Mycology For The Clinical Laboratory

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reston publishing company, inc.
a prentice-hall company
reston, virginia

Library of Congress Cataloging in Publication Data

Moore, Gary S
Mycology for the clinical laboratory.

Includes bibliographies and index.
1. Fungi, Pathogenic. 2. Medical mycology.
3. Medical technologists. I. Title.
QR245.M66 616.01'5 78-31377
ISBN 0-8359-4771-8

A Prentice-Hall Company
Reston, Virginia 22090

10 9 8 7 6 5 4 3 2 1

Printed in the United States of America

preface

Medical technologists, microbiologists, and students in medical mycology will find this text most useful for the study and laboratory diagnosis of fungi pathogenic to men and animals. It is not intended to provide detailed information on the pathology, symptomatology, treatment, or prognosis of fungal diseases, since there are several excellent books available on these topics.

This text is designed for use at the laboratory bench by both experienced and inexperienced mycologists. There is an expanded section on methods of acquiring and processing clinical specimens for mycological examination. Another section that we feel will be welcome describes the opportunistic fungi (also referred to as saprobes or contaminants). This section features photographs that emphasize the diagnostic microscopic features of the opportunists and these are paired with labeled line drawings of the same organisms. The fungi are described and illustrated in sufficient detail so that the inexperienced reader can easily identify them.

There is a section on yeasts that emphasizes the microscopic morphologies on chlamydospore agar and details the most recently published methods for their rapid identification.

In general, we have collated, from several books and manuals, information that is relevant to the laboratory identification of medically important fungi so that it is no longer necessary to go to a number of references before an answer to a specific mycologic problem can be found. Although this text is certainly not exhaustive, it does contain most of the relevant information routinely sought in the mycology laboratory.

In addition to the sections described above, there are sections for each of a wide variety of fungal diseases, with information on the clinical specimens likely to contain the organism, procedures for microscopic examination of the specimens, cultural procedures, biochemical tests, and animal inoculations where applicable.

how to use this book

Mycology is usually a totally new and strange subject to medical technologists and presents difficulties to students that are apparent when they are identifying a newly isolated colony. Even though the procedures for isolation and direct examination have been mastered, the mycologist is often left to confront a fungus culture that may be totally foreign both microscopically and macroscopically. This happens most frequently when you are new at this game, and when you least know what to do. This book is designed to reduce those hazards somewhat.

In the first place, newly isolated fungi may be morphologically abnormal because of treatments given to the patient. Therefore, if the fungus does not fit any description provided, serial transfers on Sabouraud's dextrose agar (SDA) may be necessary to restore the typical morphology.

When fungal growth is observed from a clinical specimen, the three principal characteristics to be evaluated are pigmentation, texture, and growth rate. These characteristics are described and examples given in Chapter 2 under the subheading, Gross Morphology. Using these characteristics and information provided in Table 2–4, the fungus identity can be narrowed quickly to a limited group of fungi having similar pigment, texture, and growth rate. Since textures are sometimes confusing, photographic examples of textures have been provided in the same section (Figs. 2-6 through 2-9).

Example: A fungus grows on SDA within 5 days as a flat to powdery colony with a bright green surface pigmentation. What is the likely identity?

Using Table 2-5, look down the column under Pigmentation until you see the heading "Brightly colored (yellow, green, red, orange)" and then follow across the chart until you are under the Texture heading "Granular to Powdery." Since the colony developed in 10 days or less, the organisms listed under "Intermediate to Rapid Growth" are considered likely identities for the isolated fungus.

The topography of the colony is characterized according to one of the types listed in Figs. 2-5 and 2-6, and the microscopic morphology is evaluated according to direct mounts and slide culture techniques as described in Chapter 2. This information is then used to compare this fungus with the descriptions provided in the text for each of the fungi listed in Table 2-5. The pages on which the generic descriptions of the fungi appear are listed in the Index. Since there are well-labeled photographs and drawings of most of the commonly isolated fungi, this comparison is simplified and the identity facilitated.

contents

chapter 1

fundamentals of mycology and classification of fungi

In addition to the mycotic diseases produced by the classic pathogenic fungi, the use of broad-spectrum antibiotics and immunosuppressive therapy has resulted in an increased frequency of disseminated mycoses because of opportunistic fungi. This has increased the burden on the medical laboratory to isolate and identify a much broader range of fungi.

Not only has there been an increase in the numbers and types of fungi isolated from clinical samples, but proficiency survey samples are placing more emphasis on fungal identification. Persons who are unaccustomed to the terminology and concepts used in mycology will find reading this section to be helpful. You can expect to see these terms frequently as you read through this and other texts. Therefore, if you are familiar with the terms and their meanings beforehand, your reading of the material will be more enjoyable and your comprehension improved.

CLASSIFICATION OF FUNGI

The fungi have traditionally been classified as primitive plants, and the heterotrophic fungi were included in the subdivision *Thallophyta* along with the algae. Early naturalists were concerned principally with higher plants and animals, and they recognized only the plant and animal kingdoms. As early as 1860, however, systematists proposed a third kingdom (Emmons et al., 1970) and in 1956, Copeland proposed four kingdoms. He placed the algae (except blue-green algae), protozoans, slime molds, and fungi in the kindom *Protoctista.* Whittaker (1969) extended the system to include five kingdoms, with fungi being placed in the kingdom *Fungi,* which includes eight phyla. Some systematists presently place the fungi and bacteria in a third biologic kingdom, *Protista* (Conant and Smith, 1971). The prokaryotic[1] organisms—bacteria and blue-green algae—and the eukaryotic organisms—algae, protozoans, fungi, and slime molds—would be included in the kingdom *Protista.* The more traditional view, however, (Conant and Smith, 1971), is to divide the *plant kingdom* into several divisions, which would include the fungi, bacteria, and related forms (Table 1–1).

MOLDS AND YEASTS[2]

Structure

The term *Fungus* is a general term that includes a number of diverse forms such as molds and yeasts. The terms *molds* and *yeasts* are colloquial designations and have no taxonomic significance, but are usefui in describing the two predominant forms of fungi. The terms "yeast" and "mold" are not mutually exclusive, since many fungi are molds in their normal saprobic growth but are yeastlike under modified growth conditions or in animal tissues. Further, many fungi whose predominant form is yeast or yeastlike may exhibit moldlike structures when grown under appropriate conditions.

The molds reproduce by spores, and when a spore comes in contact with a favorable substrate, the spore will send out cylindrical germ tubes that develop into long branching filaments. Each of these filaments is called a *hypha* (plural, *hyphae*). The hyphae in most molds are divided by *cross walls* or *septae* into multicellular hyphae, with each cell having one or more nuclei. The septations have "holes" in them that permit the flow of cytoplasmic material between cells. Hyphae that are so divided by transverse walls at regular intervals are said to be *septate hyphae*

[1] *Prokaryotic* refers to the lower protists (bacteria and blue-green algae), which have only a single naked chromosome without nuclear membrane-bound organelles; *eukaryotic* refers to the cells of higher protists (protozoans, fungi, and most algae) since they contain a nucleus with a nuclear membrane, multiple chromosomes, and a specialized mitotic apparatus.

[2] Information for this section was obtained from Conant and Smith (1971), Emmons et al. (1970), and Rippon (1974).

TABLE 1-1
Classification of Fungi Within the Plant Kingdom

Kingdom: Plantae
  Division: Protophyta
    Subdivision: Schizomycophyta
      Class: Schizophyceae (blue-green algae)
      Class: Schizophycetes (bacteria and related forms)
        Order: Actinomycetales
          1. Mycelium is rudimentary or absent
            Family: Mycobacteriaceae
              Genus: *Mycobacterium*
          2. True mycelium produced
            a. Vegetative mycelium fragments into bacillary or coccoid elements
            Family: Actinomycetaceae
              (1) Anaerobic or microaerophilic, non-acid-fast
              Genus: *Actinomyces*
              (2) Aerobic, partially acid-fast, or non-acid-fast, spore chains produced by some isolates
              Genus: *Nocardia*
            b. Vegetative mycelium not fragmenting with bacillary or coccoid elements
              (1) Nonmotile spores formed in chains
              Genus: *Streptomyces*
  Division: Mycota (fungi)
    Subdivision: Eumycotina (true fungi)
      Class: Zygomycetes*—most members produce aseptate, broad mycelium and form sporangia of various types with nonmotile sporangiospores. Sexual reproduction when present is by zygospores.
        Order: Mucorales
          Genus: *Rhizopus*
          Genus: *Mucor*
          Genus: *Absidia*
        Order: Entomophthorales
          Genus: *Entomophtora coronata*
          Genus: *Basidiobolus haptosporus*
      Class: Ascomycetes—the unicellular forms reproduce asexually by budding; sexually by the formation of ascospores. These are known as *true yeasts.* The mycelial forms have septate hyphae and reproduce by forming conidia of various types. Sexual reproduction is by means of ascospores born in asci.
      Class: Basidiomycetes—the mycelium septate, and sexual reproduction is by means of basidiospores.
      Class: Deuteromycetes (Fungi imperfecti)—members of this class have septate mycelium and reproduce asexually by conidia that are born in pycnidra, acervuli, or on various types of conidiophores. Sexual reproduction is usually absent. Unicellular

TABLE 1-1 *Continued*

forms reproduce by budding and sexual reproduction is usually not evident.
Order: Moniliales: The conidia are born free on conidiophores
Family: Moniliaceae—the mycelium, conidia, and conidiophores are hyaline or brightly colored.
Family: Dematiaceae—the hyphae, conidia, or conidiophores are darkly colored.
Order: Pseudosaccharomycetales—asexual reproduction by blastospores. The yeastlike fungi with or without pseudo or true mycelium
Family: Cryptococcaceae
Genus: *Candida*
Genus: *Cryptococcus*
Genus: *Rhodotorula*
Genus: *Torulopsis*
Genus: *Trichosporon*
Genus: *Geotrichum*

The phycomycetes are such a heterogeneous group of fungi that systematists now divide the "phycomycete group" into several classes. Only one class of this group contains fungi of medical importance and it is called the *zygomycetes.*

(Fig. 1–1: A–2, B–2). A few molds possess hyphae that do not have cross walls or septa, and permit the streaming of protoplasm throughout the multinucleate structure. Such hyphae are said to be *nonseptate* or *coenocytic* (Fig. 1–1: C–3). This type of mycelium is important in distinguishing the Zygomycetes from all other classes of fungi.

The continued growth and branching of the hyphae develops into a loose network of hyphae called a *mycelium* or *colony*. The term *thallus* may also be applied here if the colonial growth was derived from a single spore. A portion of the mycelium penetrates the substrate to secure the colony and absorb nutrients for growth. This is known as the *vegetative mycelium.* Fragments of this mycelium will grow and reproduce if transferred. That part of the mycelium projecting above the substrate surface is termed the *aerial mycelium.* Molds reproduce normally by sporulation, and the spores are produced from the aerial mycelium in characteristic ways. The way in which these spores are formed; the structure, size, and shape of the spores; and the morphology of the structure that bears them are the primary characteristics by which molds are identified. The aerial mycelium and spores contribute to the pigmentation, texture, and topography of the colony. These are also characteristics important in the identification of molds.

Yeast are fungi whose usual and dominant growth form is unicellular. Yeast cells are spherical, elliptical, or cylindrical with highly variable sizes. Many yeast cells are 2 to 8 micrometers (μm; 10^{-6} meters) in diameter by 3 to 15 μm in length, but some cells reach lengths of 100 μm. A

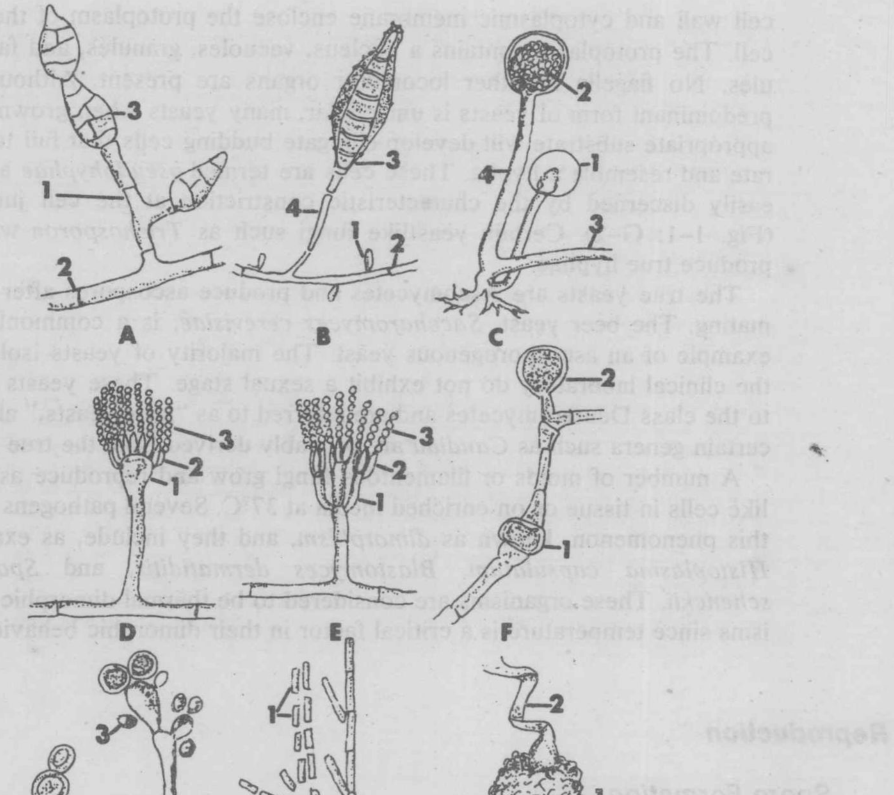

Figure 1–1. Examples of microscopic structures seen in fungi isolated in the clinical laboratory.

cell wall and cytoplasmic membrane enclose the protoplasm of the yeast cell. The protoplasm contains a nucleus, vacuoles, granules, and fat globules. No flagella or other locomotor organs are present. Although the predominant form of yeasts is unicellular, many yeasts when grown on an appropriate substrate will develop elongate budding cells that fail to separate and resemble a hypha. These cells are termed *pseudohyphae* and are easily discerned by the characteristic constriction at the cell junctions (Fig. 1–1: G–2). Certain yeastlike fungi such as *Trichosporon* will also produce true hyphae.

The true yeasts are Ascomycetes and produce ascospores after sexual mating. The beer yeast, *Saccharomyces cerevisiae,* is a commonly used example of an ascosporogenous yeast. The majority of yeasts isolated in the clinical laboratory do not exhibit a sexual stage. These yeasts belong to the class Deuteromycetes and are referred to as "false yeasts," although certain genera such as *Candida* are probably derived from the true yeasts.

A number of molds or filamentous fungi grow and reproduce as yeastlike cells in tissue or on enriched media at 37°C. Several pathogens exhibit this phenomenon, known as *dimorphism,* and they include, as examples, *Histoplasma capsulatum, Blastomyces dermatiditis,* and *Sporothrix schenckii.* These organisms are considered to be thermal dimorphic organisms since temperature is a critical factor in their dimorphic behavior.

Reproduction

Spore Formation

A spore is a resistant reproductive unit of the fungi. Fungi produce two types of spores, asexual and sexual. The fungi normally encountered in the clinical laboratory produce *asexual* spores, which develop from spore-bearing hyphae that have not undergone nuclear fusion. Asexual sporulation is seen in the Zygomycetes, Ascomycetes, and Deuteromycetes. *Sexual* spores are produced by the fusion of two nuclei. If the nuclei originate from the same thallus (vegetative part of fungus plant), the fungus is *homothallic.* If a sexual spore is produced only by fusion of two separate thalli or mating types, the fungus is said to be *heterothallic.* Fungi that reproduce sexually are known as "perfect fungi" and are represented in three classes, Zygomycetes, Ascomycetes, and Basidiomycetes. Many of these perfect fungi also reproduce asexually.

Until recently, most of the fungi of clinical importance were thought to reproduce only asexually and were assigned to the class Deuteromycetes and termed the "imperfect fungi." However, the use of special media and laboratory techniques has revealed that a sexual stage can be produced in many of these fungi in the presence of opposite mating types. The sexual stage is usually given a different generic and species name than that of its imperfect form. For example, *Trichophyton mentagrophytes* is an imperfect fungus that is identified as *Arthroderma benhamiae* in its perfect stage. However, it is convenient to know that nearly all the medically important fungi are seen normally in their asexual stage and belong to the class Deuteromycetes.

Asexual Reproduction

The imperfect fungi, or Deuteromycetes, are classified principally by the asexual spores produced. These spores, produced free by segmentation or by budding of the tips of the hyphae or from the walls of hyphae, are referred to generally as *conidia*. The hyphal structure that bears them is a *conidiophore* (Fig. 1–1: A–1, B–4). Defined more specifically, conidia are deciduous spores successively produced in conidiophores. Such spores are usually shed immediately and serve to dessеminate the species. Conidia that are single-celled conidia are termed *microconidia* (Fig. 1–1: D–3, E–3), and multicelled conidia are called *macroconidia*.

Asexual spores that are attached by a wide base to the hypha or to short lateral branches, and so are set free normally by disintegration of the mycelium, are correctly called *aleuriospores*. Microaleuriospores are small and single-celled (Fig. 1–1: D–3), and macroaleuriospores are larger and often multicelled, although single-celled types exist (Fig. 1–1: A–3, B–3). Aleuriospores that develop directly on vegetative mycelia are said to be *sessile* and *lateral* (Thyrse, French) (Fig. 1–2: E–1). The term *clustered* or *en grappe* (French) is applied to clusters of spores formed laterally on short lateral branches of hyphae (Fig. 1–2: E–2). Thyrse and en grappe formation of microaleuriospores is commonly seen in the dermatophyte, *Trichophyton mentagrophytes*.

Arthrospores (Fig. 1–1: H–1) are formed when the mycelium fragments form a chain of uniformly sized asexual spores. Arthrospore formation is common in *Geotrichum, Trichosporon,* and *Coccidioides*. Cells of the pseudomycelium of *C. albicans* or true hyphae of many filamentous fungi often enlarge to develop thick-walled, resistant, resting spores called *chlamydospores*. Such cells formed at the end of the hyphae are called *terminal chlamydospores* (Fig. 1–1: F–2, G–1) and when formed in the hyphae are designated *intercalary chlamydospores* (Fig. 1–1: F–1). Asexual reproduction in yeasts and some molds occurs by budding in which the daughter cell is abstricted from the mother cell and then enlarges itself to form a new parent cell. The spore produced may be called a *blastospore* (Fig. 1–1: G–3).

Some members of the Ascomycetes and Deuteromycetes may develop highly specialized conidiophores with very characteristic shapes and methods of conidial formation. These specialized cases permit identification of the fungus based on the structure of the conidiophore. In *Aspergillus* the conidiophore arises from a basal cell on the hypha and enlarges at the end to form a *vesicle* (Fig. 1–1: D–1). Flask-shaped structures called *sterigmata* (Fig. 1–1: D–2) (singular, *sterigma*) develop