingredients; dispense into tubes.
2. Autoclave (15 lb/in²) for 10 min.

Noting the manner in which yeasts grow in this broth can assist in their identification (see [Table 4](#) [p. 128] and [Table 5](#) [p. 130]). Sabouraud dextrose broth is also used for the detection of fungal contaminants in various products.

Starch Hydrolysis Agar

(For aerobic actinomycetes.)

Nutrient agar	23 g
Potato starch	10 g
Demineralized water	1,000 ml

1. Dissolve agar in 500 ml of water by boiling.
2. Dissolve starch in 250 ml of water by boiling.
3. Combine and add 250 ml of water.
4. Dispense into screw-cap tubes.
5. Autoclave (15 lb/in²) for 30 min.
6. Allow to cool as butts; melt down and pour into petri plates as needed.

Test Procedure

1. Inoculate a 10-mm-diameter round area of agar heavily with a pure culture of the organism to be tested.
2. Incubate at optimal growth temperature until good growth occurs.
3. Flood the area around the growth with Gram iodine.

Starch hydrolysis is demonstrated by a colorless (complete hydrolysis) or red (partial hydrolysis) area around the growth.

Unhydrolyzed starch in the medium will produce a deep blue to purple color in the presence of iodine (see [Table 2](#) [p. 115]).

Trichophyton Agars

The set of seven media tests the growth factor requirements of the species of *Trichophyton*. They are often helpful in differentiating the species. It is seldom necessary to utilize the entire battery; ordinarily, no. 1 and no. 4 are the most commonly required.

Trichophyton agars are available commercially, either prepared or in dehydrated form. Their compositions are as follows.

- No. 1, casein agar base (vitamin free)
- No. 2, casein agar base plus inositol

- No. 3, casein agar base plus inositol and thiamine
- No. 4, casein agar base plus thiamine
- No. 5, casein agar base plus nicotinic acid
- No. 6, ammonium nitrate agar base
- No. 7, ammonium nitrate agar base plus histidine

Inoculation of Media

The center of a slant of each medium must be inoculated with a small and equal-size amount of pure culture grown on routine medium with or without antibiotics. It is important that the culture be free of bacteria, for many bacteria synthesize vitamins that may invalidate the test. Care must also be taken not to transfer any of the source medium with the organism, as this may supply carryover nutrients and cause false reactions.

The following method of inoculation dilutes possible carryover nutrients and ensures that each slant receives an equal inoculum.

1. Make a homogeneous suspension of fuzzy or granular colonies in sterile saline or water.
2. Place 2 drops of the suspension on each slant of medium.

Test Procedure

1. Incubate tubes at 25–30°C for 2 weeks, preferably in a reclining position for the first few days so that the inoculum remains evenly dispensed over the surface of the agar.
2. Examine periodically for growth.
3. The tube that shows maximum growth is recorded as 4+. Other tubes are graded by comparison.

For interpretation of results, see [Table 23](#) (p. 271) and [Table 24](#) (p. 277).

Tyrosine, Xanthine, or Hypoxanthine Agar

(For differentiation of *Nocardia* spp. and characterization of the “black yeasts.”)

Nutrient agar	23 g
Tyrosine	5 g
(or xanthine	4 g)
(or hypoxanthine	5 g)
Distilled water.....	1,000 ml

1. Dissolve the agar in the distilled water by boiling (swirl frequently).
2. Add tyrosine, xanthine, or hypoxanthine, taking care to distribute the crystals evenly throughout the agar.
3. Adjust to pH 7.0; autoclave at 15 lb/in² for 15 min.

If the agar is too hot when poured, the time required for solidification will be long enough to permit settling out of the tyrosine, xanthine, or hypoxanthine granules. To avoid this, the medium should be allowed to cool to 45–48°C before being poured and the flask should be mixed well while the plates are poured to ensure an even distribution

of the crystals. If the medium is to be stored for a long period, pour well-mixed medium, with evenly distributed crystals, into tubes. Allow to solidify as butts and refrigerate.

Test Procedure

1. When needed, place tubes of agar in boiling water bath to melt, cool to 45–48°C, mix well to resuspend crystals, pour into petri plates, and allow to solidify.
2. Heavily inoculate an area of agar 10 mm in diameter with a pure culture.
3. Incubate at the temperature at which the isolate grows best, i.e., at 25–30°C or at 35–37°C, for 2–3 weeks.
4. Examine every 3 or 4 days for clearing of the medium, around or directly beneath the colony, which indicates hydrolysis. (Color Plate 167.)

For interpretation of results, see Table 2 (p. 115) and Table 20 (p. 227).

Urea Agar

This medium is used for the differentiation of the yeastlike fungi and also in the identification of aerobic actinomycetes, yeasts, and *Trichophyton* species. It is the same as used in bacteriology and is commercially available in prepared or dehydrated form.

- A.** Urea agar base (Christensen)..... 29 g
Distilled water.....100 ml

Dissolve the powder in the water and sterilize by filtration.

- B.** Agar..... 15 g
Distilled water.....900 ml

1. Dissolve the agar in the water, and sterilize by autoclaving at 15 lb/in² for 15 min.
2. Cool the agar to ~50°C.
3. Add the 100 ml of sterile urea agar base.
4. Mix well; dispense aseptically into sterile tubes.
5. Allow to cool in slanted position to form butt about 1 in. (2.5 cm) deep and slant ~1.5 in. (3.8 cm) long.

Urease-positive organisms produce an alkaline reaction indicated by a pink-red color.

Water Agar

Nutritionally deficient media are known to enhance production of spores and conidia. Water agar is used for that purpose.

- Agar.....20 g
Distilled water.....1,000 ml

1. Mix reagents; bring to boil to dissolve agar.
2. Dispense into tubes.
3. Autoclave at 15 lb/in² for 15 min.
4. Cool in slanted position or store as butts to be melted down and used as slants or plates when needed.

Yeast Extract-Phosphate Agar with Ammonia

(For isolation of *Histoplasma capsulatum* and *Blastomyces dermatitidis* from contaminated specimens.)

Phosphate Buffer

Na ₂ HPO ₄	4 g
KH ₂ PO ₄	6 g
Distilled water	30 ml

Mix well to dissolve; adjust pH to 6.0 with 1 N HCl or 1 N NaOH.

Yeast Extract Solution

Yeast extract	1 g
Agar	20 g
Chloramphenicol (optional)	50 mg
Distilled water	1,000 ml

1. Mix; bring to boil while stirring frequently.
2. Add 2 ml of phosphate buffer to the liter of yeast extract solution. Mix well.
3. Dispense 17–18 ml into screw-cap tubes.
4. Autoclave at 15 lb/in² for 15 min.

Test Procedure

1. As needed, melt and pour two tubes of medium (total, ca. 35 ml) into a sterile petri plate.
2. Spread ~0.5 ml of concentrated (centrifuged) contaminated specimen over surface of solidified agar.
3. Place 1 drop (0.05 ml) of concentrated NH₄OH (ammonium hydroxide) off center on the agar surface. Allow it to diffuse.
4. Incubate at 25–30°C.

The diffusing NH₄OH inhibits bacteria, yeasts, and many moulds while allowing slow-growing dimorphic fungi to grow.

Cycloheximide is not added to the medium, because the ammonium hydroxide will inactivate it.

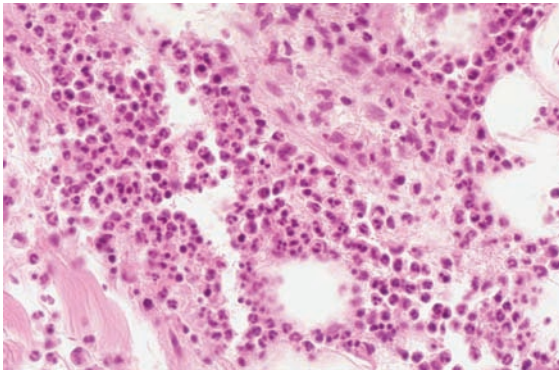
Color Plates

The photographs on the following pages are presented to exhibit color where it will help in gaining a better visual image and understanding of descriptions that appear in the text of this book. The first 37 images pertain to direct microscopic examination of specimens. The remaining 130 pertain to fungal cultures and tests that contribute to identification.

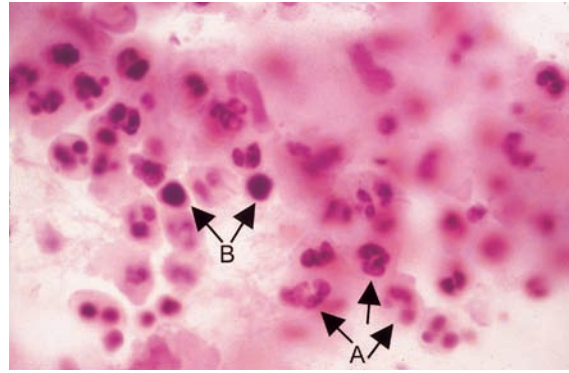
Many of the photographs of fungal colonies are from stock cultures. It is imperative that the reader be cognizant of the fact that many fungi may vary somewhat in their colony pigment and texture, depending mainly on the strain of the organism and sometimes on the medium on which it is cultured. Initial isolates from patient specimens usually produce the most classic colony morphology. Stock cultures may lose some of the typifying characteristics, especially outstanding pigment production; proficiency test samples, unfortunately, often demonstrate this loss.

The colonies represented are either on Sabouraud dextrose agar (SDA) or potato dextrose agar (PDA); the medium is indicated in each instance. Although most subcultured isolates grow well on SDA, it is not always supportive as a primary culture medium or as a medium for the propagation of conidia.

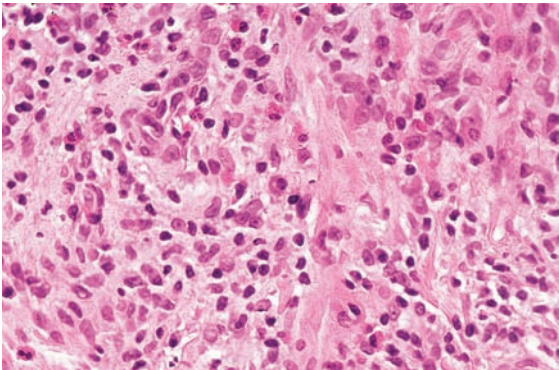
Stain abbreviations: GMS, Gomori methenamine silver; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff.



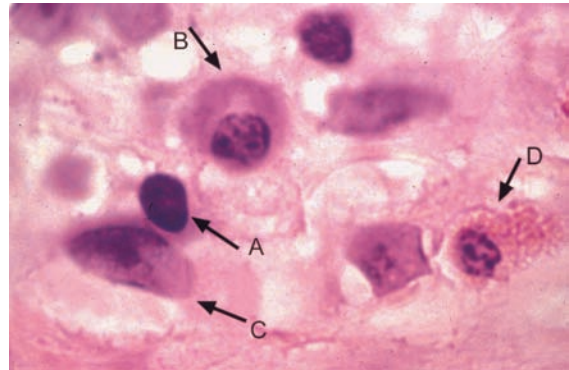
1. Suppurative inflammation/microabscess. Low magnification. H&E.



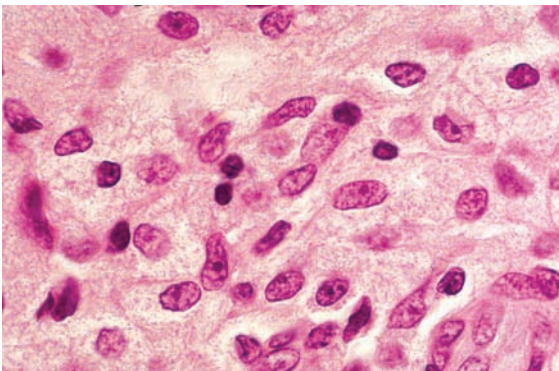
2. Suppurative inflammation: (A) polymorphonuclear leukocytes (neutrophils); (B) few lymphocytes. High magnification. H&E.



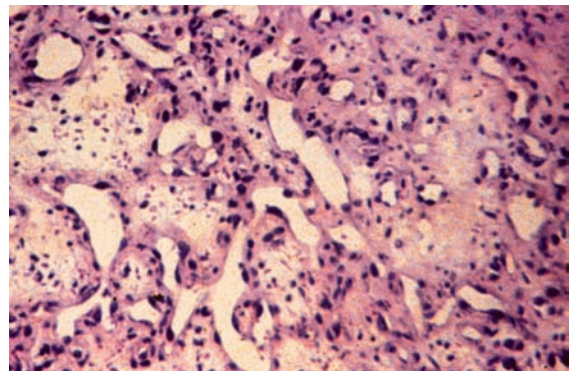
3. Chronic inflammation. Low magnification. H&E.



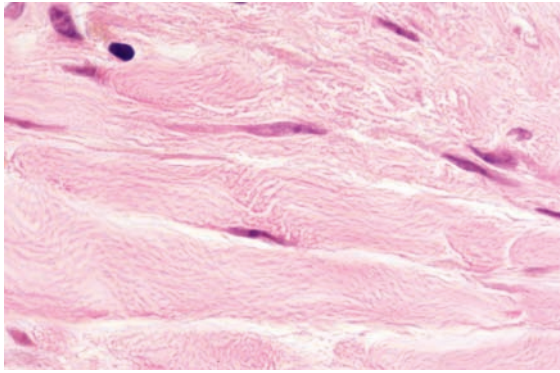
4. Chronic inflammation showing (A) lymphocyte, (B) plasma cell, (C) histiocyte, (D) eosinophil. High magnification. H&E.



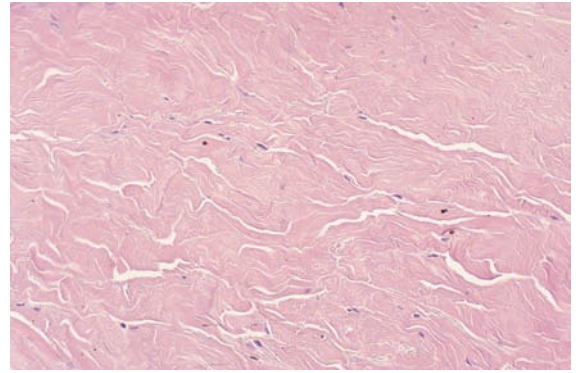
5. Epithelioid histiocytes and few lymphocytes. H&E.



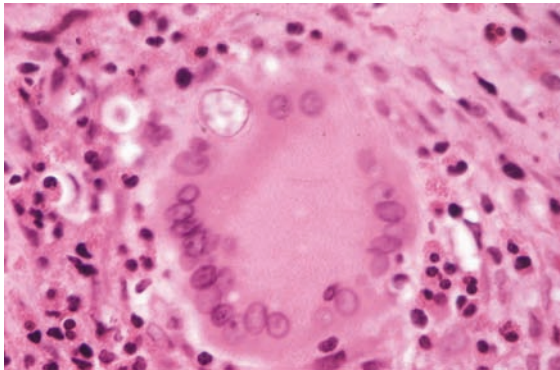
6. Granulation tissue. H&E.



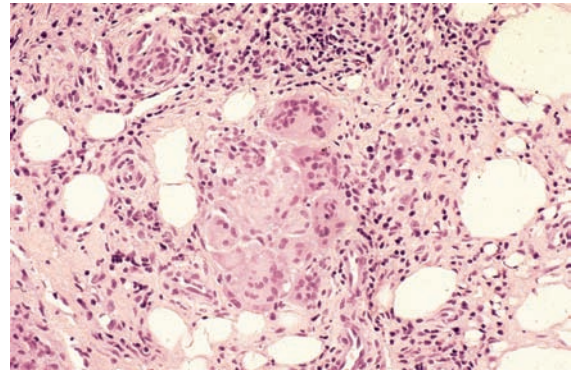
7. Collagen and fibroblasts. High magnification. H&E.



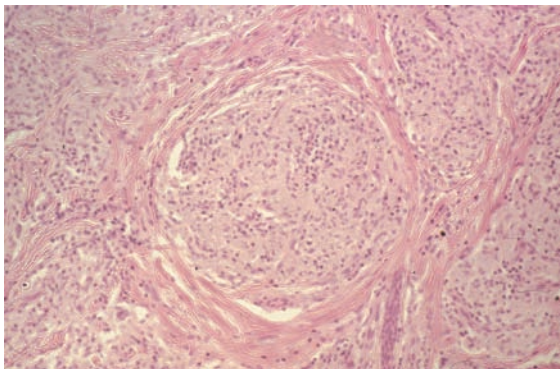
8. Fibrosis. Low magnification. H&E.



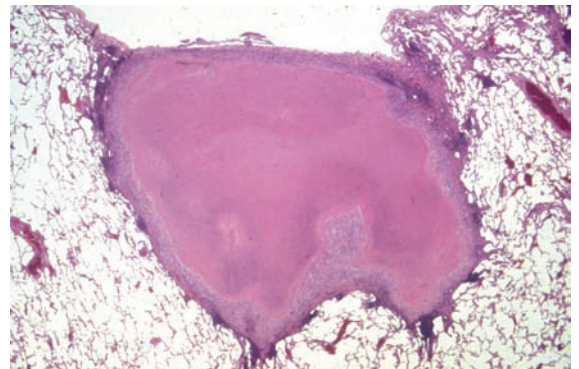
9. Langhans giant cell with engulfed spherule of *Coccidioides*. H&E. (Courtesy of Joan Barenfanger.)



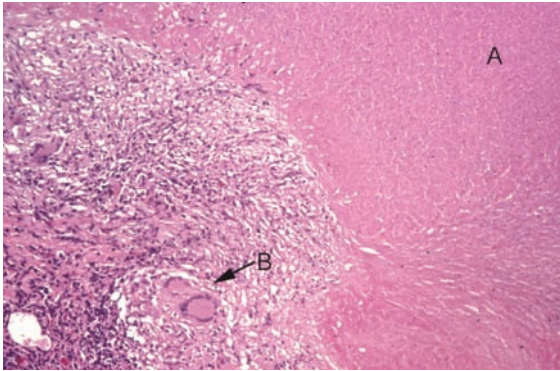
10. Granuloma composed of giant cells. H&E.



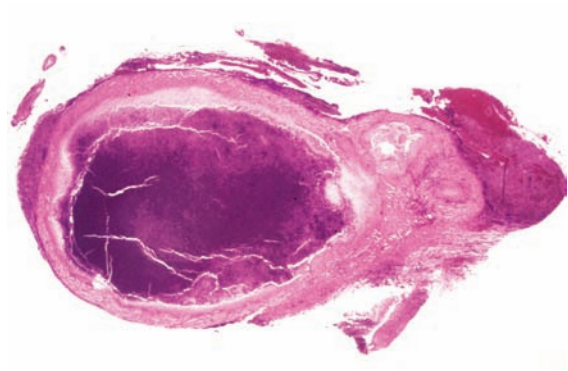
11. Granuloma with epithelioid histiocytes. H&E.



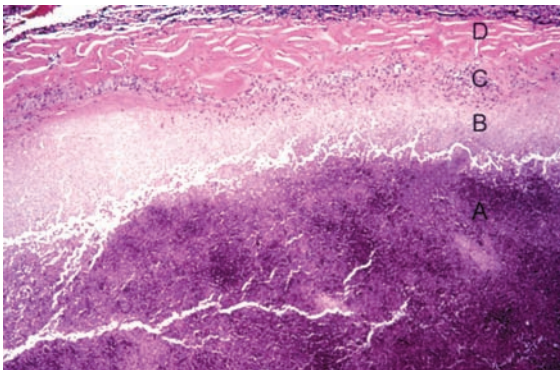
12. Granuloma with caseous necrosis in center. H&E. Low magnification. (Courtesy of Douglas Flieder.)



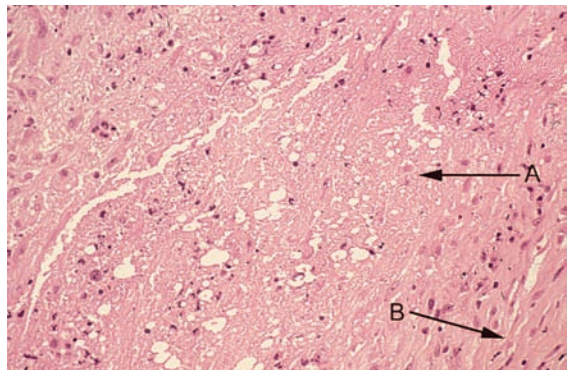
13. Granuloma with caseous necrosis (A); higher magnification of Color Plate 12. Giant cell (B, at arrow) in rim. H&E. (Courtesy of Douglas Flieder.)



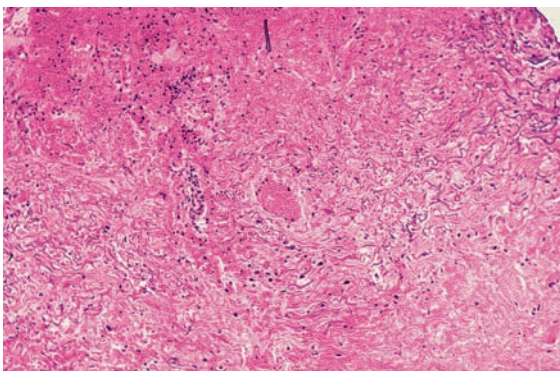
14. Calcified granuloma in a case of histoplasmosis. Low magnification. H&E. (Courtesy of Douglas Flieder.)



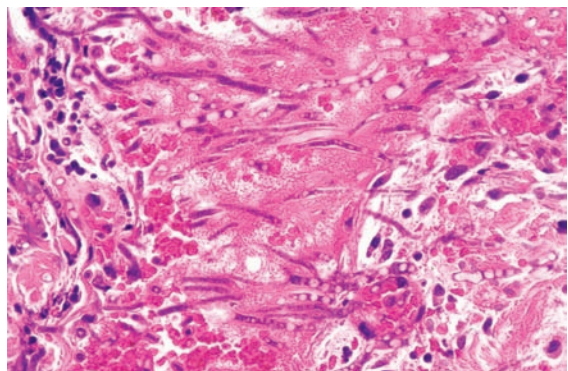
15. Calcified granuloma; higher magnification of Color Plate 14. (A) Calcification; (B) necrosis; (C) histiocytes; (D) fibrosis. H&E. (Courtesy of Douglas Flieder.)



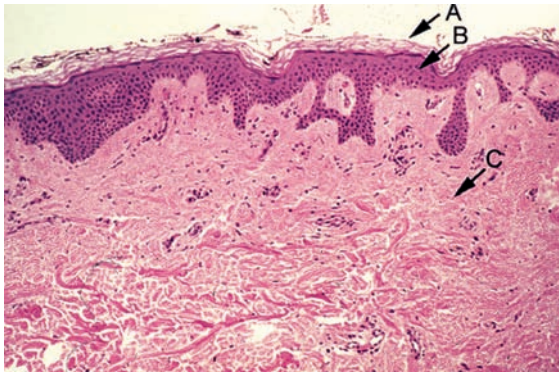
16. (A) Necrotic tissue. (B) Nonnecrotic tissue. H&E.



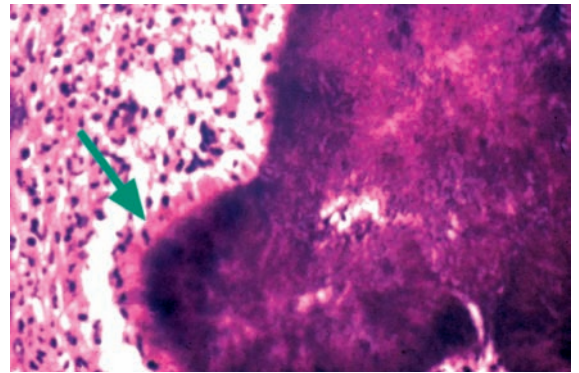
17. Angioinvasion and necrosis. Low magnification. H&E.



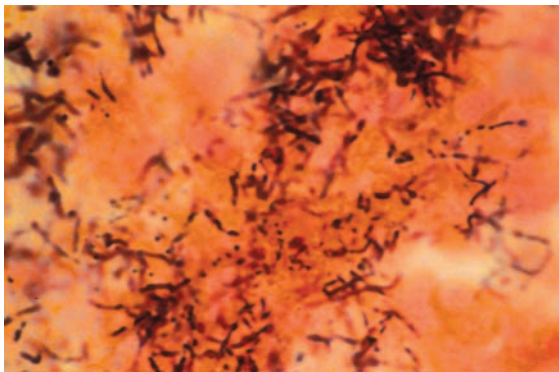
18. Angioinvasion and infarction. High magnification of Color Plate 17. H&E.



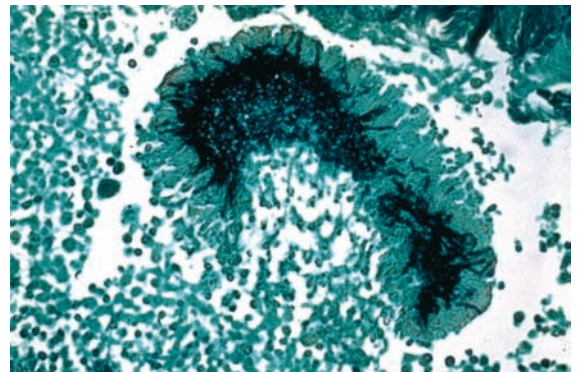
19. Skin: (A) stratum corneum; (B) epidermis; (C) dermis. H&E.



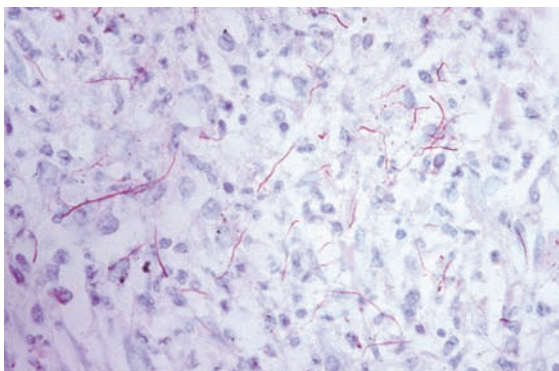
20. Splendore-Hoeppli phenomenon (at arrow) surrounding an actinomycotic granule. (Courtesy of Joan Barenfanger.)



21. *Actinomyces* in liver aspirate. Gram stain. (Courtesy of Joan Barenfanger.)



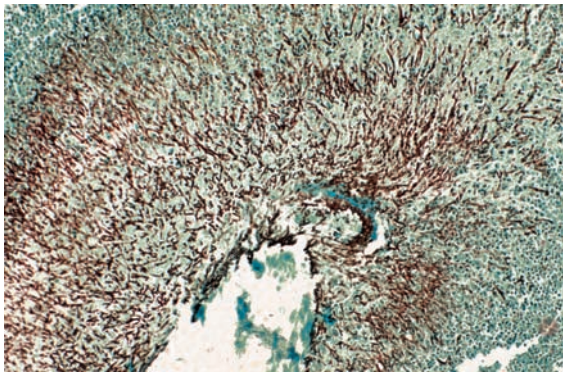
22. Eumycotic mycetoma granule. GMS. (Courtesy of Evelyn Koestenblatt.)



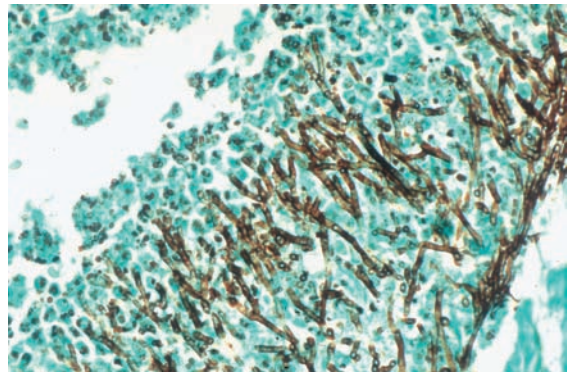
23. *Nocardia* in lung. Coates-Fite acid-fast stain. (Courtesy of Ron C. Neafie.)



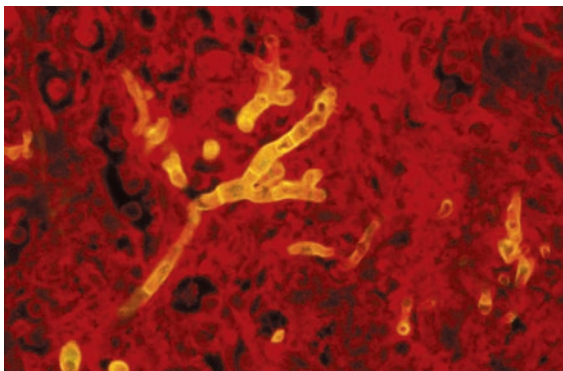
24. Mucormycete in tissue. GMS. (Courtesy of Joan Barenfanger.)



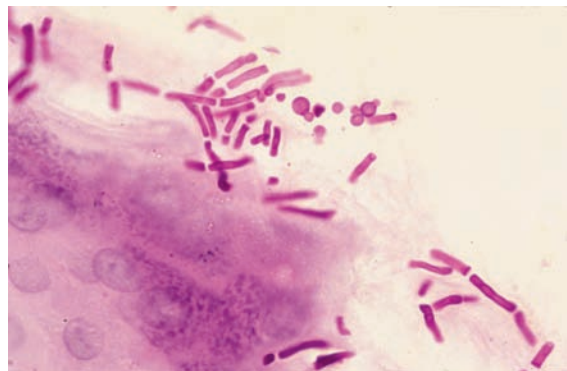
25. *Aspergillus* in lung. Low magnification. GMS.



26. *Aspergillus* in lung. High magnification. GMS.



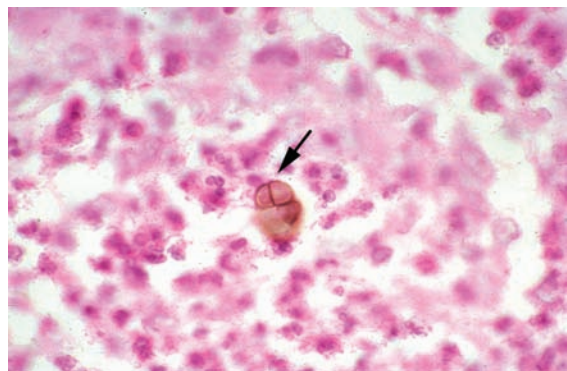
27. *Aspergillus* in lung. Oil immersion magnification. Calcofluor white stain.



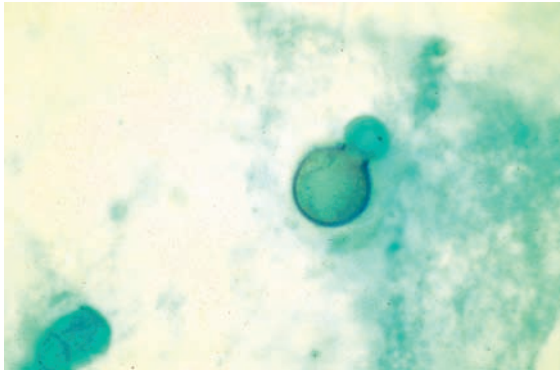
28. Tinea versicolor. PAS.



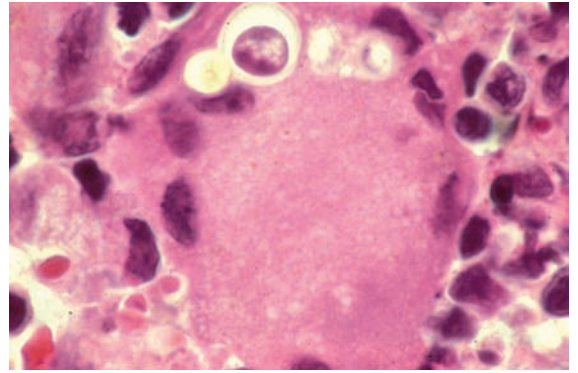
29. Fontana-Masson stain. *Cladosporium* in sputum smear.



30. Chromoblastomycosis; brownish sclerotic body in center. H&E. (Courtesy of Ron C. Neafie.)



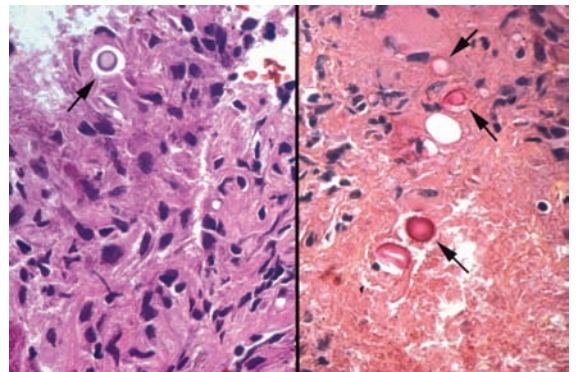
31. *Blastomyces dermatitidis*. GMS. (Courtesy of Joan Barenfanger.)



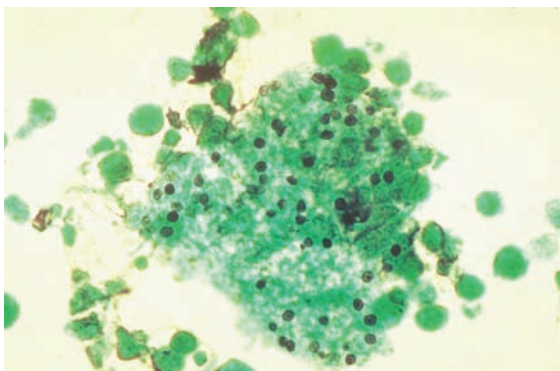
32. *Blastomyces dermatitidis* in giant cell. H&E. (Courtesy of Joan Barenfanger.)



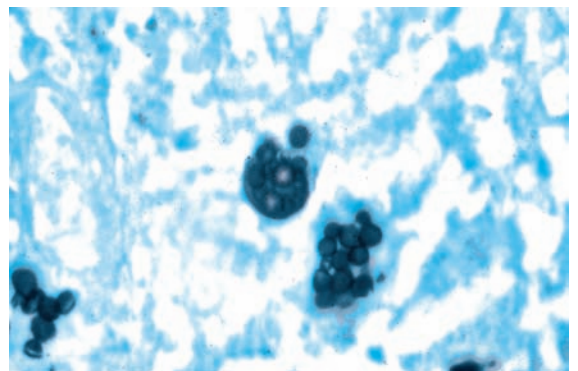
33. *Cryptococcus neoformans* in cerebrospinal fluid. India ink preparation.



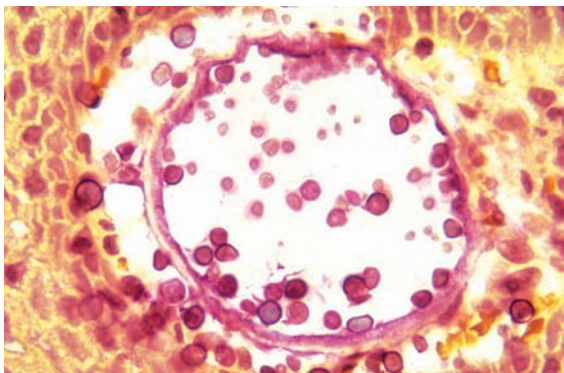
34. *Cryptococcus neoformans* (at arrows) in tissue. H&E (left). Mucicarmine (right). (Courtesy of Douglas Flieder.)



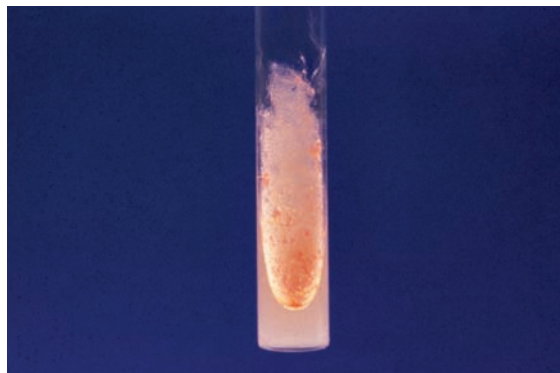
35. *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) in lung. GMS.



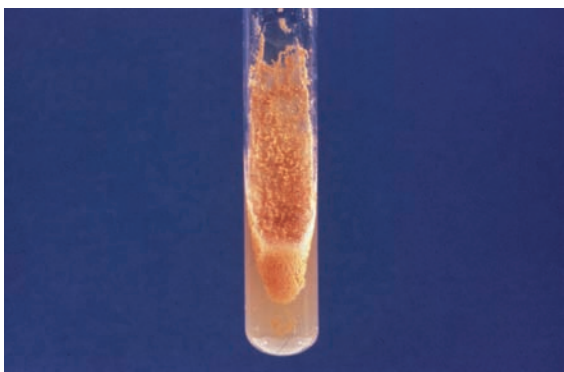
36. *Coccidioides* sp. in lung. GMS.



37. *Rhinosporidium seeberi* in nasal tissue. Mucicarmine.
(Courtesy of Douglas Flieder.)



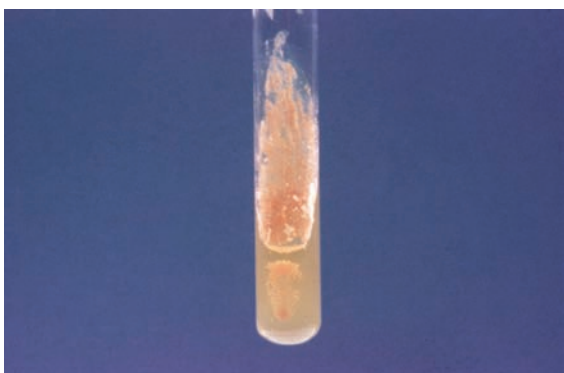
38. *Nocardia* sp. on slant. SDA, 30°C, 9 days.



39. *Nocardia brasiliensis* on slant. SDA, 30°C, 9 days.



40. *Nocardia brasiliensis*. SDA, 30°C, 9 days.



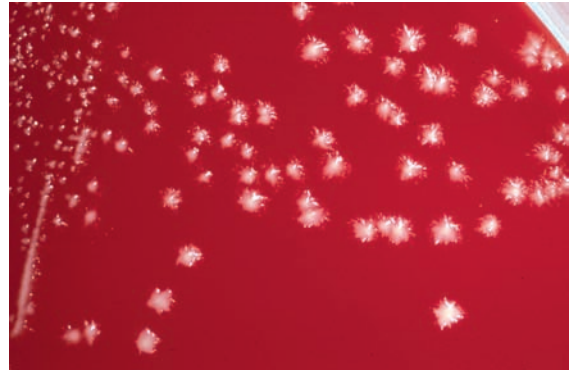
41. *Nocardia otitidiscaviarum* on slant. SDA, 30°C, 9 days.



42. *Streptomyces* sp. on slant. SDA, 30°C, 9 days.



43. *Streptomyces* sp. SDA, 30°C, 9 days.



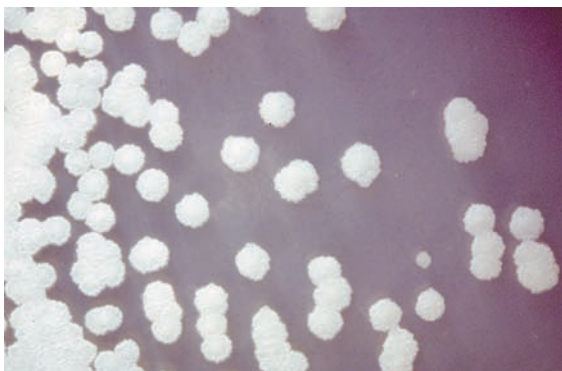
44. *Candida albicans*. Colonies with "feet" on sheep blood agar, 35°C, 2 days.



45. CHROMagar: green colonies, *C. albicans*; blue colonies, *C. tropicalis*; pink, dry colonies, *C. krusei*.



46. *Candida tropicalis*. SDA, 30°C, 2 days.



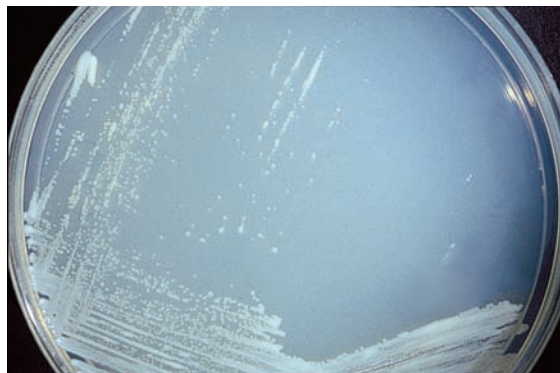
47. *Candida krusei*. SDA plate, 30°C, 2 days. Dry, rough colonies.



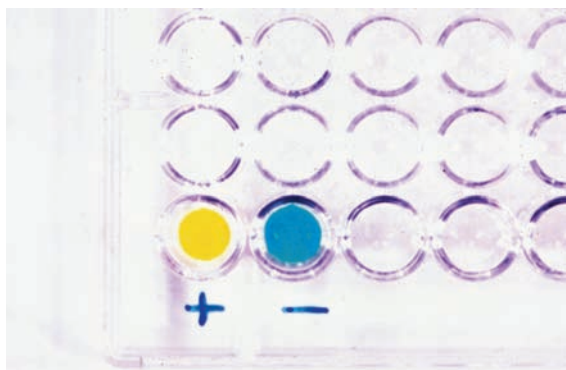
48. *Candida krusei*. SDA slant, 30°C, 3 days. Note film of growth on side of tube.



49. *Candida rugosa* on CHROMagar Candida.



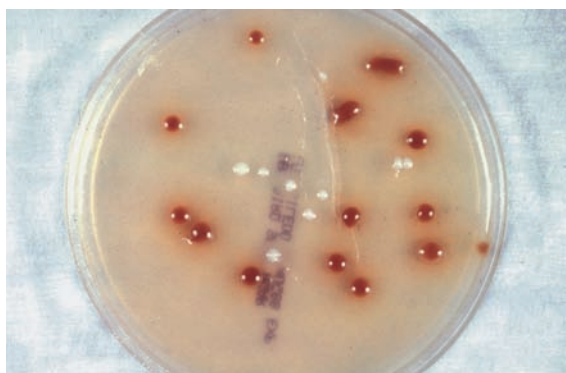
50. *Candida glabrata*. SDA, 30°C, 3 days. Note tiny colonies as compared to *C. tropicalis*.



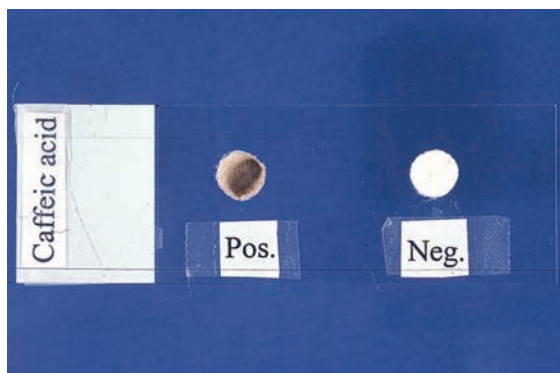
51. Rapid assimilation of trehalose (RAT) test, Mayo Clinic method: *C. glabrata* (positive) and *C. tropicalis* (negative); 37°C, 1 h (see p. 418).



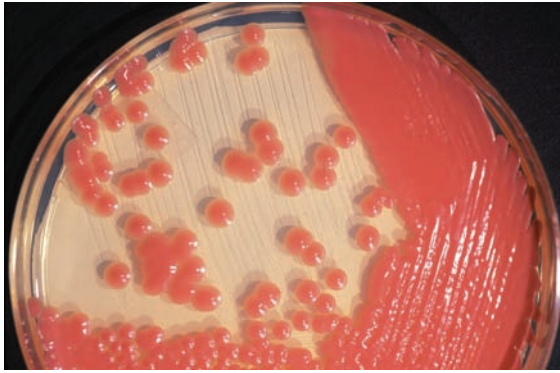
52. *Cryptococcus neoformans*. SDA plate, 30°C, 3 days. Note mucoid nature of colonies.



53. Birdseed agar. Room temperature, 3 days. The brown colonies could be *C. neoformans* or *C. gattii* (see p. 403).



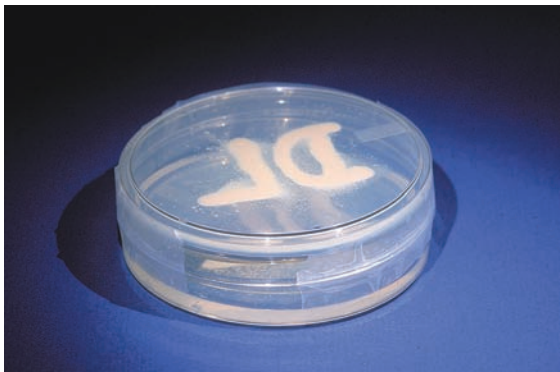
54. Caffeic acid test, 30°C, 4 h. *C. neoformans* or *C. gattii* (positive) and other *Cryptococcus* sp. (negative); see p. 379.



55. *Rhodotorula* sp. SDA, 30°C, 3 days.



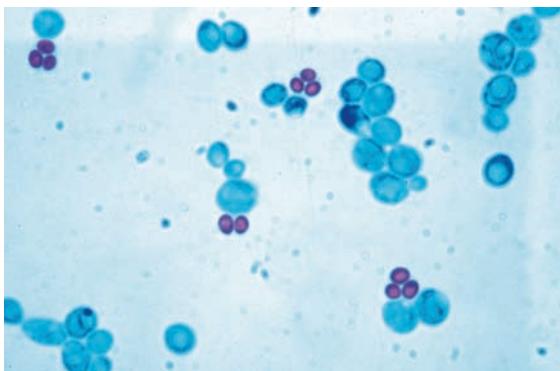
56. *Sporobolomyces salmonicolor*. SDA, 30°C, 5 days. Note small satelliting colonies (due to ballistoconidia).



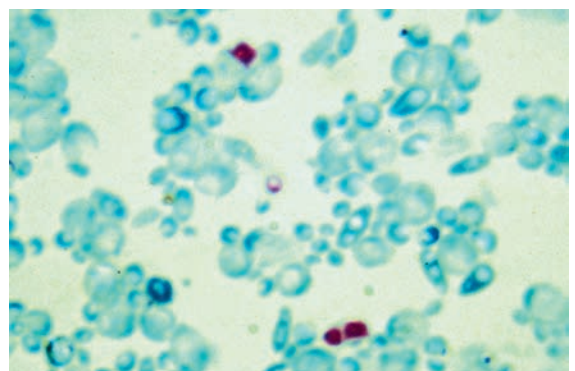
57. SDA plates taped together to best demonstrate ballistoconidia formation (see p. 154).



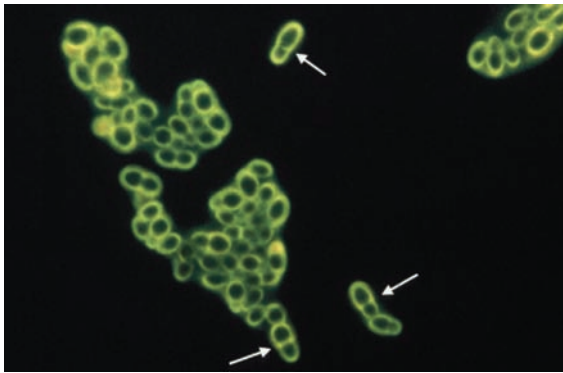
58. SDA plates separated after being taped together for 5 days at room temperature. Note mirror-image colonies of *S. salmonicolor* formed by ballistoconidia.



59. *Saccharomyces cerevisiae*. Kinyoun stain of ascospores (red) after growth on ascospore agar.



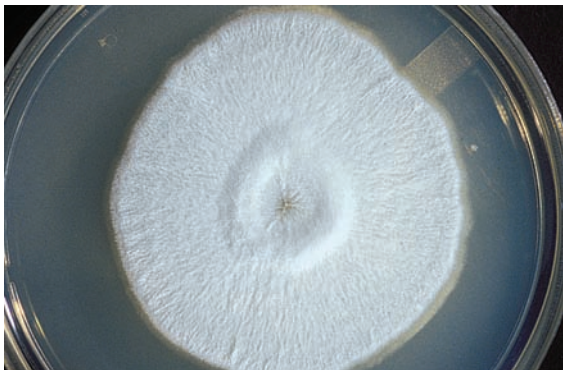
60. *Wickerhamomyces (Pichia) anomalus*. Kinyoun stain of ascospores (red) after growth on ascospore agar. Note brimmed-hat shape of ascospores.



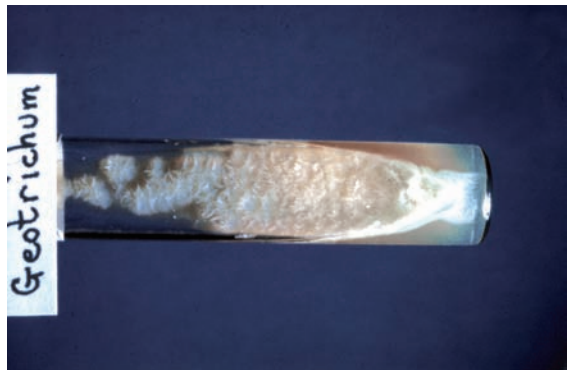
61. *Malassezia* sp. Calcofluor white stain of organism from culture. Arrows indicate bluntness at point of conidiation.



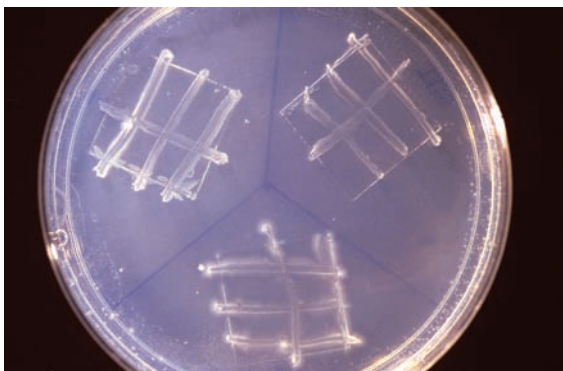
62. *Trichosporon asahii*. PDA, 30°C, 8 days.



63. *Geotrichum candidum*. SDA plate, 30°C, 3 days. Powdery to cottony strain.



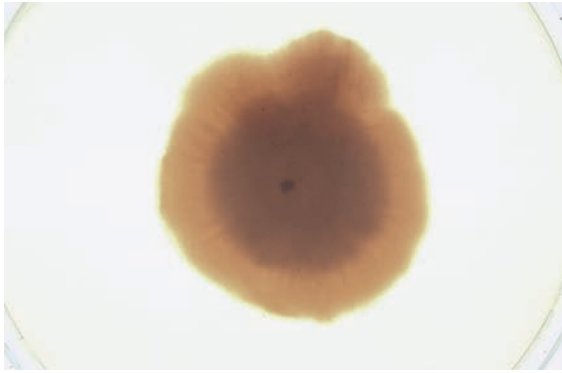
64. *Geotrichum candidum*. SDA slant, 30°C, 3 days. Rough, yeastlike strain.



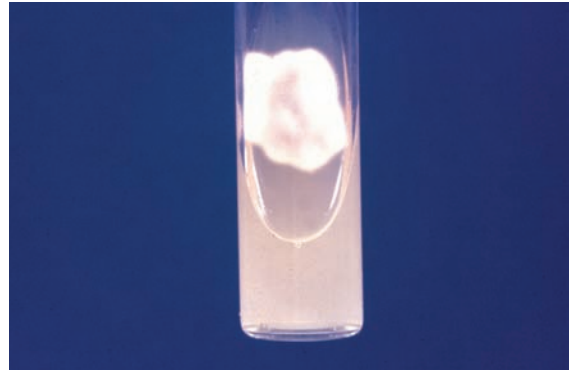
65. Dalmat plate. Three yeastlike fungi inoculated onto cornmeal-Tween 80 agar (see p. 409).



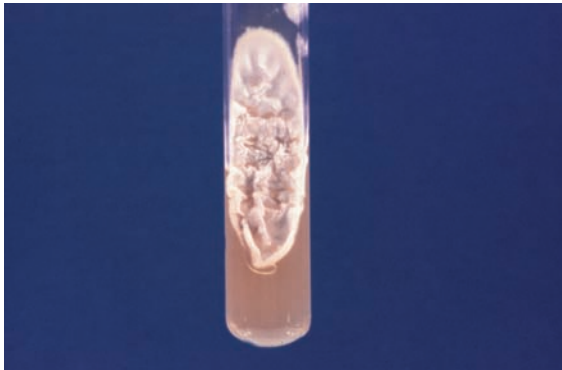
66. *Sporothrix schenckii*. Mould phase, SDA, 30°C, 6 days. Surface of colony.



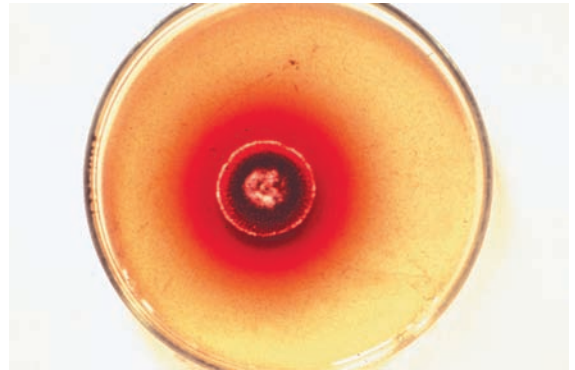
67. *Sporothrix schenckii*. Mould phase, SDA, 30°C, 6 days. Reverse of colony.



68. *Histoplasma capsulatum* slant. SDA, 30°C, 18 days.



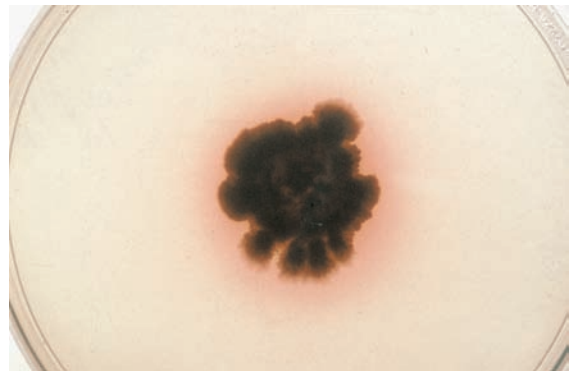
69. *Blastomyces dermatitidis* slant. SDA, 30°C, 18 days.



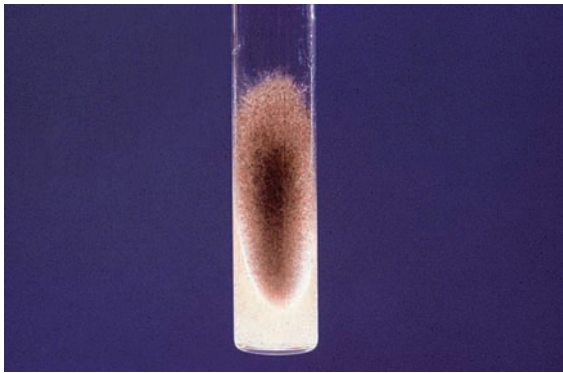
70. *Talaromyces marneffei*. SDA, 30°C, 4 days. Surface of colony. Note strong red diffusing pigment of primary isolate. (Courtesy of William Merz.)



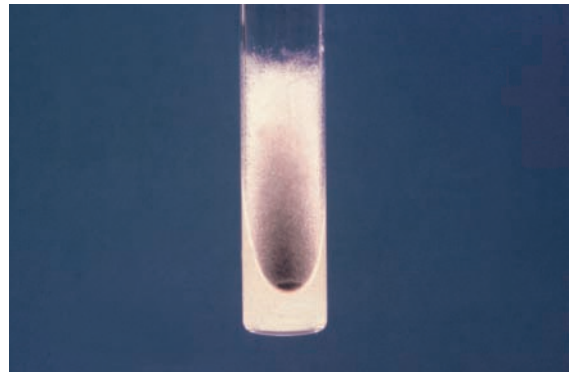
71. *Talaromyces marneffei*. PDA, 30°C, 3 days. Surface of colony. Note pale red diffusing pigment of stock culture.



72. *Talaromyces marneffei*. PDA, 30°C, 3 days. Reverse of colony. Note pale red diffusing pigment of stock culture.



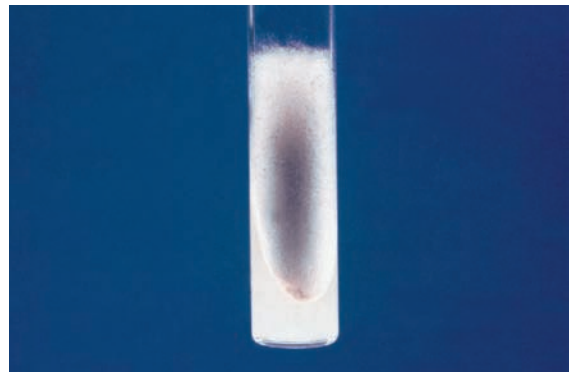
73. *Rhizopus* sp. slant. SDA, 30°C, 4 days. Note robust cotton candy-like growth, typical of many zygomycetes, order Mucorales.



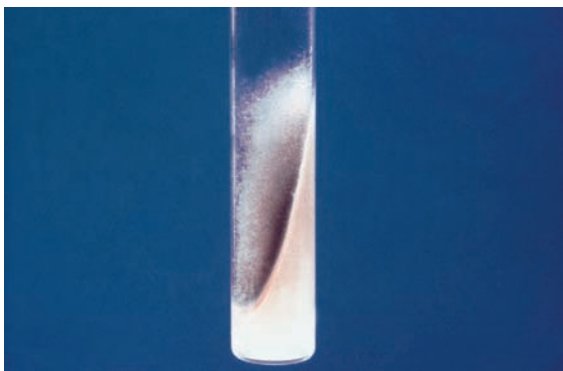
74. *Lichtheimia (Absidia) corymbifera*. SDA, 30°C, 4 days. Cotton candy-like growth.



75. *Cokeromyces recurvatus*. PDA, 30°C, 5 days. Note lack of woolly growth.



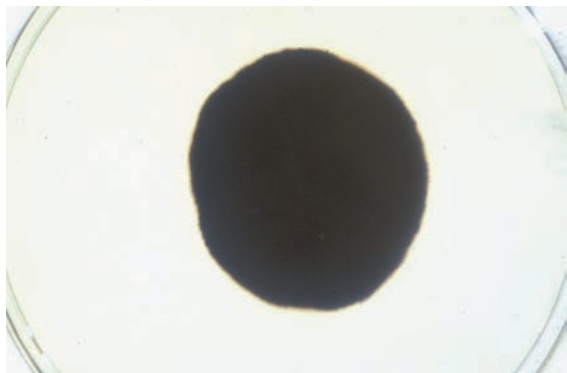
76. *Cunninghamella bertholletiae* slant. SDA, 30°C, 4 days. Cotton candy-like growth.



77. *Syncephalastrum racemosum* slant. SDA, 30°C, 3 days. Cotton candy-like growth.



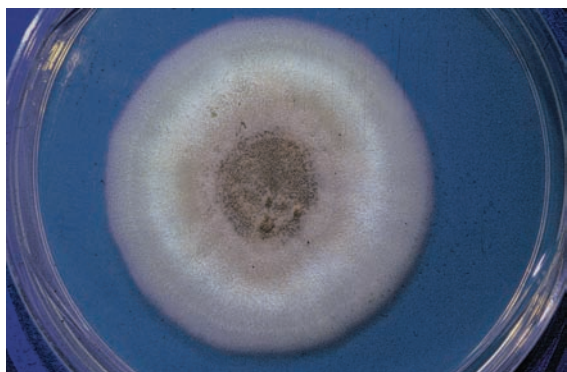
78. *Fonsecaea pedrosoi*. SDA, 30°C, 14 days. Surface of colony.



79. *Fonsecaea pedrosoi*. SDA, 30°C, 14 days. Reverse of colony, typical of completely dark, dematiaceous fungi.



80. *Phialophora verrucosa*. SDA, 30°C, 12 days.



81. *Phaeoacremonium parasiticum*. PDA, 30°C, 7 days.



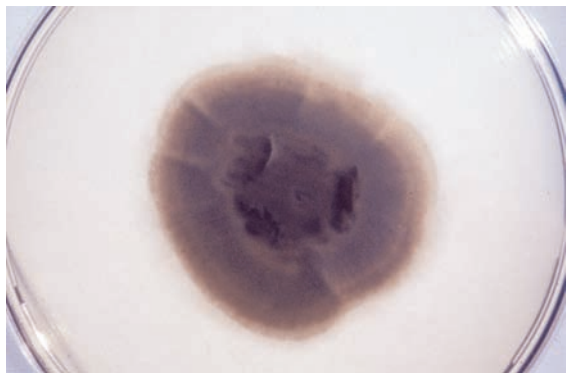
82. *Cladosporium* sp. SDA, 30°C, 7 days.



83. *Cladophialophora carrionii*. SDA, 30°C, 14 days. Surface of colony.



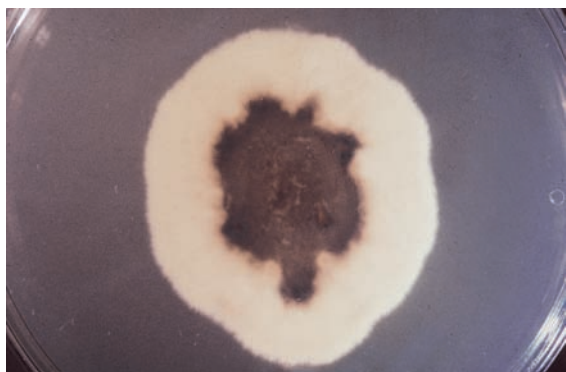
84. *Pseudallescheria boydii*/*Scedosporium boydii*. SDA, 30°C, 7 days. Surface of colony.



85. *Pseudallescheria boydii/Scedosporium boydii*. SDA, 30°C, 7 days. Reverse of colony.



86. *Lomentospora prolificans*. SDA, 30°C, 7 days. Surface of colony.



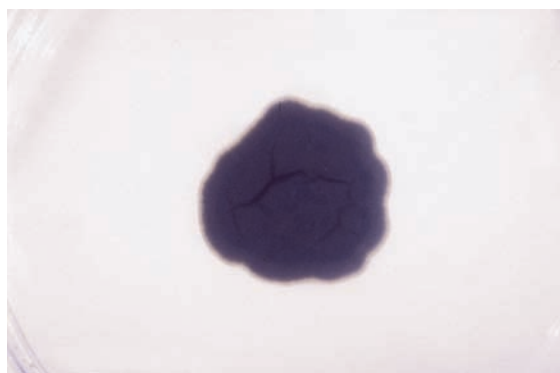
87. *Lomentospora prolificans*. SDA, 30°C, 7 days. Reverse of colony.



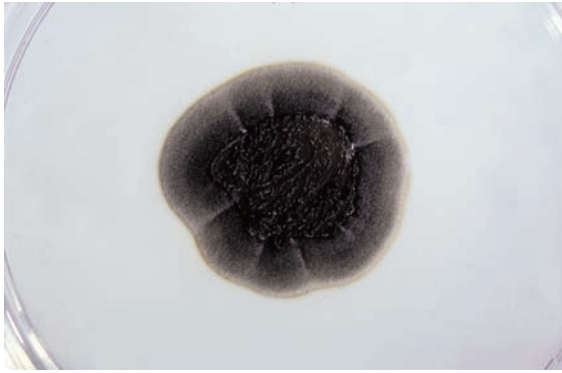
88. *Verruconis gallopava*. SDA, 30°C, 7 days.



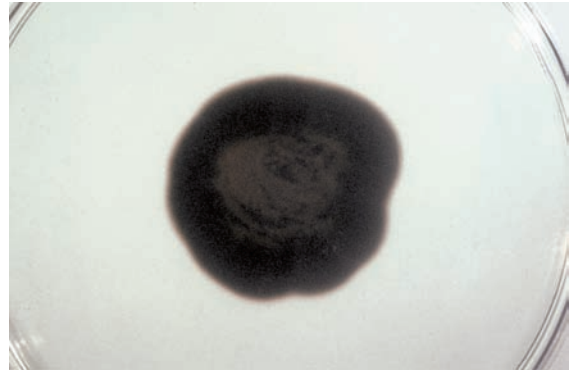
89. *Exophiala jeanselmei* complex. SDA, 30°C, 14 days. Surface of colony.



90. *Exophiala jeanselmei* complex. SDA, 30°C, 14 days. Reverse of colony.



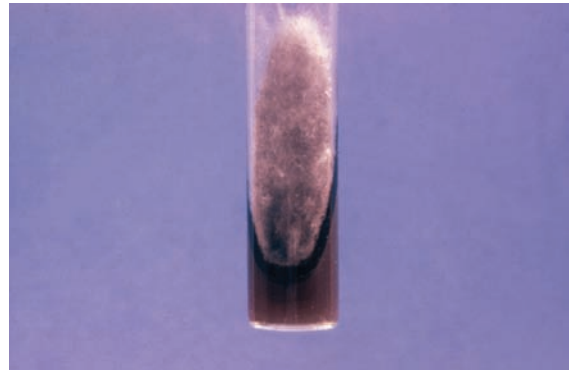
91. *Exophiala dermatitidis*. SDA, 30°C, 21 days. Surface of colony.



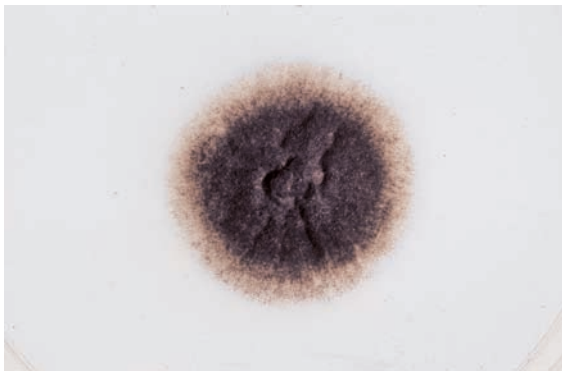
92. *Exophiala dermatitidis*. SDA, 30°C, 21 days. Reverse of colony.



93. *Aureobasidium pullulans*. SDA, 30°C, 7 days.



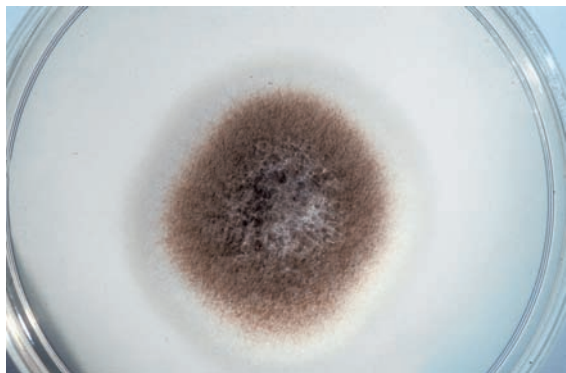
94. *Neoscytalidium dimidiatum* slant. SDA, 30°C, 3 days.



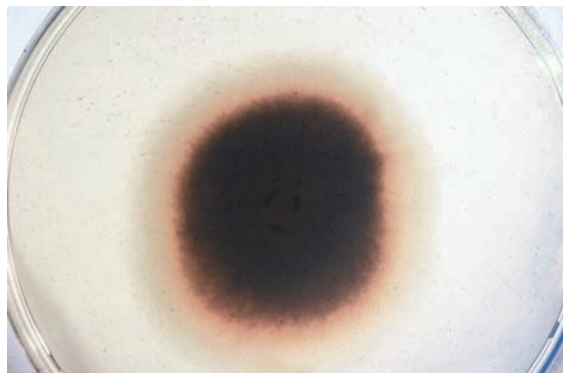
95. *Stachybotrys chartarum*. SDA, 30°C, 7 days.



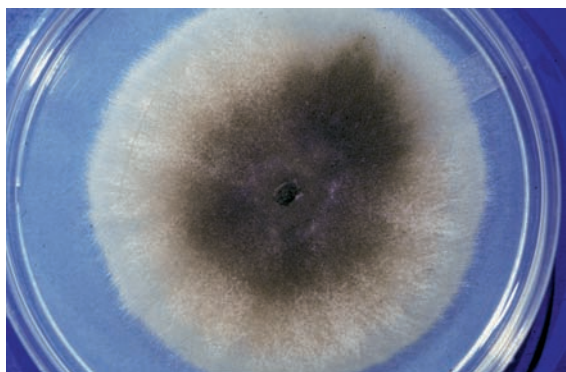
96. *Curvularia* sp. SDA, 30°C, 5 days.



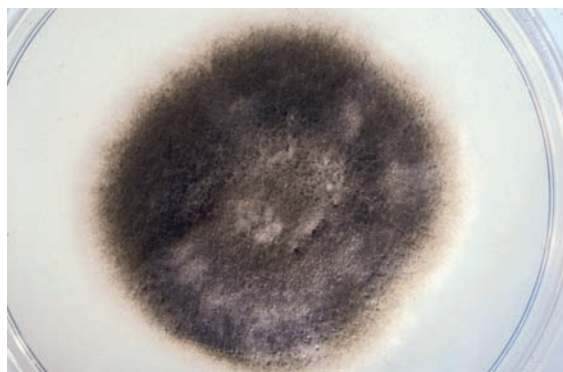
97. *Bipolaris* sp. SDA, 30°C, 5 days. Surface of colony.



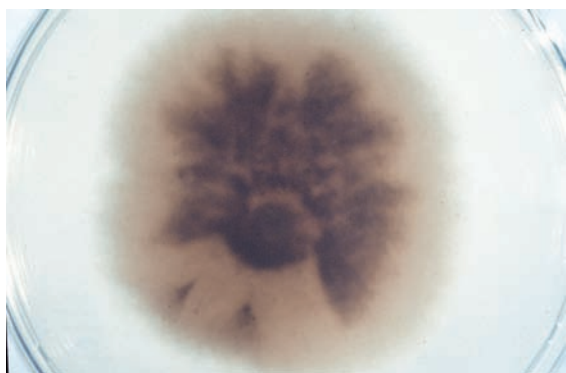
98. *Bipolaris* sp. SDA, 30°C, 5 days. Reverse of colony.



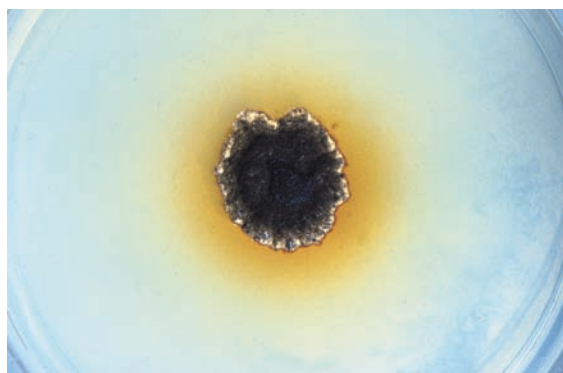
99. *Exserohilum* sp. SDA, 30°C, 7 days.



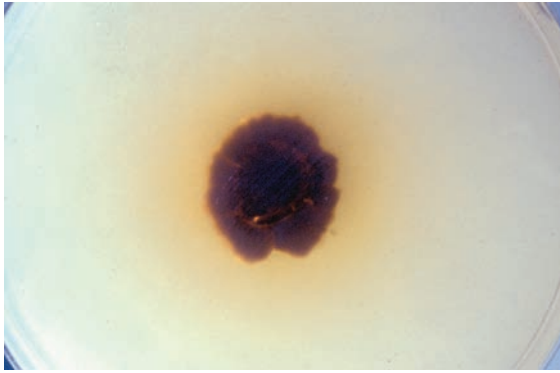
100. *Alternaria* sp. SDA, 30°C, 5 days. Surface of colony.



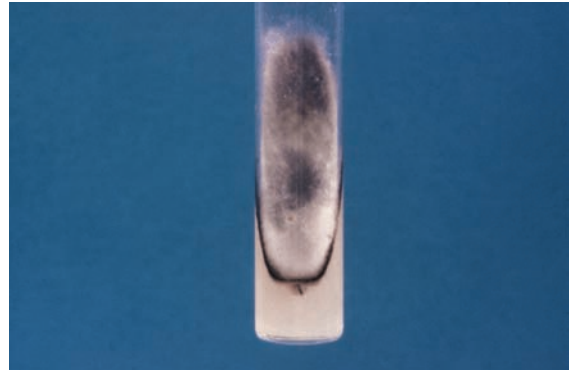
101. *Alternaria* sp. SDA, 30°C, 5 days. Reverse of colony.



102. *Epicoccum* sp. PDA, 30°C, 7 days. Surface of colony. Note yellow-orange diffusing pigment.



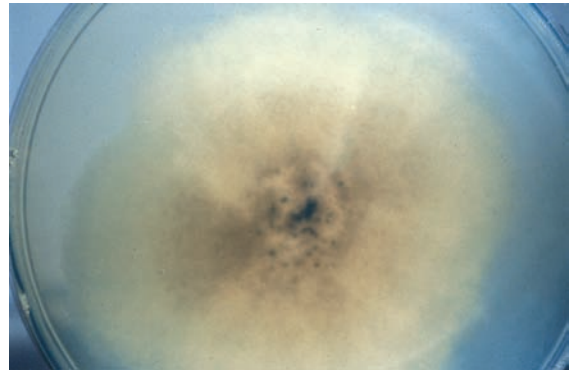
103. *Epicoccum* sp. PDA, 30°C, 7 days. Reverse of colony. Note yellow-orange diffusing pigment.



104. *Nigrospora* sp. slant. SDA, 30°C, 4 days.



105. *Chaetomium* sp. PDA, 30°C, 5 days. Surface of colony.



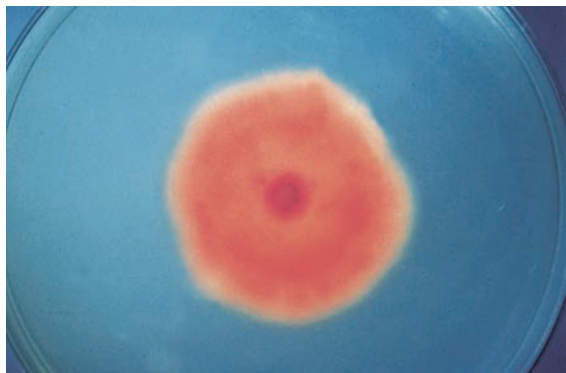
106. *Chaetomium* sp. PDA, 30°C, 5 days. Reverse of colony.



107. *Phoma* sp. SDA, 30°C, 5 days.



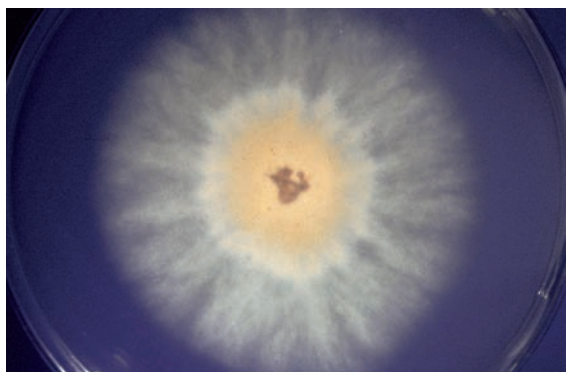
108. *Microsporum audouinii*. PDA, 30°C, 7 days. Surface of colony.



109. *Microsporium audouinii*. PDA, 30°C, 7 days. Reverse of colony.



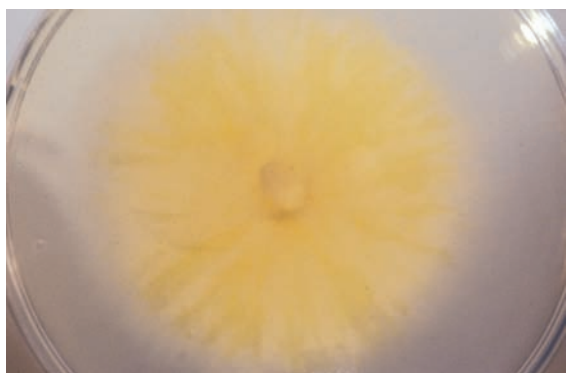
110. *Microsporium canis* var. *canis*. PDA, 30°C, 7 days. Surface of colony.



111. *Microsporium canis* var. *canis*. PDA, 30°C, 7 days. Reverse of colony.



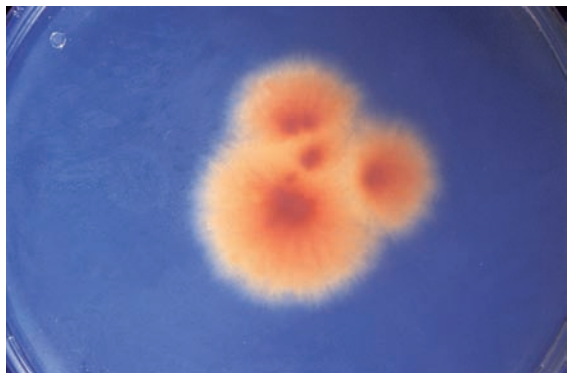
112. *Microsporium gypsum* complex. PDA, 30°C, 6 days. Surface of colony.



113. *Microsporium gypsum* complex. SDA, 30°C, 6 days. Reverse of colony.



114. *Microsporium nanum*. SDA, 30°C, 7 days. Surface of colony.



115. *Microsporum nanum*. SDA, 30°C, 7 days. Reverse of colony.



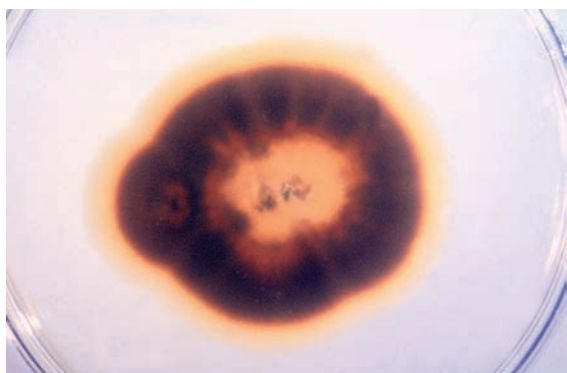
116. *Trichophyton mentagrophytes*. PDA, 30°C, 7 days. Surface of colony.



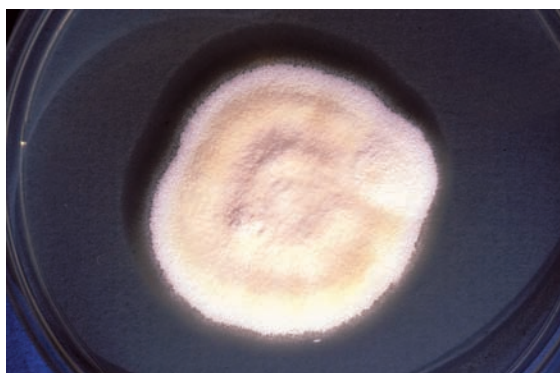
117. *Trichophyton mentagrophytes*. PDA, 30°C, 7 days. Reverse of colony.



118. *Trichophyton rubrum*. PDA, 30°C, 7 days. Surface of colony.



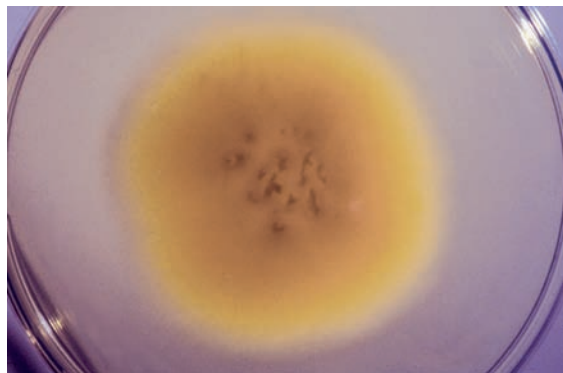
119. *Trichophyton rubrum*. PDA, 30°C, 7 days. Reverse of colony.



120. *Trichophyton tonsurans*. SDA, 30°C, 7 days. Surface of colony, yellowish rose strain.



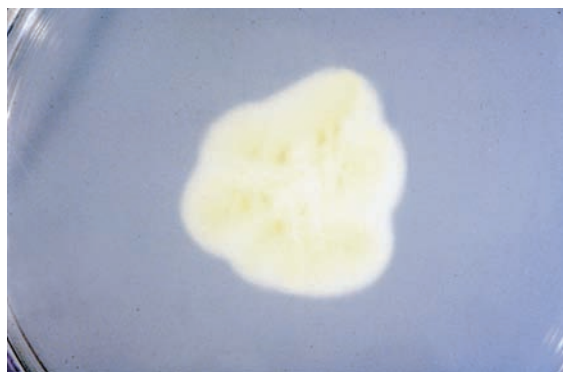
121. *Trichophyton tonsurans*. SDA, 30°C, 7 days. Surface of colony, white and yellow strain.



122. *Trichophyton tonsurans*. SDA, 30°C, 7 days. Reverse of colony.



123. *Trichophyton terrestre*. SDA, 30°C, 7 days. Surface of colony.



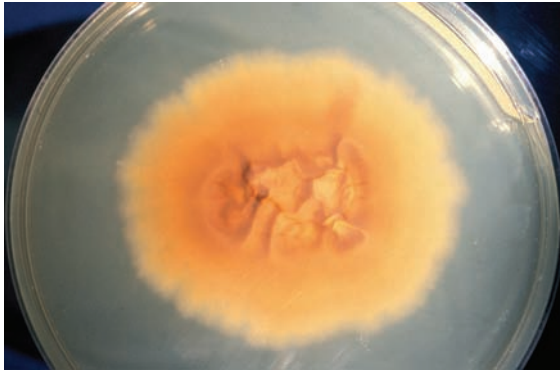
124. *Trichophyton terrestre*. SDA, 30°C, 7 days. Reverse of colony.



125. *Trichophyton soudanense*. (Courtesy of Joel Mortensen.)



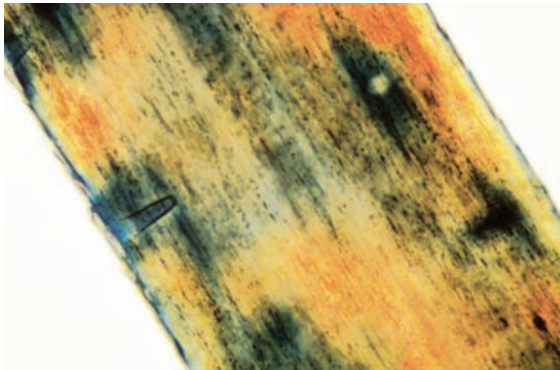
126. *Epidermophyton floccosum*. PDA, 30°C, 12 days. Surface of colony.



127. *Epidermophyton floccosum*. PDA, 30°C, 12 days. Reverse of colony.



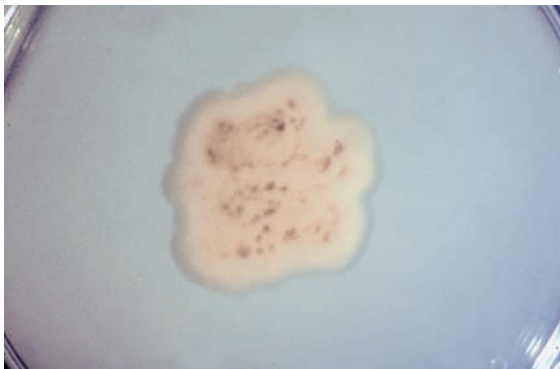
128. Dermatophyte test medium. Dermatophytes turn medium red (see p. 409).



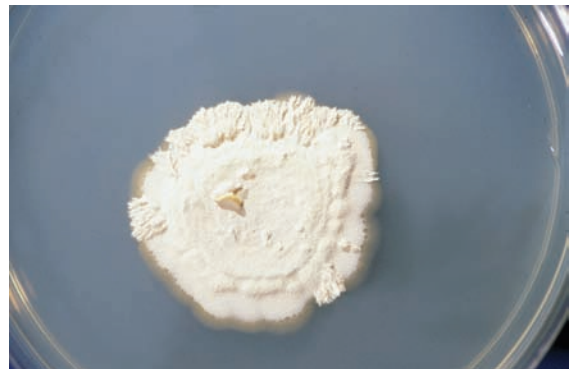
129. *In vitro* hair perforation test. Positive result. Note wedge-shaped perforations (see p. 382).



130. *Malbranchea* sp. PDA, 30°C, 7 days. Surface of colony.



131. *Malbranchea* sp. PDA, 30°C, 7 days. Reverse of colony.



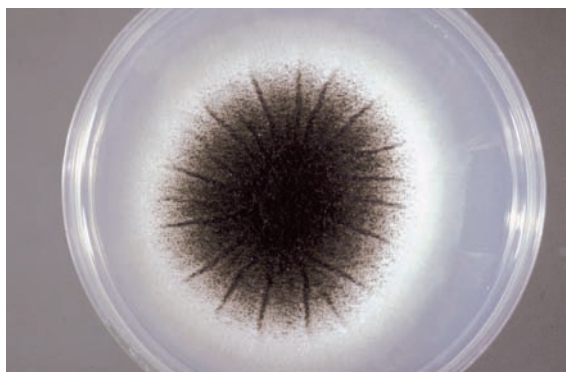
132. *Arthrographis kalrae*. PDA, 30°C, 8 days.



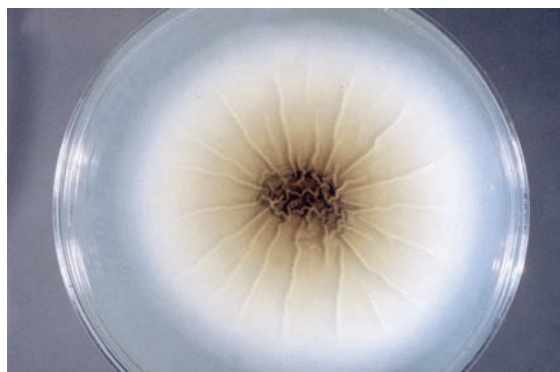
133. *Aspergillus fumigatus*. SDA, 30°C, 4 days. Surface of colony.



134. *Aspergillus fumigatus*. SDA, 30°C, 4 days. Reverse of colony.



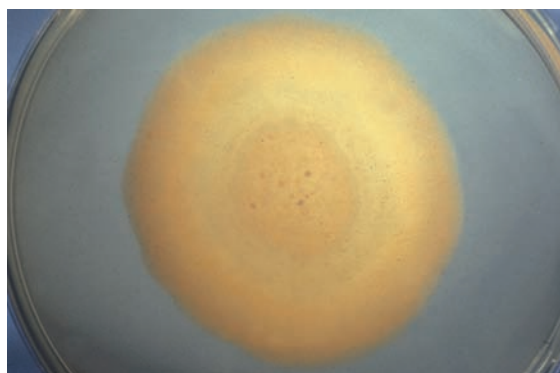
135. *Aspergillus niger*. SDA, 30°C, 4 days; surface of colony.



136. *Aspergillus niger*. SDA, 30°C, 4 days. Reverse of colony.



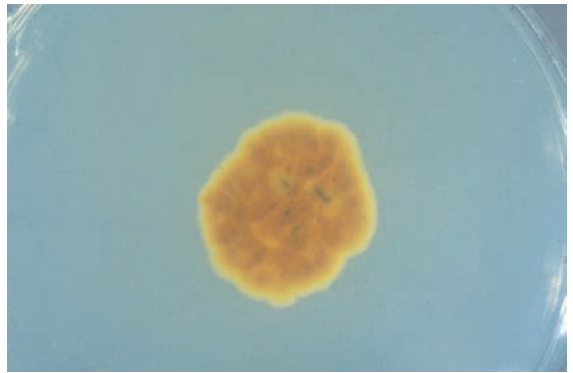
137. *Aspergillus flavus*. PDA, 30°C, 4 days. Surface of colony.



138. *Aspergillus flavus*. PDA, 30°C, 4 days. Reverse of colony.



139. *Aspergillus versicolor*. SDA, 30°C, 4 days. Surface of colony.



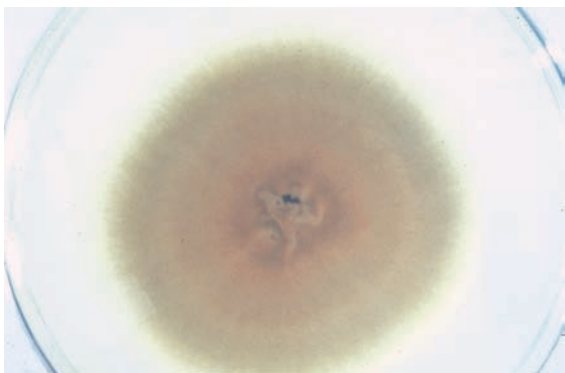
140. *Aspergillus versicolor*. SDA, 30°C, 4 days. Reverse of colony.



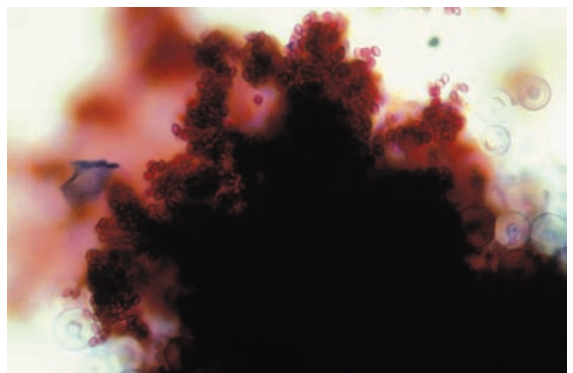
141. *Aspergillus calidoustus*. SDA, 30°C, 4 days.



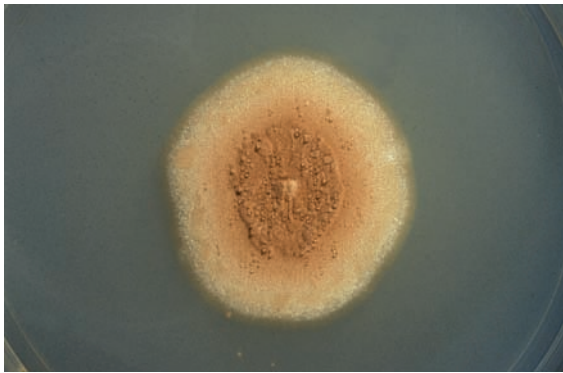
142. *Aspergillus nidulans*. SDA, 30°C, 4 days. Surface of colony.



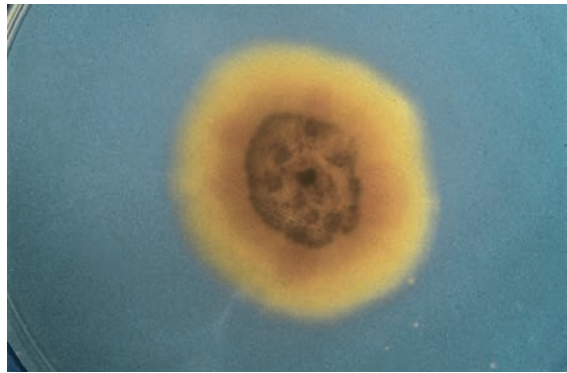
143. *Aspergillus nidulans*. SDA, 30°C, 4 days. Reverse of colony.



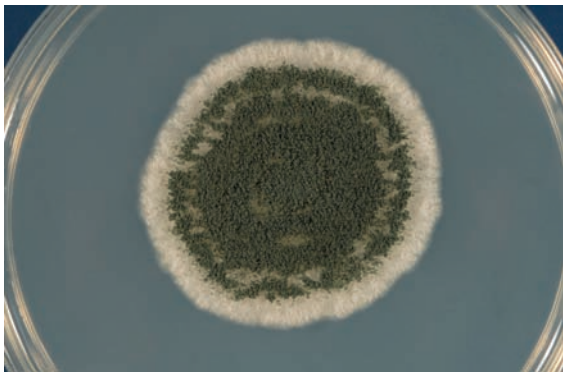
144. *Aspergillus nidulans*. Red ascospores and hyaline Hülle cells.



145. *Aspergillus terreus*. PDA, 30°C, 4 days. Surface of colony.



146. *Aspergillus terreus*. PDA, 30°C, 4 days. Reverse of colony.



147. *Aspergillus clavatus*. PDA, 30°C, 4 days.



148. *Penicillium* sp. SDA, 30°C, 4 days.



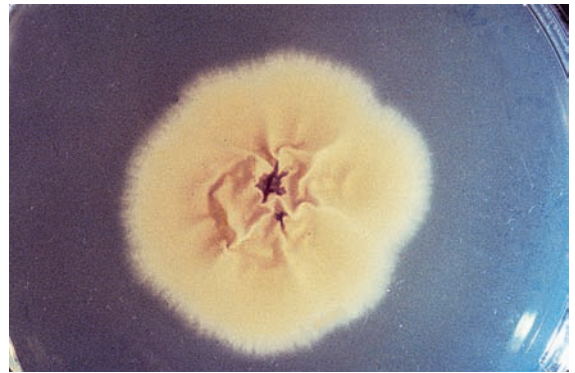
149. *Paecilomyces variotii*. SDA, 30°C, 4 days.



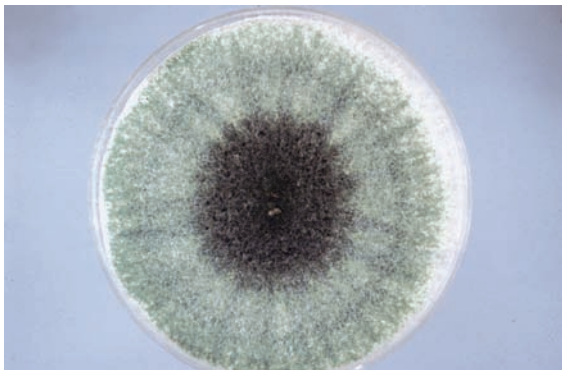
150. *Purpureocillium lilacinum*. PDA, 30°C, 4 days. (Courtesy of Victoria Ruppert.)



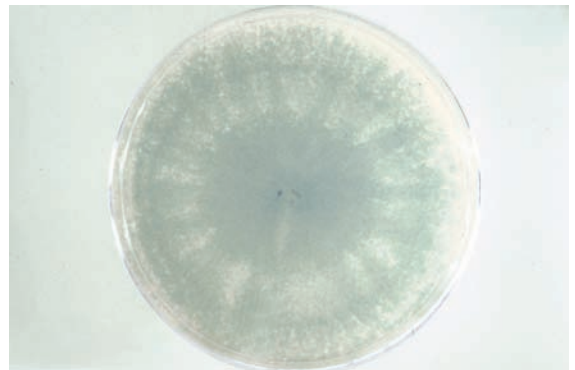
151. *Scopulariopsis brevicaulis* sp. SDA, 30°C, 5 days. Surface of colony.



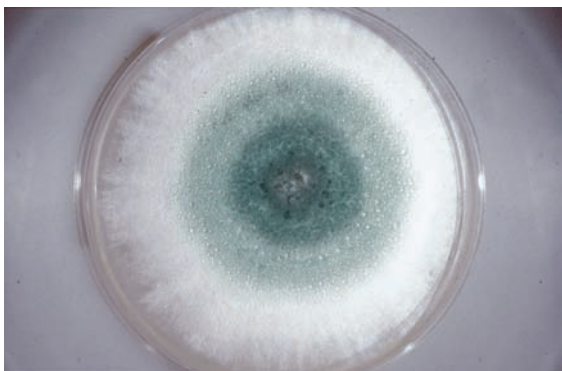
152. *Scopulariopsis brevicaulis* sp. SDA, 30°C, 5 days. Reverse of colony.



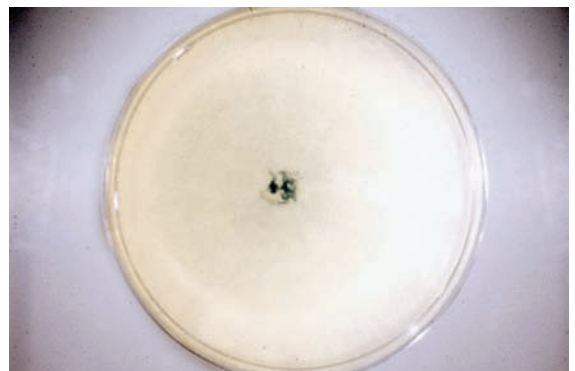
153. *Gliocladium* sp. SDA, 30°C, 5 days. Surface of colony.



154. *Gliocladium* sp. SDA, 30°C, 5 days. Reverse of colony.



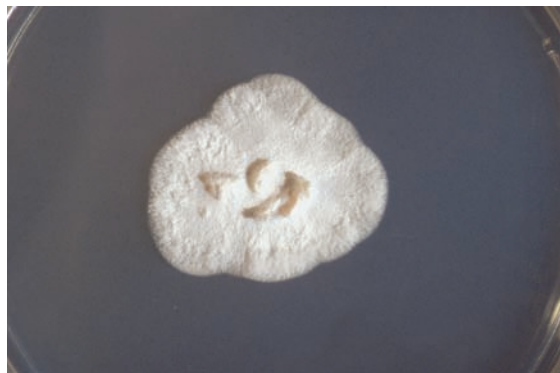
155. *Trichoderma* sp. SDA, 30°C, 5 days. Surface of colony.



156. *Trichoderma* sp. SDA, 30°C, 5 days. Reverse of colony.



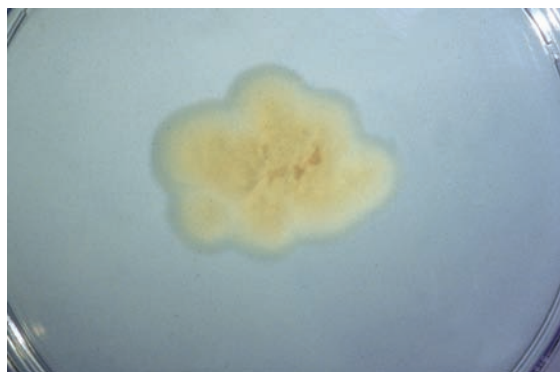
157. *Beauveria* sp. SDA, 30°C, 4 days.



158. *Verticillium* sp. SDA, 30°C, 4 days.



159. *Acremonium* sp. SDA, 30°C, 5 days. Surface of colony.



160. *Acremonium* sp. SDA, 30°C, 5 days. Reverse of colony.



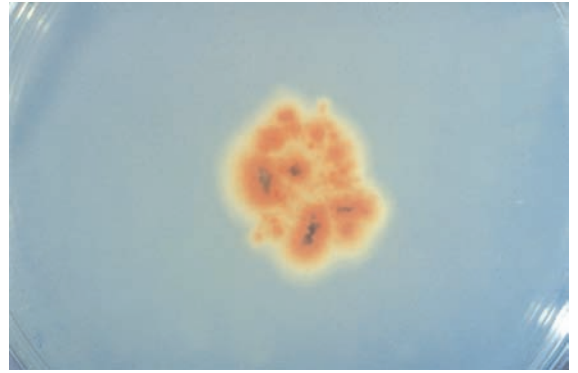
161. *Fusarium* sp. SDA, 30°C, 4 days.



162. *Trichothecium roseum*. PDA, 30°C, 8 days. The extended incubation was required for pigment production.



163. *Chrysosporium* sp. PDA, 30°C, 6 days. Surface of colony.



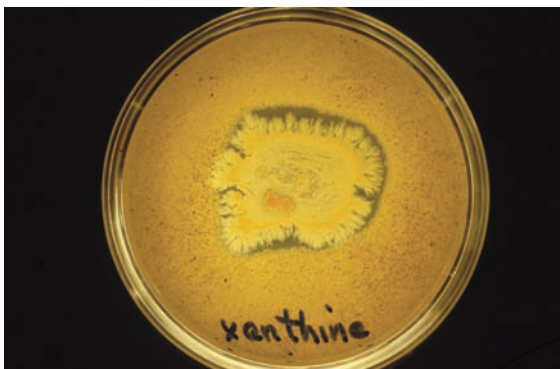
164. *Chrysosporium* sp. PDA, 30°C, 6 days. Reverse of colony.



165. *Sporotrichum pruinosum*. SDA, 30°C, 6 days. Colony spreads to cover entire agar surface.



166. *Sepedonium* sp. SDA, 30°C, 6 days.



167. Xanthine hydrolysis test. Positive result (see p. 423).

Glossary

Abscess Localized collection of pus in cavity formed by dissolution of tissue.

Aerial hyphae Hyphae above the agar surface.

Aerobic Able to grow in the presence of atmospheric oxygen.

Aleurioconidium (pl. *aleurioconidia*) A conidium produced by the extrusion from the lateral aspect of a conidiophore or hyphal structure that is released by rupture of the attachment base.

Anaerobic Able to grow in the absence of free or atmospheric oxygen.

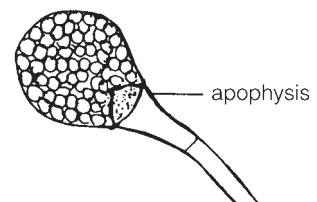
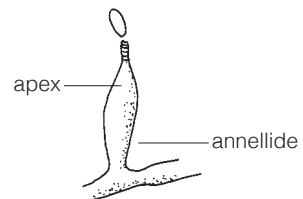
Anamorph An asexual form of a fungus.

Anellide A cell that produces and extrudes conidia; the tip tapers, lengthens, and acquires a ring of cell wall material as each conidium is released; oil immersion magnification may be required to see the rings.

Anthropophilic Pertaining to dermatophytes that preferentially colonize and infect humans.

Apex (pl. *apices*) The tip or the top (often referring to the upper part of a conidia-bearing structure).

Apophysis The swelling of a sporangiophore immediately below the columella.



Arthroconidium An asexual spore formed by the breaking up of a hypha at the point of septation. The resulting cell may be rectangular or barrel shaped and thick or thin walled, depending on the genus.

Ascospore A sexual spore produced in a saclike structure known as an *ascus*.

Ascus (pl. *asci*) A round or elongate saclike structure usually containing two to eight ascospores. The asci are often formed within a fruiting body, such as a cleistothecium or perithecium.

Asexual Reproduction of an organism by division or redistribution of nuclei, but without nuclear fusion, i.e., not by the union of two compatible haploid nuclei. Also known as the *imperfect state*. This form of the fungus is called an *anamorph*.

Assimilation The ability of a fungus to use a specific carbon or nitrogen source for growth; assimilation is read by the presence or the absence of growth.

Ballistospore A spore that is forcibly discharged from a fungal cell.

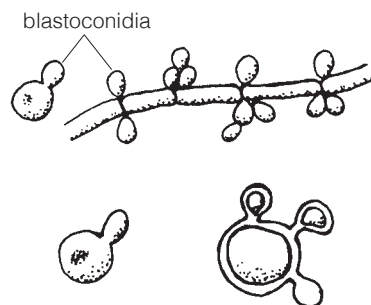
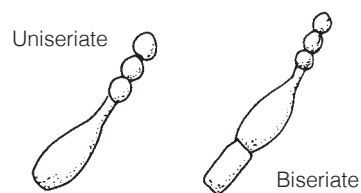
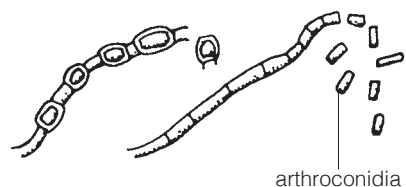
Basidiospore A sexual spore formed on a structure known as a *basidium*. Characteristic of the class Basidiomycetes.

Biseriate With reference to the genus *Aspergillus*, the phialide is supported by a metula as opposed to a uniseriate phialide, which forms directly on the vesicle. (See "Uniseriate.")

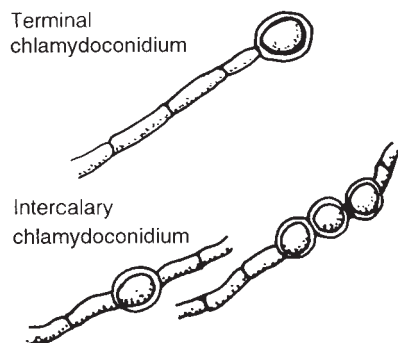
Blastoconidium A conidium formed by budding along a hypha, pseudohypha, or single cell, as in the yeasts.

Budding A process of asexual reproduction in which the new cell develops as a smaller outgrowth from the older parent cell. Characteristic of yeasts or yeastlike fungi.

Capsule A colorless, transparent mucopolysaccharide sheath on the wall of a cell.



Chlamydoconidium An enlarged, rounded conidium that is thick walled and contains stored food, enabling it to function as a survival propagule. It may be located at the end of the hypha (terminal) or inserted along the hypha (intercalary), singly or in chains. Characteristically, it is greater in diameter than the hypha on which it is borne. Unlike other conidia, it does not readily separate from the hypha.



Chlamydospore The misnomer applied to the thick-walled vesicle formed by *Candida albicans* and *Candida dubliniensis*. The more accepted term is *chlamydoconidium*.

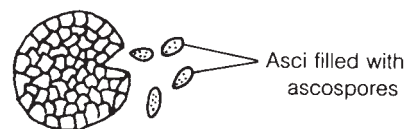
Chloramphenicol An antibiotic produced by *Streptomyces venezuelae* but usually prepared synthetically. It is a useful additive to mycology media, as it inhibits the growth of many bacteria that might contaminate the cultures.

Clade A group of organisms having similar features inherited from a common ancestor.

Clamp connection A specialized bridge over a hyphal septum in the Basidiomycetes. During the formation of a new cell, it allows postmitosis nuclear migration.

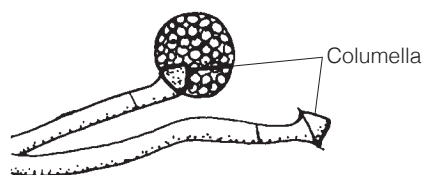


Cleistothecium A large, fairly round, closed, many-celled structure in which asci and ascospores are formed and held until the structure bursts.



Coenocytic Describing mucormycetes with pauciseptate hyphae containing multiple nuclei within the cytoplasm.

Columella The enlarged, dome-shaped tip of a sporangio-phore that extends into the sporangium. Often the sporangium bursts, leaving the columella bare and readily visible upon microscopic examination.



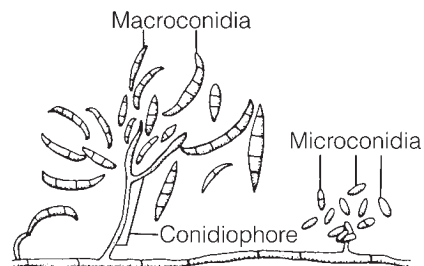
Columnar Forming a column-like arrangement; the term is most commonly used to describe the phialides that form fairly parallel to the axis of the conidiophore in some species of *Aspergillus*, rather than radiating in many directions.

Conidiogenous cell The cell that produces the conidia.



Conidiophore A specialized hyphal structure that serves as a stalk on which conidia are formed. The shape and arrangement of the conidiophores and the conidia are generally characteristic of a genus. The suffix *phore* means “carrying” and is added to the word that denotes what it is carrying; e.g., conidiophores bear conidia and sporangiophores bear sporangia. (See next page for illustration.)

Conidium (pl. *conidia*) An asexual propagule that forms on the side or the end of the hypha or conidiophore. It may consist of one or more cells, and the size, shape, and arrangement in groups are generally characteristic of the organism. It is always borne externally, i.e., not enclosed within a saclike structure such as a sporangium. If a fungus produces two types of conidia, those that are small and usually single celled are referred to as *microconidia*, whereas the larger *macroconidia* are usually segmented into two or more cells.



Contaminant An environmental organism that is growing on a patient's culture, but is not thought to be actively involved in the infection. Its source could be the laboratory, but it is far more likely to have been on the patient, at or near the infection site, when the specimen was collected.

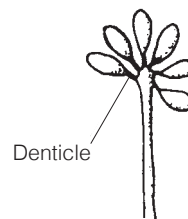
Cryptic species An organism that is morphologically identical to another species but can be distinguished by molecular methods.

Cutaneous Pertaining to the skin.

Cycloheximide An antibiotic (proprietary name, Actidione) used in selective mycology media to inhibit the growth of saprophytic fungi. Because it is also known to inhibit some pathogenic fungi, it must be used in conjunction with a medium without antibiotics.

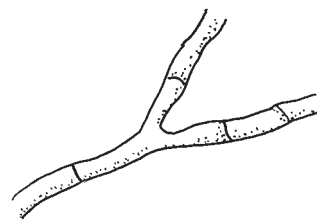
Dematiaceous Having structures that are brown to black; this is due to a melanotic pigment in the cell walls.

Denticle Short, narrow projection bearing a conidium.



Dermatophyte A fungus belonging to the genus *Trichophyton*, *Microsporum*, or *Epidermophyton* with the ability to obtain nutrients from keratin and infect skin, hair, or nails of humans or animals.

Dichotomous Branching (of hyphae) into two equal branches that are each equal in diameter to the hypha from which they originated.



Dimorphic Having two distinct morphological forms. In this guide, it refers to temperature-dependent changes in the organism on artificial culture media, i.e., fungi having a mould phase when cultured at 25–30°C and a yeast phase when cultured at 35–37°C and/or viewed directly in the host's specimen. Other sorts of dimorphism are not dependent on temperature, but exhibit different forms (i) in tissue versus artificial medium, regardless of temperature; (ii) or on routine media versus when grown under a coverslip (reducing the oxygen available).

Ectothrix Dermatophyte invasion of hair characterized by destruction of the cuticle, hyphae within the hair shaft, and a sheath of arthroconidia on the outer surface.

Endothrix Dermatophyte invasion of hair characterized by arthroconidia inside the hair shaft; the outer cuticle remains intact.

Favic chandeliers Terminal hyphal branches that are irregular, broad, and antlerlike in appearance. Especially characteristic of *Trichophyton schoenleinii*.



Fermentation The ability of a fungus to utilize a specific carbohydrate in the presence of other organic compounds, resulting in the production of gas. Therefore, the production of gas is the only indicator of a positive fermentation reaction; acid production (color change in indicator) may simply indicate that the carbohydrate has been assimilated. All carbohydrates fermented by a fungus are also assimilated, but many compounds that are assimilated are not necessarily fermented.

Filamentous Long, cylindrical, and threadlike; hyphae forming.

Floccose Cottony; like raw, fuzzy cotton.

Foot cell The base of the conidiophore, where it merges with the hyphae, giving the impression of a foot; typically seen in *Aspergillus* spp.

Fragmentation Breaking of the hyphae into pieces, each of which is capable of forming a new organism. Arthroconidia are formed in this manner.



Fungus (pl. *fungi*) An organism that is either filamentous or unicellular and lacks chlorophyll. It has a true nucleus enclosed in a membrane and chitin in the cell wall.

Fusiform Spindle shaped, i.e., being wider in the middle and narrowing toward the ends.

Geniculate Bent like a knee.

Genotype The genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms; deciphered by molecular testing.

Geophilic Pertaining to dermatophytes that grow in soil.

Germ tube A tubelike outgrowth from a conidium or spore; the beginning of a true hypha. A germ tube is not constricted at its point of origin from the parent cell (in contrast to a pseudohypha, which is constricted at its point of origin).

Glabrous Smooth; without or almost without aerial hyphae.

Grain or granule An organized mass of hyphae or actinomycetous bacterial filaments in a mycetoma.

Hilum (pl. *hila*) Scar of attachment; it appears at the point(s) where the conidium was formerly attached to the conidiophore and/or another conidium.

Host The animal or plant that supports a parasite.

Hülle cells Thickened, large, sterile cells with a small lumen; they are associated with cleistothecia produced by the sexual stage of some *Aspergillus* spp. Droplets of exudate on the surface of a colony may indicate the site of Hülle cells.

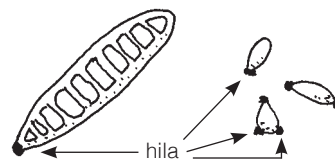
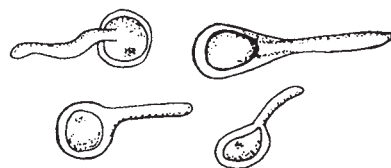
Hyaline Clear, transparent, colorless.

Hypha (pl. *hyphae*) A filamentous structure of a fungus. Many together compose the mycelium.

Hyphomycete An asexual fungus that produces mycelium that may be colorless (hyaline) or darkly pigmented (dematiaceous).

Inflammation A local protective response of the body; characterized by redness, pain, heat, and swelling.

Intercalary Situated along the hypha, not at its end.



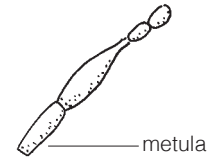
Intracellular Within cells.

Keratin A scleroprotein containing large amounts of sulfur, such as cystine; the primary component of skin, hair, and nails.

Keratitis Inflammation of the cornea of the eye.

Macroconidium (pl. *macroconidia*) The larger of two types of conidia in a fungus that produces both large and small conidia; may be single celled but usually is multicelled. (See “Conidium.”)

Metula (pl. *metulae*) The separate structural portion of the conidiophore that supports the phialide (much like a pedestal) in genera such as *Aspergillus*, *Penicillium*, and *Paecilomyces*.



Microconidium (pl. *microconidia*) The smaller of two types of conidia in a fungus that produces both large and small conidia; usually single celled and round, ovoid, pear shaped, or club shaped. (See “Conidium.”)

Monomorphic In this guide, refers to fungi having the same type of morphology in culture at both 25–30°C and 35–37°C (i.e., if growth occurs at both temperature ranges; some saprophytes are inhibited at 35–37°C).

Monophyletic Describing a group of organisms that share a close common ancestor.

Mould A filamentous fungus composed of filaments that generally form a colony that may be fuzzy, powdery, woolly, velvety, or relatively smooth.

Muriform Having transverse and longitudinal septations.



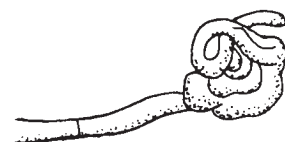
Mycelium (pl. *mycelia*) A mat of intertwined hyphae that constitutes the colony surface of a mould.

Mycetoma A localized, chronic, cutaneous or subcutaneous infection classically characterized by swollen tumorlike lesions that yield granular pus through draining sinuses.

Mycology The study of fungi and their biology.

Mycosis (pl. *mycoses*) A disease caused by a fungus.

Nodular body A round, knotlike structure formed by intertwined hyphae; seen especially in some dermatophytes.



Onychomycosis Fungal infection of the nail caused by any fungus (a dermatophyte or other fungus). Tinea unguium differs by referring only to nail infection caused by dermatophytes.

Ostiole A mouth or opening.

Pathogen Any disease-producing microorganism.

Pectinate Resembling a comb.

Pellicle A firm or buttonlike mass formed on liquid medium by some fungi.

Perithecium (pl. *perithecia*) A large, round or pear-shaped structure usually having a small, rounded opening (which differentiates it from a cleistothecium; the opening is called an *ostiole*) and containing asci and ascospores.

Phaeo- A prefix meaning dark (brownish or blackish).

Phaeohyphomycosis A subcutaneous or systemic disease caused by a variety of black fungi that develop in tissue as dark hyphae and/or yeastlike cells.

Phenotype The observable physical or biochemical characteristics of an organism.

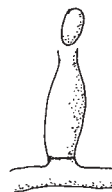
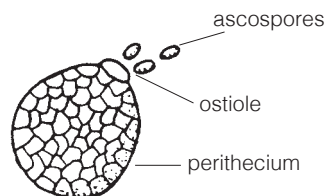
Phialide A cell that produces and extrudes conidia without tapering or increasing in length with each new conidium produced. It is usually shaped like a flask, vase, or tenpin.

Phyletic Relating to the evolutionary development or relationship of a group of species.

Phylogenetic Based on the lines of descent or evolutionary development of the organism.

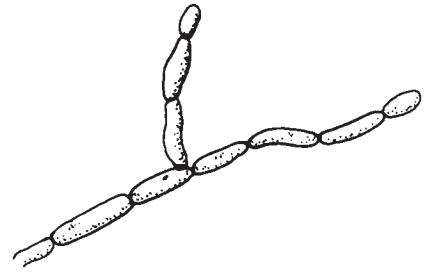
Pleomorphism The occurrence of two or more forms in the life cycle of an organism. Also refers to the occurrence of a form of dermatophyte that ceases to produce conidia (becomes sterile).

Polyphyletic Describing a group of organisms that do not share a close common ancestor.



Propagule A unit that can give rise to another organism.

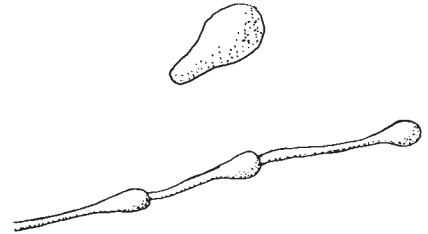
Pseudohypha Chain of cells formed by budding that, when elongated, resembles a true hypha; differs from true hyphae by being constricted at the septa, forming branches that begin with a septation, and having terminal cells smaller than the other cells.



Pycnidium (pl. pycnidia) A large, round or flask-shaped fruiting body containing conidia. Pycnidia usually have an opening (an ostiole).

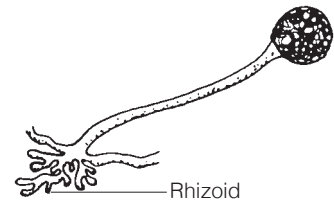
Pyriform Pear shaped.

Racquet hypha A hypha with club-shaped cells, the larger end of one cell being attached to the smaller end of an adjacent cell.



Radiate Spreading out in all directions from a common center.

Rhizoid Rootlike, branched hypha extending into the medium.



Ringworm Superficial skin disease caused by dermatophytes. Term derived from the ringlike, circular form of the lesions and from the belief that these infections were caused by wormlike organisms. The current accepted term is *tinea*.

Saprobe/Saprophyte An organism that uses dead organic matter as a source of nutrients.

Septate Having cross walls.



Sexual state The portion of the life cycle in which the organism reproduces by the union of two compatible haploid nuclei. Also known as the *perfect state*. This form is called the *teleomorph*.

Sino-orbital Pertaining to the paranasal sinuses and adjacent socket of the eye (often used in describing mucormycosis, aspergillosis, or other mould infection involving these structures).

Species complex Composed of phylogenically related species that are essentially indistinguishable by their morphology.

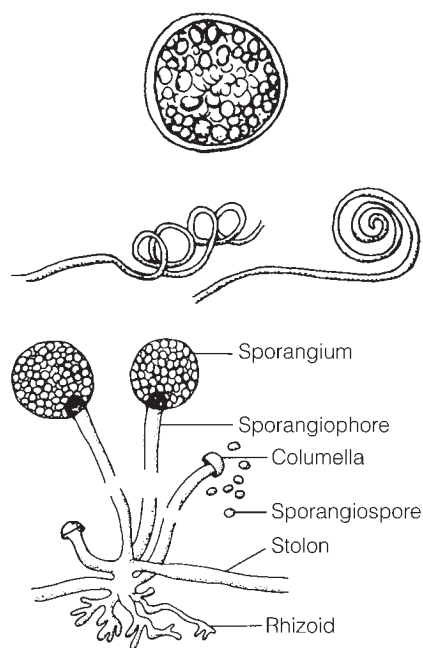
Spherule Large (20–100 μm), round, thick-walled structure containing spores; characteristic of *Coccidioides immitis* in infected host material under direct microscopic examination. Spherules do not grow on routine artificial mycology media.

Spiral hypha Hypha forming coiled or corkscrewlike turns.

Sporangiophore A specialized hyphal branch or stalk bearing a sporangium.

Sporangiospore An asexual spore produced in a sporangium.

Sporangium (pl. *sporangia*) A closed, saclike structure in which asexual spores (sporangiospores) are formed by cleavage.



Spore Propagule that develops by sexual reproduction (ascospore, basidiospore, or zygospore) or by asexual means within a sporangium (sporangiospore). Those most commonly seen in the clinical laboratory are usually enclosed in a saclike structure (as opposed to conidia, which are free, not enclosed).

Sporodochium (pl. *sporodochia*) A cushion-shaped mat of hyphae covered with conidiophores.

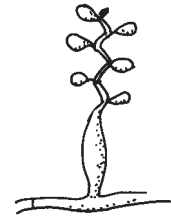
Sterigmata Term formerly used to denote phialides of *Aspergillus* and other genera. More accurately refers to denticles produced by Basidiomycetes.

Stolon A horizontal hypha, or runner, that grows along the surface of the medium, often bearing rhizoids that penetrate the medium and sporangiophores that ascend into the air (see drawing at Sporangiphore).

Subcutaneous Situated or occurring directly under the skin.

Suppurative Producing pus.

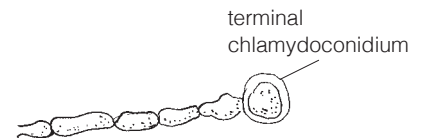
Sympodial growth Conidiogenous structure that continues to increase in length by forming a new growing point just below each new terminal conidium, often resulting in a geniculate (bent) appearance.



Synanamorph One of two or more anamorphs of the same fungus; e.g., the *Graphium* form of *Scedosporium apiospermum* is considered a synanamorph.

Teleomorph The sexual form of a fungus; demonstrates sexual structures, such as ascospores or large fruiting bodies, e.g., cleistothecia.

Terminal At the end.



Thallus The vegetative body of a fungus; the colony surface.

Tinea barbae Infection of the bearded areas of the face and neck.

Tinea capitis Infection of the scalp and hair shaft.

Tinea corporis Infection of the glabrous skin on body parts not otherwise specified (usually the trunk of the body).

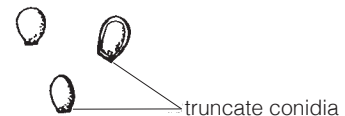
Tinea cruris Infection of the groin, perineum, and perianal region.

Tinea manuum Infection of the hand.

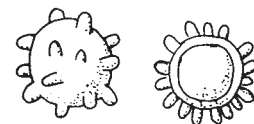
Tinea pedis Infection of the feet.

Tinea unguium Infection of the nails (onychomycosis is infection of the nail caused by any fungus, not necessarily caused by a dermatophyte).

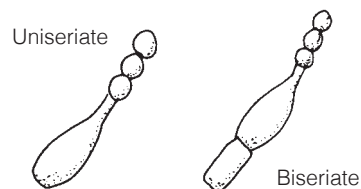
Truncate Cut off sharply; ending abruptly with a flattened edge.



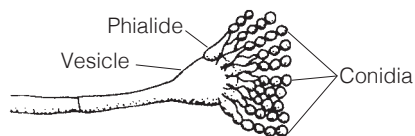
Tuberculate Having knoblike projections.



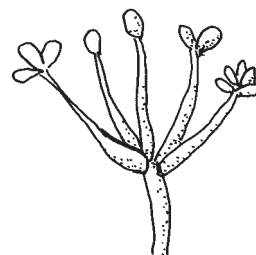
Uniseriate With reference to the genus *Aspergillus*, the phialide forms directly on the vesicle; a biseriate phialide is supported by a metula. (See “Biseriate.”)



Vesicle Enlarged structure at the end of a conidiophore or sporangiophore. In *Aspergillus* spp. it bears the phialides, which in turn bear the conidia.



Whorl A group of cells radiating from a common point.



Yeastlike colony A soft, pasty, smooth colony; usually no filamentous (fuzzy) growth can be observed macroscopically.

Zoospores Pertaining to dermatophytes that colonize and cause disease in animals.

Zygospore Sexual spore that is characteristic of the zygomycetes. They are usually large, dark, and rough walled.

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Selected Websites

Adelaide Mycology Online

<http://www.mycology.adelaide.edu.au/>

Maintained by David Ellis of the University of Adelaide in Australia. A wealth of information including, among other gems, a free download of the book *Descriptions of Medical Fungi*, 3rd ed. (Kidd et al., 2016).

The Aspergillus Website

<http://www.aspergillus.org.uk/>

Everything you ever wanted to know about *Aspergillus*.

Barcode of Life Data Systems (BOLD)

<http://www.boldsystems.org/views/login.php>

For collection, management, analysis, and use of DNA barcodes.

CBS Fungal Biodiversity Centre

<https://www.gbif.org/publisher/6a430540-7f09-11d8-a1a0-b8a03c50a862>

Maintained by the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; an excellent broad resource.

DNA Data Bank of Japan

<http://www.ddbj.nig.ac.jp/intro-e.html>

The sole nucleotide sequence data bank in Asia.

Doctor Fungus

<http://msgercdoctorfungus.com>

Website of the Mycoses Study Group. Loaded with information about the clinically encountered fungi, but not always up to date.

EMBL Nucleotide Sequence Database (EMBL-Bank)

<https://www.ebi.ac.uk>

European nucleotide sequence resource.

<https://www.ebi.ac.uk/ena>

European Nucleotide Archive (ENA).

Fusarium-ID

<http://www.westerdijkinstituut.nl/fusarium/>

Database for identification of *Fusarium* spp., including MLST database.

Index Fungorum

<http://www.indexfungorum.org/names/names.asp>

International Commission on the Taxonomy of Fungi

<http://www.fungaltaxonomy.org>

ISHAM

<http://www.isham.org>

Website of the International Society for Human and Animal Mycology.

Japan Society of Culture Collections (JSCC)

<http://www.nbrc.nite.go.jp/jscc/idb>

Database search. "JSMRS On-Line Database"

<http://www.nbrc.nite.go.jp/jscc/idb/snalpha?Org=Fungus>

Catalogue of cultures.

Medical Mycology Research Center, Chiba University, Japan

<http://www.pf.chiba-u.jp/eng/>

Overview.

<http://www.pf.chiba-u.jp/english/gallery.html>

Photos and microphotographs of many fungi and a few aerobic actinomycetes.

MLST website, Imperial College London, United Kingdom

<http://www.mlst.net/>

Multilocus sequence typing (MLST); data analysis, database, and more.

Molecular Mycology Research Laboratory, University of Sydney, Australia

<http://www.mycologylab.org/DefaultInfo.aspx?Page=Home>

Database and resource center.

Mycobank

<http://www.mycobank.org/>

Databases, nomenclature and species bank, online taxonomic novelties submission, and more. Administered by the International Mycological Association.

NCBI (Includes GenBank)

<http://www.ncbi.nlm.nih.gov>

Website of the National Center for Biotechnology Information. Provides access to extensive biomedical and genomic information.

PHYLIP (Phylogeny Inference Package)

<http://evolution.genetics.washington.edu/phylip/software.html>

Programs available for phylogenetic analysis.

Species Fungorum

<http://www.speciesfungorum.org/names/names.asp>

For nomenclature and taxonomy.

Westerdijk Fungal Biodiversity Institute

<http://www.westerdijkinstitute.nl/>

TUTORIALS**Cycle Sequencing: Dolan DNA Learning Center, Cold Spring Harbor Laboratory**

<http://www.dnalc.org/view/15923-Cycle-sequencing.html>

Sanger sequencing; animated presentation with written explanation.

High-Throughput Sequencing

<http://www.sumanasinc.com/webcontent/animations/content/highthroughput2.html>

Pyrosequencing, high-throughput using microbeads; animated and narrated.

The Polymerase Chain Reaction (PCR) (Sumanas Inc.)

<http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

Animated and narrated explanation of PCR.

Principle of Pyrosequencing Technology (Qiagen)

<https://www.qiagen.com/us/resources/technologies/pyrosequencing-resource-center/technology-overview/>

The Sanger Method (Sarah Obenrader)

http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Obenrader/sanger_method_page.htm

Illustrated description of the basic and modified method of Sanger sequencing.

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