Laboratory Handbook of Medical Mycology

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Preface

This laboratory handbook summarizes the concepts dealing with the laboratory aspects of medical mycology. Owing to the increased importance of fungi in medicine, there is a pressing need to discuss important topics such as laboratory safety and emergency procedures, quality control of media and equipment, new isolation techniques, susceptibility testing, and modern concepts for the identification of fungi. The information included in this handbook is intended to assist laboratory technologists, microbiologists, and mycologists in safely isolating and accurately identifying fungi of medical importance.

In contrast to other mycological works dealing with medically important fungi, the laboratory aspects of mycoserology and the identification of the actinomycetes have been excluded. The actinomycetes were omitted because they are bacteria, not fungi. Study of the actinomycetes is rapidly becoming a highly specialized field of study. To include them as a minor component of this handbook would be an injustice to this important and intriguing group of organisms. The techniques used for the serodiagnosis of the mycoses have been omitted because any attempt to include these techniques would be a duplication of what is available in other books.

The approach that I have taken in writing this book is to utilize as much contemporary mycological thought as possible. The key to understanding any field of science necessitates a thorough understanding of its language. It is, therefore, extremely important that the first chapter of this book be read first. Each of the terms, concepts and techniques of special interest to medical mycologists is clearly and concisely defined. These descriptions are supplemented with a large glossary, as well as a large number of photomicrographs. Many of the newer terms and concepts, especially those associated with conidiogenesis, may at first be difficult to grasp totally; however once they are clearly understood, most laboratorians will find medical mycology much easier.

The emphasis of this handbook is directed toward the identification of fungi that are commonly encountered in the medical mycology laboratory. This emphasis on identification is important since the ultimate function of each diagnostic and reference mycology laboratory is to identify fungi rapidly and accurately. This handbook will be a valuable asset in achieving this goal.

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Chapter 1

Basic Terminology and Classification

Ultrastructural, genetic and biochemical studies of many different types of living organisms have revealed that the prokaryote–eukaryote dichotomy is far more basic than the traditional animal–plant dichotomy. This new understanding of living organisms and their relationships to each other has stimulated a reevaluation of our current phylogenetic concepts. As a result, it is now realized by some that at least five kingdoms are necessary to accommodate all living things (Table 1.1). In this new classification scheme, the fungi are placed in the Kingdom Fungi, which contains the Chytridiomycetes (chytrids), Zygomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti (Deuteromycetes) and lichens. Organisms previously considered to be fungi, that is, mycetozoans, labyrinthulids, thraustochytrids, plasmodiophorids, and oomycetes are now classified by many in the Kingdom Protista.

Mycology is that branch of biology which deals with the study of fungi. Medical mycology is a specialized area concerned with the study of fungi that are able to incite disease in man and animals. Fungi (sing. fungus) are eukaryotic organisms that usually grow in a filamentous, or yeastlike form, or both. Their nucleus, like that of other eukaryotic organisms, contains a nucleolus and several chromosomes that are bound by a nuclear membrane. The latter usually persists during nuclear division. The term coenocytic is used to describe the condition of a cell, nonseptate hypha or other structural unit when it contains many nuclei. Hyphal cells in septate hyphae may be uninucleate, binucleate, or multinucleate. For the most part, cellular and nuclear division are independent events, especially with respect to vegetative growth. As in other eukaryotic organisms, fungi have mitochondria, 80 S ribosomes, and centrioles. Flagellate cells are produced

only by the chytrids. These motile, 1-celled, asexual spores called zoospores, have a single whiplash-type posterior flagellum, which is of the 9 + 2 fibril construction.

The cell wall of fungi primarily consists of chitin, chitosan, glucan and mannan, and rarely cellulose, in various combinations. Fungi are carbon heterotrophs and therefore require preformed organic compounds as a carbon source. Unlike plants, fungi cannot use carbon dioxide as a sole carbon source because they do not have chlorophyll. When supplied a carbon source such as glucose, fungi can synthesize their own proteins and usually most amino acids and vitamins if nitrogen and the essential minerals are available. Many fungi must have some vitamins, amino acids, or both supplied, or at least their precursors. They all synthesize lysine by the L-α-adipic acid pathway.

Table 1.1. The Five Kingdoms of Organisms[a]

Kingdoms	Principal features	Representative members
Monera	Prokaryotic; absorptive nutrition; mitotic apparatus absent; asexual reproduction by binary fission	Bacteria and blue-green algae; 14 phyla
Protista	Eukaryotic; ingestive, absorptive or photoautotrophic nutrition; mitotic reproduction; DNA and RNA; unicellular or multicellular; flagella or cilia with microtubules in 9 + 2 pattern	Protozoans, plasmodiophorids, labyrinthulids, hyphochytrids, mycetozoans, oomycetes, etc.; 30 phyla
Fungi	Eukaryotic; absorptive nutrition; zygotic meiosis; unicellular or filamentous; posterior whiplash-type flagella present (chytrids), cilia absent; cell walls chitinous; lysine synthesis by the L-α-adipic acid biosynthetic pathway	Chytridiomycetes, zygomycetes, ascomycetes, basidiomycetes, fungi imperfecti, lichens; 5 divisions
Plantae	Eukaryotic; photoautotrophic nutrition; tissue highly differentiated; diploid phase arising from embryophyte	Liverworts, mosses, vascular plants; 9 divisions
Animalia	Eukaryotic; heterotrophic nutrition; gametic meiosis; multicellular; develop from diploid blastula, gastrulation occurs	Sponges, coelenterates, worms, arthropods, echinoderms, mammals, etc.; 32 phyla

[a] Modified from Margulis (1974).

Actinomycetes (Kingdom Monera) superficially resemble fungi because of their filamentous nature. The actinomycetes are prokaryotic, Gram-positive, filamentous bacteria. Their nucleus consists of a continuous single chromosome that is not enveloped by a nuclear membrane. The cell wall of the actinomycetes consists of a glycosaminopeptide complex. The actinomycetes can serve as hosts to bacteriophages, whereas the fungi cannot serve as their hosts. These organisms are sensitive to antibacterial agents, such as penicillin, but not to antimycotic agents such as amphotericin B. The converse holds true for the fungi. As a more practical method to distinguish actinomycetes and fungi, medical mycologists usually consider an organism with Gram-positive filaments 0.5–1.0 μm in diameter to be an actinomycete. Care must be exercised in using size, since fungi may also be Gram-positive and some fungi, such as members of the genus *Fusidium,* are morphologically similar to the actinomycetes.

VEGETATIVE GROWTH

Fungi pathogenic to man can be conveniently separated into two basic groups, moulds and yeasts. Moulds consist of those fungi that grow in a filamentous form, whereas yeasts are characterized by a unicellular morphology that reproduces by budding. Some mycologists prefer to restrict the term yeast to unicellular budding fungi that have the ability to reproduce by sexual means, and the term "yeastlike" to similar fungi that reproduce only asexually. In this handbook, the term yeast will be used to encompass all unicellular budding fungi, regardless of how they reproduce. A number of fungi (*Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Sporothrix schenckii*, and *Paracoccidioides brasiliensis*) are dimorphic. That is, except for *C. immitis,* they can grow as a mould at room temperature and as a yeast at 37°C or in tissue. *Coccidioides immitis* grows as a mould at room temperature and as spherules producing endospores in tissue or on specialized media at 37°C.

Each vegetative filament or element of a fungus is called a hypha. A number of these filaments are referred to as hyphae, and a large amount of hyphae is known as mycelium. Since mycelium can be either single or collective, there is no need to use the expression mycelia. Hyphae are the actively growing assimilative phase of fungi. New growth occurs as linear elongation originating in a zone immediately behind the growing tip of the hypha. As a result of this linear elongation, the walls of hyphae tend to be parallel. As the hypha develops, it becomes divided into compartments or cells by the development of cross walls called septa (sing. septum). Such hyphae are referred to as being septate. The septa may either be partial,

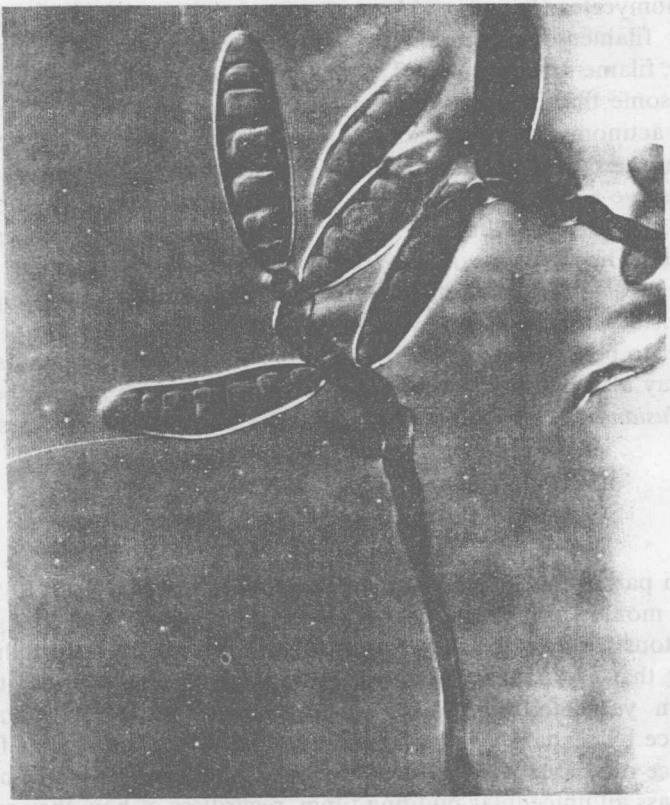


Fig. 1.1 *Drechslera* sp. The conidia have pseudosepta.

complete, or perforated. Partial septa (pseudosepta) result as thickenings of the lateral walls of the hyphae. They may be abortive cross walls. Complete septa result from centripetal growth of the lateral walls until the ingrowing septal walls meet each other, usually leaving a minute pore(s). Perforated septa, which may be uniperforate or multiperforate, develop in the same manner as complete septa, but have a wider central pore. This type of septum is typically seen in the Ascomycetes and Fungi Imperfecti. If pores are present, then nuclei have the potential to move from one cell to the next. Pores may be plugged in a numbers of manners. Woronin bodies are organelles that commonly plug septal pores in some fungi. In some hyphae, septa are rarely formed. For example, in the Zygomycetes septa are formed to wall off empty cells and to separate asexual and sexual structures from the rest of the hypha. The septa in this group of fungi are variable; nuclei do not migrate from one cell to the next. For hyphae that

contain occasional septa, the expression sparsely septate is preferred to the terms aseptate or nonseptate. Some fungi, such as species of *Drechslera*, develop pseudosepta (partial septa) in their conidia. Pseudosepta are thick outgrowths from the inner cell wall layer that grow toward the center of the conidium (asexual propagule), but do not completely wall off each compartment (Fig. 1.1).

The diameter of the hyphae is a useful characteristic in distinguishing hyphae of the Zygomycetes from other fungi. The Zygomycetes typically develop hyphae that can be characterized as being sparsely septate, branching irregularly, and having a diameter of approximately 10–15 μm. In general, excluding the Zygomycetes and fungi like *Fusidium*, most hyphae are approximately 1.5–3.5 μm in diameter.

Hyphal cells may be quite variable in shape and size. Many fungi when grown under unfavorable conditions develop swollen hyphal cells called vesicles (Fig. 1.2). Vesicles, unlike chlamydospores (Fig. 1.3), do not function as reproductive propagules but as cells for the storage of food substrates or materials toxic to the fungus. They may be thick-walled; this can result in a superficial resemblance to chlamydospores. The dermatophytes, as well as many other fungi, occasionally develop hyphae that

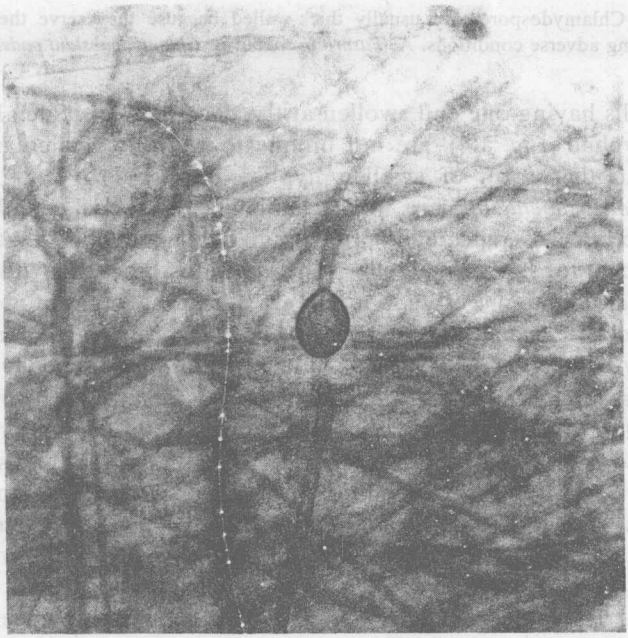
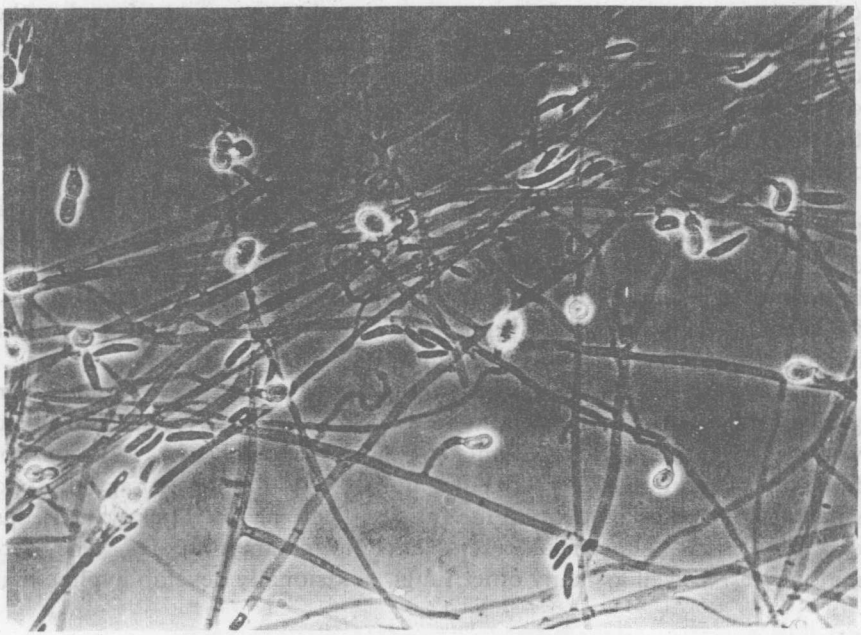


Fig. 1.2 A vesicle is a swollen vegetative cell.



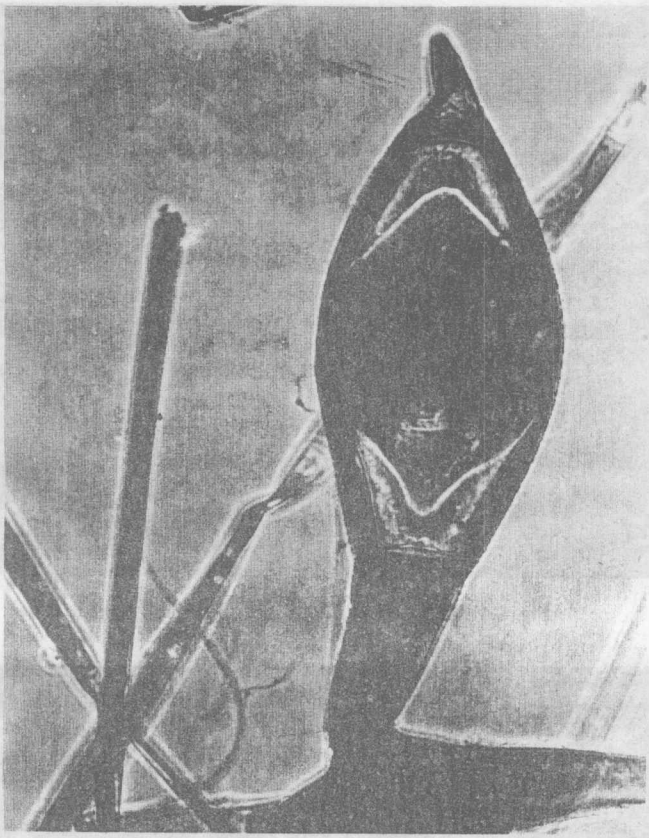
A

Fig. 1.3 Chlamydospores are usually thick-walled because they serve the function of survival during adverse conditions. A. *Fusarium solani*. B. *Chlamydoabsidia padeni*.

contain cells having one end swollen at the distal portion. Such hyphae, or racquet hyphae (Fig. 1.4), are not unique to any one particular genus or species. Another form of swollen hyphae produced by fungi, such as *Trichophyton schoenleinii*, are favic chandeliers. A favic chandelier (Fig. 1.5) is a cluster of repeatedly branching, swollen hyphae that have the overall appearance of a chandelier. These various types of swollen hyphae have no real taxonomic value, but they do occasionally aid in identifying a fungus. A classic example is the terminal vesicles of *Microsporum audouinii*, which may have a very distinctive spinelike terminal appendage (Fig. 1.6).

When harsh environmental conditions occur, some fungi form a sclerotium. A sclerotium (pl. sclerotia) is a complex mass of hyphae or cells that is organized into a rounded resistant structure that contains reserve food materials. Sclerotia may be either large, that is, approaching several millimeters in diameter (Fig. 4.8), or relatively small. Some species of *Aspergillus* (especially *A. flavus*), *Fusarium, Rhizoctonia*, and similar fungi commonly develop these structures.

One final vegetative structure that must be discussed is the pseudohypha (pl. pseudohyphae, pseudomycelium). A pseudohypha represents, in essence, a series of blastoconidia that have remained attached to each other,



B

Fig. 1.3 Continued

forming a hyphalike filament. The confusion between true hyphae and pseudohyphae (Fig. 1.7) becomes most apparent when the cells of pseudohyphae are extremely elongated. The major differences between hyphae and pseudohyphae are set forth in Table 1.2.

ASEXUAL REPRODUCTION

Fungi reproduce by asexual means, sexual means, or both. Asexual reproduction is either an increase in the vegetative phase of the fungus or the development of asexual propagules. In either case, such reproduction does not involve the union of nuclei or gametes. The vegetative phase is commonly referred to as the thallus and usually consists of hyphae being aggregated into a colony.