Basic Mycology

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Introduction

The mycology laboratory plays a vital role in the diagnosis of fungal infections through the recovery and identification of the etiologic agent. Specimen collection from appropriate sites is critical, as is the proper transport, storage, and processing of samples. Fungal elements seen by direct microscopy often provide the first clues to a fungal infection, and are the basis upon which empiric therapy is initiated. To ensure recovery of the fungus, a sufficient number and type of media should be utilized for primary isolation based upon the clinical history and the possible organisms expected. Accurate fungal identification, in combination with antifungal susceptibility testing, provides the basis for appropriate organism-directed antifungal therapy and is essential for conducting epidemiologic investigations.

Human and/or animal pathogens historically considered to be fungal are now placed in three kingdoms: *Fungi, Straminipila* (*Stramenopila*), containing the Oomycete *Pythium* [1], and *Protoctista,* with the bulk of the human pathogens in the kingdom Fungi [2]. Organisms within this kingdom are eukaryotic (have cells containing a membrane-bound nucleus), heterotrophic (lack chlorophyll or other pigments capable of photosynthesis for making food, therefore must obtain nourishment from an external food source), may be unicellular or filamentous, and have cells surrounded by cell walls containing glucan, chitin, or both. Unlike animals, fungi possess cell walls, but unlike plants, the major cell wall component is not cellulose. In the past, medical problems attributed to these organisms, in comparison to those caused by the bacteria, viruses, and parasites, have been relatively few, and included allergic symptoms, mushroom poisoning, mycotoxicoses from ingested fungal toxins, and occasional fungal infections [2]. However, with the advent of modern medical advances utilizing immunosuppressive regimens, and with an increase in diseases/underlying conditions significantly altering the human immune system, fungal infections (mycoses) have increased significantly over the past couple decades. The recovery of these organisms from host tissue and their identification is often critical to the diagnosis and treatment of mycotic disease and is the classic method for documentation of pathogenicity. Histopathology, and other adjunctive tools, such as antigen or antibody assays and molecular techniques, addressed elsewhere in this text, may also be relied upon for empiric/preemptive therapeutic decisions, when cultures are either not available or fail to provide unequivocal information. The proper collection, transport, and processing of specimens; selection of fungal stains and preliminary direct microscopy techniques; and use of appropriate media and incubation conditions are all important to the accurate identification of fungal infection. This chapter provides a cursory review of the laboratory fundamentals as they relate to medical mycology. It also reviews basic taxonomy, classification, and nomenclature regarding the kingdom fungi and changes resulting from the Melbourne Code implemented in January 2013 [3]. Also included are descriptions of mycologic terms/features common to the most frequently recovered etiologic agents in the teleomorphic (sexual) phyla Ascomycota, Basidiomycota, and Glomeromycota (formerly Zygomycota) [4] as well as those seen in the anamorphic (asexual) fungi. Fungi without known sexual states are referred to as "mitosporic" (based upon their reproductive mitotic processes). The mitosporic fungi are the most common etiologic agents of human and animal disease.

Specimen Collection, Transport, and Processing

The likelihood of recovering a fungal etiologic agent is directly proportional to the quality of methods employed in the collection, transport, and processing of clinical

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Table 2.1 Common specimen collection sites for fungal cultures[a]

Collection site	Comments
Abscesses, subcutaneous sites	Aspirate abscess; sample base of subcutaneous lesions
Blood	Use maximum amount of blood recommended for the system being used
Bone marrow	Pediatric Isolator™ recommended[b]
CSF	Do not refrigerate
Draining sinus tracts	Search for granules of eumycotic mycetoma; wash several times with saline containing antibiotics
Ear	Rotate swab firmly in outer ear
Eye	Inoculate corneal scrapings directly onto plates in a "C" shape
Hair	Use forceps to collect several hairs with shaft intact and sample any active lesions
Intravenous catheters	Use Maki ("roll") method
Lower respiratory	Process promptly for dimorphic pathogens (BAL, brush, aspirate, wash, sputum)
Medical devices (valves, hardware, etc.)	Dislodge any biofilms before inoculation into liquid medium
Nails	For dermatophytes, agents of dermatomycoses, and *Candida* spp.; clean with 70% alcohol; collect subungual debris and clip affected nails
Nasal sinus	Surgical collection, commonly ethmoid and maxillary sinuses
Open wound	Aspirate or swab vigorously
Prostatic fluid	Primarily for blastomycosis
Skin	For dermatophytes; clean with 70% alcohol and scrape vigorously
Sterile body fluids	May be concentrated by centrifugation or syringe filtration
Tissue	Surgical collection; use punch biopsies for skin lesions
Urine	Early morning midstream collection
Vagina	Primarily for refractory vaginal candidiasis
Vitreous fluid	Needle aspiration
Upper respiratory (oral)	Swab lesions, use selective media for yeasts

CSF cerebrospinal fluid, *BAL* bronchoalveolar lavage

[a] This list is not all inclusive. Also see Appendix A in reference [5]

[b] Wampole Laboratories, Princeton, NJ

specimens, and with the 2012 publication of *Principles and Procedures for Detection of Fungi in Clinical Specimens—Direct Examination and Culture*: *Approved Guideline, M54-A,* by the Clinical and Laboratory Standards Institute (CLSI), this information is now condensed into a readily available and user-friendly document addressing all aspects of these processes for the improved recovery of fungal isolates [5]. For all disease processes, recovery is highest from an active site of infection. Common (but not all inclusive) specimen types include those from the respiratory tract [6], draining sites, aspirated abscess fluids, normally sterile body fluids, urine [7], vaginal secretions [8], corneal scrapings [9], surgical tissue specimens, intravenous catheter tips (obtained by the Maki roll method [10]), and various surgically removed medical devices [11]. Although tissue may be homogenized for the recovery of *Histoplasma capsulatum,* when the patient history suggests infection with a member of the *Mucorales* or other filamentous fungi, tissue grinding should be avoided as it may be deleterious to the growth in culture of fragile fungal hyphae [4, 11]. Specimens peripheral to the site of infection, such as blood or bone marrow, may be diagnostic in disseminated disease or when foci are not easily accessible. Several blood culture systems reliably recover yeast pathogens [12]. If manual blood cultures are used, consider a broth/agar biphasic system in which an agar paddle is attached to the bottle (Septi-Chek, BD Diagnostic Systems, Sparks, MD). Several automated, continuously monitored blood culture systems are available with instruments designed and scalable for both high- and small-volume laboratories. These include the VersaTREK (Trek Diagnostic Systems/Thermo Scientific, Cleveland, Ohio), BacT/Alert (bio Mérieux, Durham, NC), and BACTEC (BD Diagnostic Systems, Sparks, MD) systems [13–19]. Always follow manufacturer's recommendations for the specific system, using the maximum amount of blood samples recommended. The ratio of blood to broth is the most critical factor in fungal recovery, and should be near 1:5 in most systems [20]. Lysis centrifugation methods, either commercially available as the Isolator™ system (Alere, Waltham, MA) or manual methods [21, 22], are recommended for dimorphic fungal pathogens and filamentous fungi [23, 24]. Intravascular catheter tips are also frequently submitted, and should be cultured according to the semiquantitative method of Maki [10]. Blood cultures should also be drawn at the time of catheter removal to correlate catheter colony counts and organisms recovered with catheter-related septicemia. Catheter colony counts of less than 15 are less likely to be associated with infection. Specimens should be maintained at room temperature and transported to the laboratory as soon as possible, ideally within 2 h. Exceptions include storage of central nervous system specimens at 30 °C, and 4 °C extended storage for specimens likely to have bacterial contamination. Hair, skin, and nails

Table 2.2 Useful direct microscopy methods for the routine mycology laboratory[a]

Method	Comments
Calcofluor white	Requires fluorescence microscope; can be used with KOH to detect all fungi, including *Pneumocystis*
Gram stain	Detects most fungi, which are present; however, *Cryptococcus* spp. may exhibit only faint staining
Giemsa stain	Several modifications; detects intracellular *H. capsulatum* and intracystic bodies and trophozoites of *Pneumocystis*
India ink stain	Commonly used from demonstration of capsular material of *Cryptococcus neoformans* in CSF
Potassium hydroxide	Clears debris so fungi are more readily observed; stains may be added for better visualization of fungal elements
Wright stain	Useful to detect intracellular *H. capsulatum* in bone marrow and peripheral smears

CSF cerebrospinal fluid
[a] Additional fungal stains are available through the histopathology laboratory. This list is not all inclusive

Table 2.3 Media useful for primary isolation and identification[a]

Medium	Uses/Comments
Sabouraud dextrose agar (SDA)	For yeasts
	Usually adequate for aspergilli
	Poor color and conidiation for black moulds
	Classic morphologic descriptions for dermatophytes
CHROMagar Candida[b]	Contains chromogenic substrates and antimicrobial agents; for isolation and identification of yeasts
Albicans ID[c]	As above
Potato dextrose agar (PDA)	Useful for all mould recovery/identification
Potato flakes agar (PFA)	
Brain heart infusion (BHI) agar	
Inhibitory mould agar (IMA)	
Yeast extract phosphate medium	
Sabhi agar	
Mycosel agar™ [§] or Mycobiotic agar	SDA with chloramphenicol and cycloheximide
Dermatophyte test medium (DTM)	
Dermatophyte identification medium (DIM)	

[a] This list is not all inclusive. All are commercially available. Please also see Sect. 10.2 in reference [5]
[b] CHROMagar Microbiology, Paris, France
[c] bioMérieux, Marcy l'Etoile, France
[d] BD Diagnostic Systems, Sparks, MD

may be transported in clean paper envelopes. Several sources provide specific guidelines for the collection, transport, and processing of various types of specimens for fungal culture [5, 11, 25, 26]. See Table 2.1 for common collection sites and consult Appendix A in the CLSI M54-A document [5] for more detailed information/procedures.

Prior to receipt in the mycology laboratory, a portion of all tissue samples submitted for culture should also be placed in formalin for submission to the histology laboratory. Histopathologic examination together with appropriate stains are usually necessary to document fungal invasion. These may include the routine hematoxylin and eosin (H&E) stain, Gomori methenamine silver (GMS) stain, periodic acid–Schiff (PAS) stain, and others. Discussion of the use of histopathology and of mycological stains is found in Chap. 4 of this book. See also Appendix C of the CLSI M54-A document [5]. As a part of routine processing, the mycology laboratory should also examine a portion of the specimen directly by microscopy, typically with the use of a potassium hydroxide (KOH) preparation, gram stain, calcofluor white fluorescent stain, India ink stain (limited to cerebrospinal fluid examination for *Cryptococcus neoformans*), or some other method (Table 2.2). Observation of fungal structures by direct microscopy and/or histopathology is essential to corroborate organism recovery in culture (rule out contamination). Table 9.4 in Sect. 9.1.1 of the CLSI M54-A document [5] provides a useful listing/description of yeasts and yeast-like fungi seen in clinical specimens.

The media used for primary isolation may vary according to personal preferences; however, certain basic tenets apply to all media used for primary recovery. Material from nonsterile sites should be cultured on media that will support fungal growth but also inhibit bacteria. Antibacterial agents, alone or in combination, are added for this purpose.

Common choices include chloramphenicol (<16 μg/ml), gentamicin (5–100 μg/ml), penicillin (20 μg/ml), streptomycin (40 μg/ml), and ciprofloxacin (5 μg/ml). These agents should not be included, however, when actinomycetes are suspected. Media may also be made selective by the addition of the eukaryotic protein synthesis inhibitor cycloheximide at 0.5 μg/ml. This may be useful in the detection of dimorphic fungi and dermatophytes; however, many clinically significant saprobic fungi may be suppressed, leading to failure in recovering opportunistic etiologic agents in compromised hosts. Therefore, media with and without this agent should routinely be employed. Enriched media with 5–10 %, sheep erythrocytes may be incorporated into the battery for fastidious thermally dimorphic fungi such as *H. capsulatum* and *Blastomyces dermatitidis*. Peptone-based versus plant-based media may also be a consideration. Many of the opportunistic filamentous fungi prefer plant-based media, producing more typical colony morphologies and more diagnostic structures, thus increasing the potential to make identification possible from primary plates. Plant-based media may also be made selective with antibacterial agents or cycloheximide. Table 2.3 lists several commercially available media that may be used for both primary isolation and identification. Please consult Sects. 10.2 and 10.3 in the CLSI M54-A document for additional information [5]. The choice of tubed versus plated media is made based upon space constraints, personal preference, and safety. The greater surface area provided by plates is preferred by many laboratorians (and always preferred by the fungi!), as manipulation of cultures, isolation procedures, etc. are more easily performed on plates. When used, plate lids should be firmly attached with an air-permeable material or plates sealed in air-permeable bags to avoid cross-contamination or laboratory worker exposure.

Optimally, cultures should be incubated at 30 °C (± 1 °C). If this temperature is not available, room temperature near 25 °C should be used. Seven-day incubation is generally adequate when screening for yeasts from oropharyngeal or vaginal sites. Although 4-week incubation times have been traditionally recommended, studies suggest that 3 weeks are adequate to detect fungal growth from most other specimens, excluding those from skin, hair, and nails, and in cultures requested specifically to attempt to recover dimorphic pathogens [27]. The time required for development of diagnostic structures, particularly for some coelomycetes and ascomycetes, may be considerably longer, up to several weeks [28, 29].

Examining Cultures

One recommendation for culture examination is every day for the first 3 days and twice a week thereafter. Alternate reading schedules can be found in the CLSI M54-A document, Sect. 11.1 [5], depending upon the source of the isolate. Cultures of yeasts are typically creamy to waxy, while moulds appear velvety to woolly to cottony. Some safety precautions common to both yeasts and moulds include the careful handling of plates and tubes so as not to create aerosols of infectious material and the prevention of contamination of patient cultures with ubiquitous fungi from the work surroundings.

Phenotypic Yeast and Yeastlike Organism Identification

Yeast cultures, consisting of unicellular organism that replicate by budding, may be handled on the open bench, adhering to the same safety precautions as for bacteria. Yeast and yeastlike fungi should be examined for their colony color (white to cream to pink; brownish-black for the yeast synanamorph of *Exophiala* species when observed on Sabouraud dextrose agar; blue to green to pink for *Candida* species on CHROMagar Candida™ (CHROMagar Microbiology, Paris, France)), growth rate, temperature requirements (or preferences), macroscopic morphology (smooth, wrinkled, glabrous, moist, dry, etc.), and microscopic morphology (size and shape, presence of blastoconidia, capsules, germ tubes, pseudohyphae, true hyphae, chlamydoconidia, etc.). Yeast morphology is most reliably observed on a cornmeal agar plate using the Dalmau method [30]. This technique involves streaking a very small amount of yeast onto a plate in two parallel lines, streaking back and forth over these lines for better isolation, and covering the area with a flame-sterilized coverslip. The plate is incubated at room temperature for 18–24 h and then examined microscopically for diagnostic structures. Tease mounts may also provide useful information. Additional procedures that may be required for the identification of yeasts include the reduction of nitrate to nitrite, urease activity, the ability of the organism to grow on media containing cycloheximide, and assimilation and fermentation patterns. Many commercial systems, both manual and automated, are available to assist in yeast identification.

Phenotypic Mould Identification

Any filamentous organisms recovered on culture should be examined and manipulated in a biological safety cabinet. While moulds can be recovered on a variety of media, conidiation/sporulation is generally enhanced on plant-based media. If not used in primary isolation, plant-based media should be employed in the identification process. Moulds should be examined for their growth rate, temperature requirements, and macroscopic morphology to include color

(hyaline to brightly colored or phaeoid (brownish to blackish)), texture (velvety, woolly, granular, cottony, etc.), and the observation of any diagnostic features visible to the naked eye. The microscopic detail may be studied using tease mounts or temporary tape mounts (clear tape only) in lactophenol cotton blue. The preferred technique to demonstrate diagnostic structures and methods of conidiogenesis for most filamentous fungi is the slide-culture method. Additionally, this method can provide a permanent mount that can be preserved in a slide collection for future studies and is extremely useful for comparison with other similar isolates or atypical strains. Members of the order *Mucorales* (which includes *Rhizopus, Lichtheimia* (formerly *Absidia*), *Mucor,* and several other genera) may rapidly overgrow slide cultures, making this method less than optimal for studying these fungi.

Slide cultures should also not be set up on Moulds where the clinical history suggests a dimorphic pathogen such as *H. capsulatum, B. dermatitidis, Coccidioides* species, *Paracoccidioides brasiliensis* (not commonly seen in the USA), or *Talaromyces* (*Penicillium*) *marneffei* (usually restricted to HIV-infected individuals from endemic areas of Southeast Asia) [31]. Tease mounts should be prepared for these isolates in a mounting fluid known to kill the fungus, such as lactophenol cotton blue. *Sporothrix schenckii,* and other *Sporothrix* spp. [32], also dimorphic organisms, pose less of an exposure risk, and may be examined by slide culture. *H. capsulatum* and *B. dermatitidis* may be definitively identified using the DNA Gen-Probe® (AccuProbe, San Diego, CA) methodology. This method, which confirms a *Coccidioides* species, does not, however, differentiate between *Coccidioides immitis* and *Coccidioides posadasii*.

Molecular Fungal Identification

While this work contains a chapter on diagnostic molecular biology (Chap. 3), information about fungal identification would be incomplete without brief mention here of the most commonly used molecular method for the identification of clinical isolates. The method most often cited is sequencing with identification of the isolates by comparative sequence analysis [33–35]. Briefly, genomic DNA is extracted, an optimal gene target is amplified and sequenced, and the data are aligned and analyzed. The most common targets useful for a wide range of fungi are within the ribosomal DNA (rDNA) and include the internal transcribed spacer (ITS1 and ITS2) regions and the D1/D2 regions of the large ribosomal subunit. Sequence results are then compared against type strains or other credible deposits in a database using the BLASTn algorithm. GenBank is a commonly used database; however, it is non-curated, so there is no assurance that the sequence data deposited is from a correctly identified isolate, thus making it imperative that the molecular identification is compatible with the phenotypic features. While the ITS and D1/D2 regions are very useful, additional targets may be needed for some genera such as beta-tubulin (TUB), calmodulin (CAL), actin (ACT), CO1 (the mitochondrial cytochrome oxidase 1 gene), the translation elongation factor (TEF), and others [36]. Other databases that are curated such as those at the Centraalbureau voor Schimmelcultures (CBS) and the online *Fusarium* database [37] are often necessary for a definitive identification. In light of cost containment and the dwindling number of individuals trained in classical morphologic identification, more laboratories are considering adding the sequencing of isolates in conjunction with phenotypic methods or as a stand-alone method. It should be noted that sequence-alone identification of limited or non-informative targets without a comparison of the phenotypic features has the potential for misidentification of isolates that could negatively impact patient management [36].

Taxonomy, Classification, and Nomenclature

Many volumes have been dedicated to the taxonomy, classification, and nomenclature of clinically significant fungi. Herein, this work only highlights some of the basic concepts. The classification scheme accepted by most authorities will be presented for the kingdom fungi. The term classification, in the fungal sense, refers to the application of names for the categories into which the taxa (taxonomic groups) may be grouped, with some subdivisions regarding their relative order. "Taxonomy" refers to this classification in a very systematic way, and nomenclature is the assigning of names to fungi. These processes, previously regulated by the rules of the International Code of Botanical Nomenclature (ICBN), are now under the auspices of the International Code of Nomenclature for algae, fungi, and plants. The Melbourne Code, adopted in 2011, and published in 2012 [3, 38], resulted in major changes which became effective January 1, 2013. Some of the most significants are as follows: (a) the abolishment of Article 59 of the previous ICBN, permitting multiple names for the same organism (such as one name for the anamorph and one for the teleomorph, i.e., such as the use of both *Aspergillus fumigatus* and *Neosartorya fumigata*); (b) the need to determine *which* name will be used (an ongoing process); and (c) the deposition of isolate information into a recognized repository for the valid publication of a fungal name. The following is an abbreviated classification scheme for the kingdom Fungi:

Group	Group Ending
Kingdom	none
Subkingdom	none
Phylum	mycota
Subphylum	mycotina
Class	mycetes
Order	ales
Family	aceae
Genus	no specific ending
Species	no specific ending
Variety	no specific ending

The phyla in which the sexual or teleomorph forms of the majority of human/animal pathogens reside are the *Ascomycota*, *Basidiomycota* and the *Glomeromycota* (formerly the *Zygomycota*) [4]. An example of this classification scheme for the ascomycete, *Microascus cinereus,* recovered from lower respiratory sites [39], would look like this:

Kingdom	Fungi
Subkingdom	Dikarya
Phylum	Ascomycota
Subphylum	Ascomycotina
Order	Microascales
Family	Microascaceae
Genus	*Microascus*
Species	*Microascus cinereus*

M. cinereus, a sexual fungus (or the teleomorph) that produces perithecia, asci, and ascospores in culture also simultaneously produces an asexual form (the anamorph) that is microscopically quite different. Asexual fungi, previously given the prefix "form" to the classification scheme, such as form-class, form-order, etc., are now commonly known as "mitosporic" fungi, or those reproducing my mitosis rather than meiosis. The anamorphic form of *M. cinereus* is the phaeoid fungus, *Scopulariopsis cinereus* [39]. Anamorphic fungi are identified mostly on the basis of their method of conidiogenesis (how they form their reproductive structures). Asexual reproductive propagules are referred to as conidia, hence, the term conidiogenesis. Sexual fungi are mostly identified based on the method they use to form their sexual reproductive propagules (e.g., ascospores, basidiospores). Not all taxonomists have agreed that we should apply different names to the anamorph and teleomorph of the same fungus, the holomorph, or "whole fungus"; however, this has been the practice until the implementation of the Melbourne Code. As mentioned above, multiple names will no longer be permitted; however, the determinations as to which name will be used for the various genera is still ongoing. One genus that does appear to retain its anamorphic name is *Fusarium* [40]. Adding to this confusion, some multiple fungi-produced anamorph forms, such as is seen with the fungus *Pseudallescheria boydii*. *P. boydii* is the teleomorph; *Scedosporium boydii* is the anamorph (previously thought to be *S. apiospermum*) [41], and they may also produce a *Graphium* synanamorph, or another anamorphic form of the "whole fungus." Practically speaking, most etiologic agents are identified in the laboratory on the basis of structures formed by the anamorphic form of the fungus. Although many mitosporic fungi have known teleomorphs, most require two mating strains to produce the sexual form. These are referred to as heterothallic. A few clinically significant fungi require only one strain to produce the teleomorph, and these as considered homothallic. *M. cinereus* and *P. boydii,* cited above, are examples of homothallic fungi.

Fungal Identification

Yeast identification is performed in a manner similar to that for bacterial identification, and easily lends itself to various compartmentalized and automated methods that measure various physiologic characteristics. Mould identification, however, currently relies more upon the observation of macroscopic morphologies, such as color and colonial features, growth rate, temperature maximums and minimums, and microscopic structures. Some of these more common identifying characteristics are exemplified in the organisms chosen in the thumbnail sketch of the kingdom fungi as illustrated in Table 2.4.

Ascomycota

Under the phylum Ascomycota, the ascomycetous yeasts are usually identified by yeast methods, while the Mould are identified based upon the structures they produce. Some of the filamentous homothallic ascomycetes produce ascomata known as cleistothecia, perithecia, or gymnothecia in which the asci and ascospores are contained (Figs. 2.1–2.4).

Basidiomycota

Similarly, the red and white yeasts within the phylum Basidiomycota are commonly identified by yeast methodologies. The filamentous basidiomycetes pose identification dilemmas, as they frequently remain sterile in culture, producing no unique reproductive structures. *Schizophyllum commune* is one of the few that may sometimes be tentatively identified by its production of spicules along the sides of the

Table 2.4 Simplified schematic of the kingdom Fungi for some human/animal pathogens

Subkingdom: Dikarya
Phylum: Ascomycota
Class: Hemiascomycetes—yeasts
Class: Euascomycetes—mould; produce ascospores in a variety of sexual structures known as ascomata (*pl.*), ascoma (*sing.*)
Cleistothecium—round, closed ascoma
Example: *Pseudallescheria boydii*, Fig. 2.1
Perithecium—pear-shaped ascoma, with an opening or ostiole
Example: *Microascus cirrosus*, Fig. 2.2
Gymnothecium—ascoma with a loose network of hyphae
Example: *Myxotrichum deflexum*, Fig. 2.3
Asci (*pl.*), ascus (*sing.*)—within the ascoma and containing ascospores
Ascospores—various sizes, shapes, colors, ornamentation
Example: *Sporomiella* sp., Fig. 2.4
Phylum: Basidiomycota
Subphylum: **Pucciniomycotina = Urediniomycetes**
Primarily contains the rusts
Subphylum: **Ustilaginomycotina = Ustilaginomycetes**
Contains yeastlike members of smut fungi
Subphylum: Agaricomycotina = Hyenomycetes
Contains mushrooms (basidiocarps) producing yeast anamorphs (*Cryptococcus* species) and filamentous anamorphs that are frequently sterile or may produce arthroconidia
Example: *Schizophyllum commune*, a human etiologic agent, produces spicules (small protrusions) along the hyphae, Fig. 2.5
Basidiospores sometimes seen from basidiocarps of *S. commune*, Fig. 2.6
Phylum: Glomeromycota
Subphylum: ***Mucormycotina***
Order: ***Mucorales***—asexual reproduction by multi-spored or few (to one) spored sporangia (sporangiola)
Heterothallic genera (require two mating strains) include some spp. of *Rhizopus*, *Lichtheimia*, *Mucor*, and others; produce sporangiospores
Example: *Rhizopus microsporus*, Fig. 2.7
Homothallic genera/species (one mating strain required) produce zygospores
Example: *Cokeromyces recurvatus*, Fig. 2.8
Subphylum: ***Entomophthoromycotina***
Order: ***Entomophthorales***—characterized by forcibly discharged conidia. Produce asexual primary conidia and smaller secondary conidia
Example: *Conidiobolus incongruus*, Fig. 2.9
Example: *Basidiobolus ranarum*, produces zygospores
Mitosporic fungi (formerly fungi imperfecti)
Methods of conidiogenesis
Blastic—conidia blown out
Phialidic conidiogenous cell—often have discernable collarettes and produce phialoconidia
Example: *Phialophora americana,* Fig. 2.10, and *Aspergillus flavus,* Fig. 2.11
Annellidic conidiogenous cells—have rings or annellations and become longer and narrower with production of annelloconidia
Example: *Scopulariopsis cirrosus,* Fig. 2.12
Some species blow out conidia through pores on geniculate conidiophores
Example: *Curvularia hawaiiensis,* Fig. 2.13
Thallic—conidia formed from preexisting hypha
Arthroconidia produced that may or may not have intervening disjunctor cells
Example: *Coccidioides* species, Fig. 2.14, and dematiaceous arthroconidia of *Neoscytalidium dimidiatum,* Fig. 2.15
Hyphomycetes—bear their conidia free and display various colors, methods of conidiogenesis, growth rates, etc.
Example: *Aspergillus flavus,* Fig. 2.10
Coelomycetes—bear their conidia within some type of asexual structure known as a conidioma (*sing.*) (conidiomata (*pl.*)) and display various colors, methods of conidiogenesis, growth rates, etc.
Pycnidium—round conidioma with an opening (ostiole) and conidia contained within; Example: *Phoma* species, Fig. 2.16
Acervulus—flat, cup-shaped conidioma, with conidia more or less exposed; Example: *Colletotrichum* species

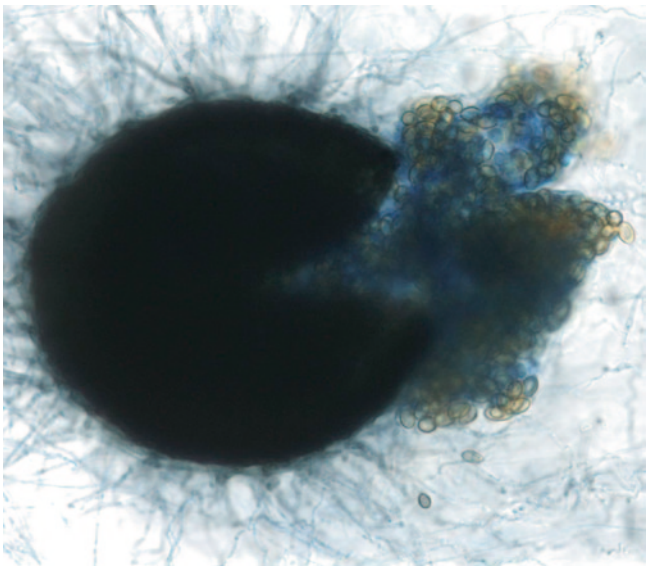

Fig. 2.1 Globose ascoma (closed cleistothecium) of *Pseudallescheria boydii*

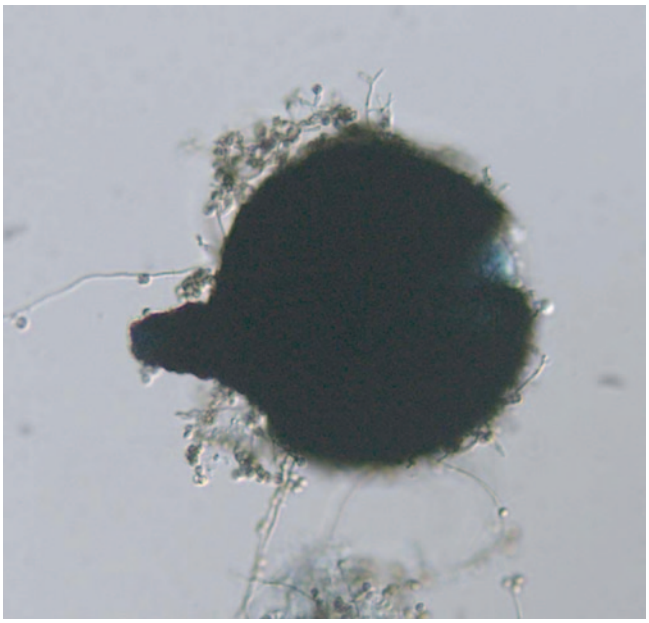

Fig. 2.2 Pear-shaped ascoma (perithecium with an opening or ostiole) of *Microascus cirrosus*

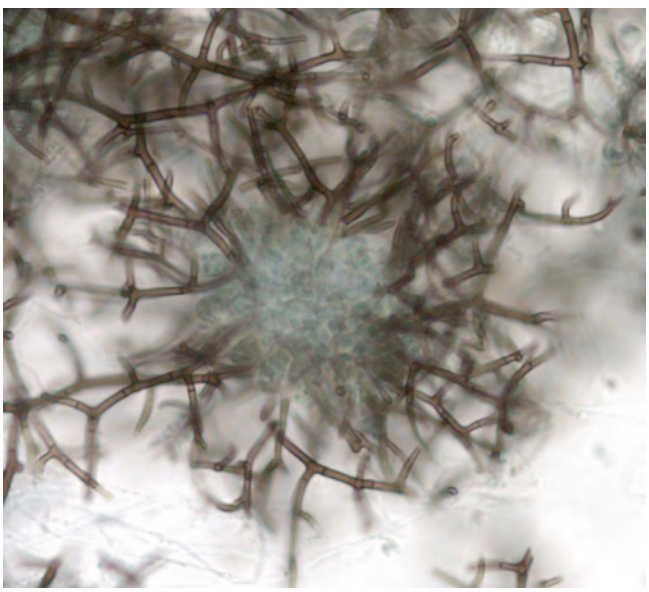

Fig. 2.3 Gymnothecium (ascoma with a loose hyphal network surrounding central ascospores) of *Myxotrichum deflexum*

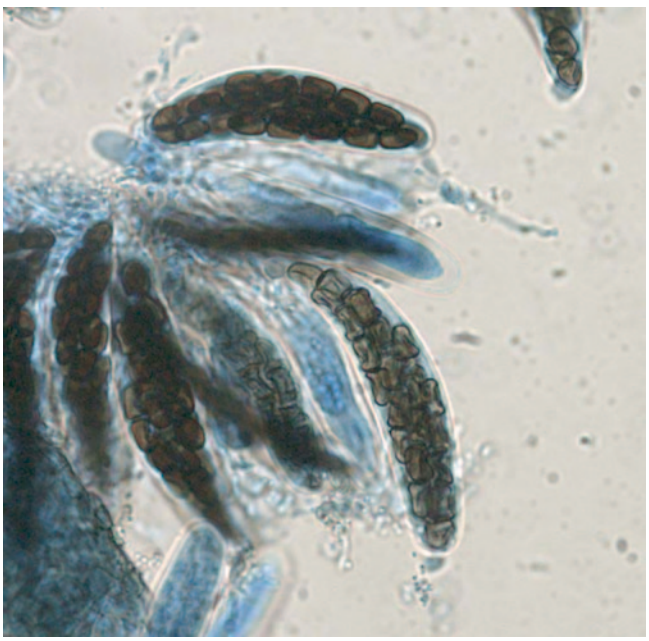

Fig. 2.4 Asci containing dark ascospores of a *Sporomiella* species

hyphae, and occasionally by clamp connections (Fig. 2.5), basidiocarps, and basidiospores (Fig. 2.6) when dikaryons (compartments of a hypha that contain two nuclei, each derived from a different parent) are present.

Glomeromycota

Human and animal pathogens in the phylum Glomeromycota (formerly Zygomycota) are contained within the subphyla *incertae sedis Mucoromycotina* containing the order *Mucorales* [42], and *Entomophthoromycotina* containing the order *Entomophthorales*. In the order, *Mucorales* are the most common mucoralean genera such as *Rhizopus* (Fig. 2.7), *Mucor, Lichtheimia* (formerly *Absidia*) [43], *Rhizomucor, Cunninghamella,* and *Cokeromyces* (Fig. 2.8), while the *Entomophthorales* encompass the less frequently seen genera

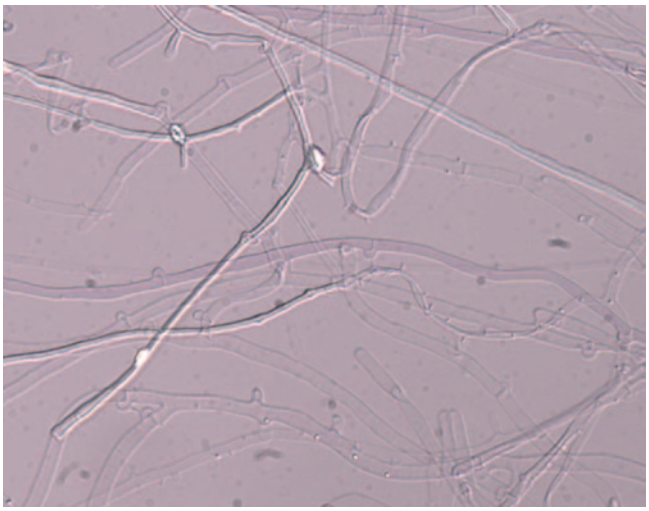

Fig. 2.5 Spicules and clamp connections on hyphae of *Schizophyllum commune*

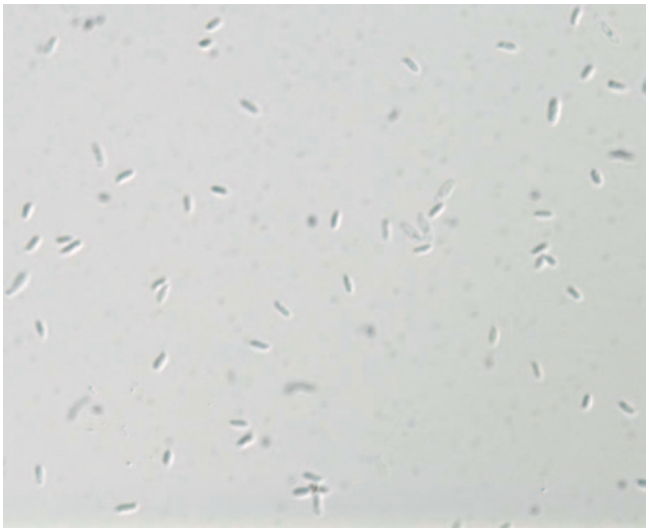

Fig. 2.6 Basidiospores produce by *Schizophyllum commune*

Fig. 2.7 Ramified rhizoids, short, dark sporangiophores, collapsed columellae, and sporangiospores of *Rhizopus microsporus*

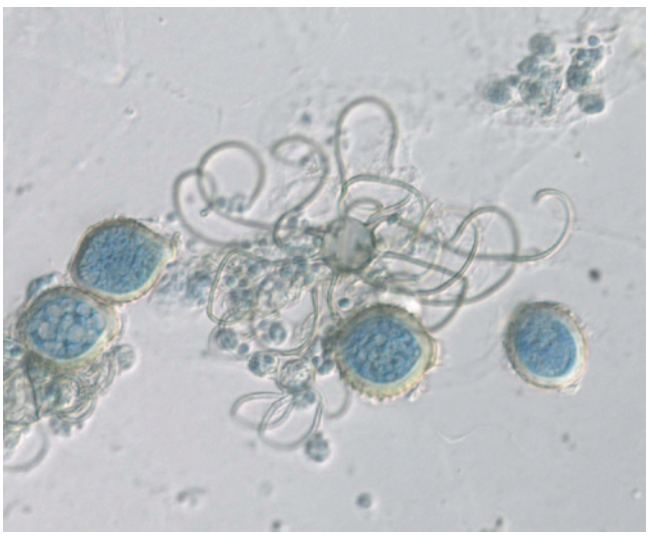

Fig. 2.8. Central vesicle, recurving stalks with terminal sporangioles containing sporangiospores, and thick-walled zygospores of *Cokeromyces recurvatus*

Conidiobolus (Fig. 2.9) and *Basidiobolus* (both characterized by forcibly discharged conidia).

Mitosporic Fungi

The group that contains the most human etiologic agents, by far, is one known as the "mitosporic fungi," or previously, the "fungi imperfecti." While these fungi may by connected to various sexual phyla, this association has not been yet demonstrated, and therefore these fungi are identified on the basis of their asexual rather than sexual reproductive propagules (method of conidial formation or conidiogenesis). Two main groups exist within the mitosporic fungi. The hyphomycetes bear their conidia free to the air, while the coelomycetes have their conidia contained within some type of enclosed to semi-enclosed structure [28, 29]. The hyphomycetes contain numerous common moniliaceous (hyaline) and phaeoid or dematiaceous (dark) genera and generally produce their conidia by either blastic or thallic methods. Blastic conidia are "blown out" of some type of conidiogenous cell. These include those produced from phialides, as in

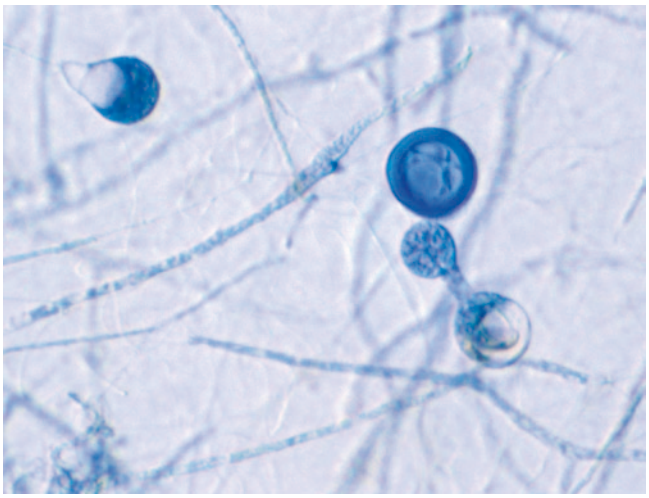

Fig. 2.9 Primary sporangiole giving rise to secondary sporangiole of *Conidiobolus coronatus*

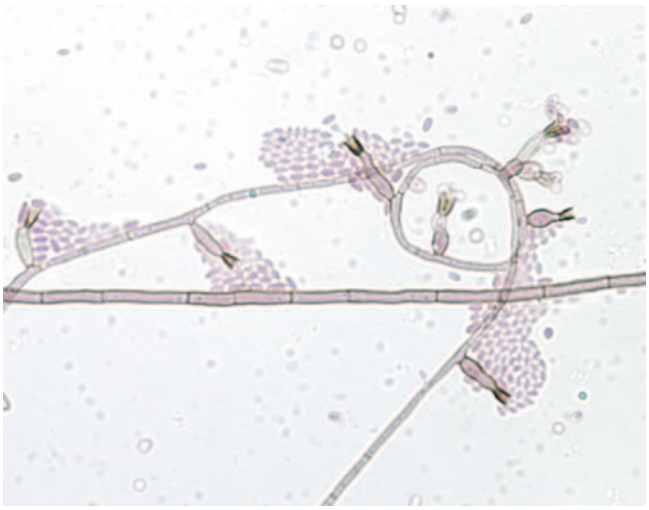

Fig. 2.10 Phialides of *Phialophora americana* with deep collarettes producing phialoconidia

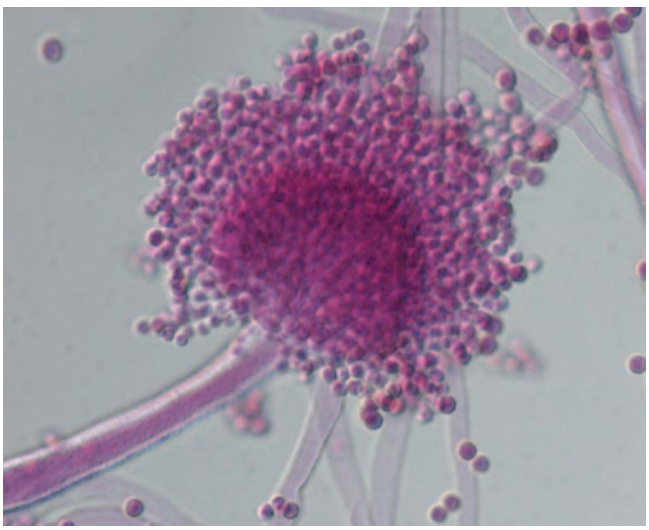

Fig. 2.11 Rough conidiophore and biseriate fruiting head of *Aspergillus flavus*

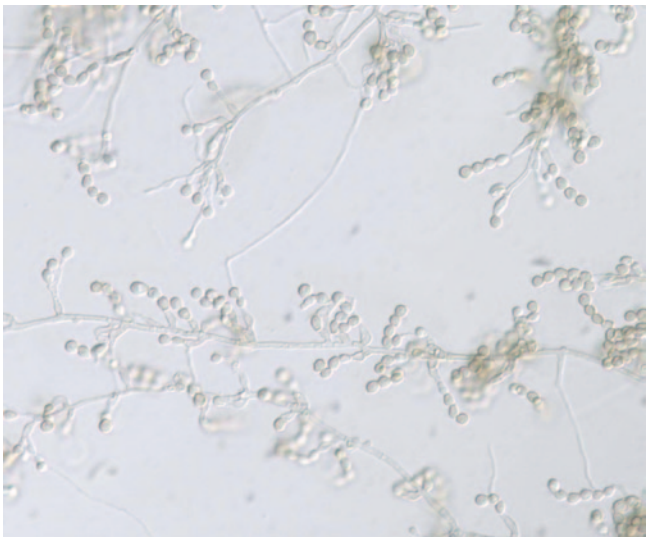

Fig. 2.12 Annellides and chains of annelloconidia produced by *Scopulariopsis cirrosus*

Phialophora species (Fig. 2.10) or *Aspergillus* species such as *Aspergillus flavus* (Fig. 2.11), or from annellides, as in *Scopulariopsis cirrosus* (Fig. 2.12). Some species blow out their conidia through pores, such as in *Curvularia* (formerly *Bipolaris*) *hawaiiensis* [44] (Fig. 2.13). Thallic conidia are formed from preexisting hyphae, as in *Coccidioides* species (Fig. 2.14), *Malbranchea* species, and *Neoscytalidium* (formerly *Scytalidium*) *dimidiatum* [45] (Fig. 2.15). The structures produced by coelomycetes to contain their conidia are known as conidioma (*sing*.) or conidiomata (*pl*.). They may be round structures with an opening or ostiole known as a pycnidium, as in *Phoma* species (Fig. 2.16), or a flat, cup-shaped, semi-enclosed structure known as an acervulus. The conidiogenous cells within both of these conidiomata may be either phialidic or annellidic. A recent review highlights coelomycete fungi seen in the clinical laboratory and salient features for their identification [29].

Fig. 2.13. Geniculate conidiophores with pores through which the conidia of *Curvularia hawaiiensis* are blown out

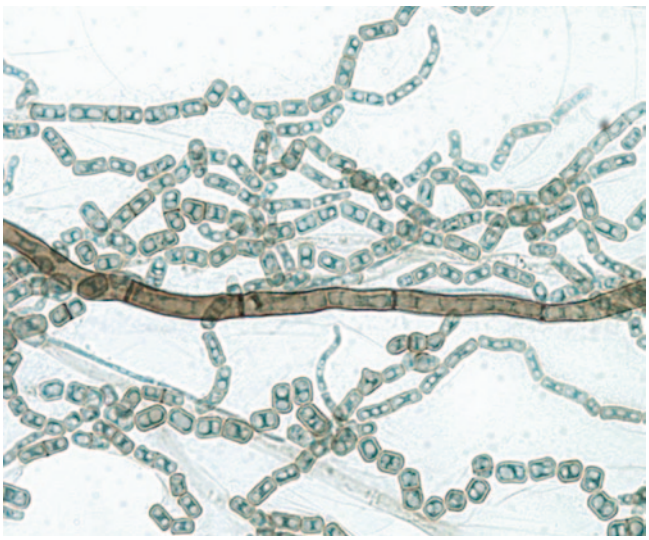

Fig. 2.15 Dematiaceous hyphae and arthroconidia of *Neoscytalidium dimidiatum* which lack disjunctor cells

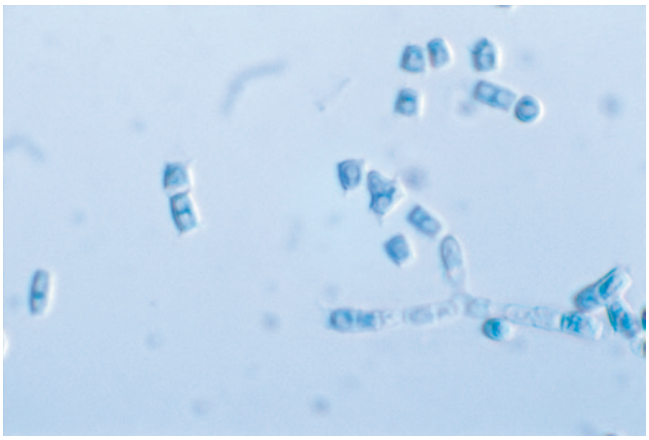

Fig. 2.14 Hyphae and arthroconidia with disjunctor cells of *Coccidioides* species

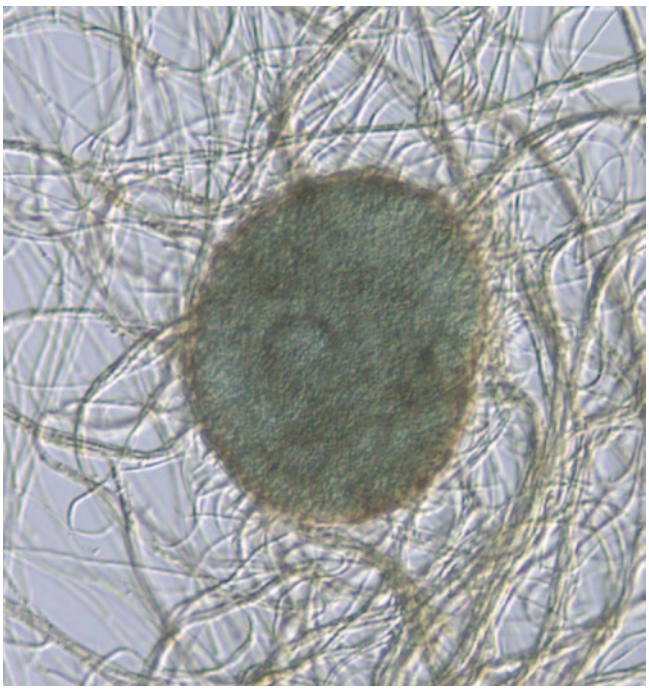

Fig. 2.16 Conidioma of a *Phoma* species containing a large central ostiole or opening.

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