

LAB 10: THE FUNGI, PART 2 - THE MOLDS

DISCUSSION

Molds are multinucleated, filamentous fungi composed of **hyphae**. A hypha is a branching, tubular structure from 2-10 μm in diameter and is usually divided into cell-like units by crosswalls called **septa**. The total mass of hyphae is termed a **mycelium**. The portion of the mycelium that anchors the mold and absorbs nutrients is called the **vegetative mycelium**; the portion that produces asexual reproductive spores is termed the **aerial mycelium** (see Fig. 1).

Molds possess a rigid polysaccharide cell wall composed mostly of chitin and, like all fungi, are eukaryotic (see Fig. 2). Molds reproduce primarily by means of **asexual reproductive spores** such as conidiospores, sporangiospores, and arthrospores. These spores are disseminated by air, water, animals or objects and upon landing on a suitable environment, germinate and produce new hyphae (see Fig. 1). Molds may also reproduce by means of sexual spores such as ascospores and zygospores, but this is not common. The form and manner in which the spores are produced, along with the appearance of the hyphae and mycelium, provide the main criteria for identifying and classifying molds.

[Review of Prokaryotic and Eukaryotic Cells from Unit 1.](#)

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A. COMMON MOLDS

To illustrate how morphological characteristics such as the type and form of asexual reproductive spores and the appearance of the mycelium may be used in identification, we will look at three common non-pathogenic molds.

The two most common types of asexual reproductive spores produced by molds are conidiospores and sporangiospores. Conidiospores are borne externally in chains on an aerial hypha called a conidiophore (see Fig. 3); sporangiospores are produced within a sac or **sporangium** on an aerial hypha called a sporangiophore (see Fig. 4).

Penicillium and *Aspergillus* are examples of molds that produce **conidiospores**. *Penicillium* is one of the most common household molds and is a frequent food contaminant. The **conidiospores** of *Penicillium* (see Fig. 5A and Fig. 5B) usually appear [grey, green, or blue](#) and are produced in chains on **finger-like projections called phialides** coming off of the conidiophore.

Aspergillus is another common contaminant. Although usually nonpathogenic, it may become opportunistic in the respiratory tract of a compromised host and, in certain foods, can produce mycotoxins. The conidiophore terminates in a ball-like structure called a vesicle. Its **conidiospores**, which typically appear [brown to black](#), are produced in chains on phialides **coming off of the vesicle** (see Fig. 6).

- Scanning electron micrograph of the conidiospores of *Penicillium*; courtesy of [Dennis Kunkel's Microscopy](#).
- Scanning electron micrograph of the conidiospores of *Aspergillus*; courtesy of [Dennis Kunkel's Microscopy](#).

Although generally harmless in most healthy individuals, *Aspergillus* species do cause allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing *Aspergillus* pneumonia (or chronic necrotizing pulmonary aspergillosis [CNPA]), aspergilloma (a mycetoma or fungus ball in a body cavity such as the lung), and invasive aspergillosis. In highly immunosuppressed individuals, however, *Aspergillus* may disseminate beyond the lung via the blood.

Rhizopus is an example of a mold that produces **sporangiospores**. Although usually nonpathogenic, it sometimes causes opportunistic wound and respiratory infections in the compromised host. At the end of its sporangiophore is dome-shaped end called a columella that extends into a **sac-like structure called a sporangium**. Its **sporangiospores**, typically [brown or black](#), are produced within the **sporangium** (see Fig. 7). Anchoring structures called **rhizoids** are also produced on the vegetative hyphae.

- Scanning electron micrograph of the conidiospores of *Rhizopus*; courtesy of [Dennis Kunkel's Microscopy](#).

Mucormycoses are infestations caused by fungi belonging to the order of Mucorales. *Rhizopus* species are the most common causative organisms. The most common infection is a severe infection of the facial sinuses, which may extend into the brain. Other mycoses include pulmonary, cutaneous, and gastrointestinal.

Rhizopus can also reproduce sexually. During sexual reproduction (see Fig. 8), hyphal tips of (+) and (-) mating type join together and their nuclei fuse to form a sexual spore called a zygospore (see Fig. 9). This gives rise to a new sporangium producing sporangiospores having DNA that is a recombination of the two parent strain's DNA.

Molds are commonly cultured on fungal-selective or enriched media such as Sabouraud Dextrose agar (SDA), Corn Meal agar, and Potato Dextrose agar.

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The **dermatophytes** are a group of molds that **cause superficial mycoses of the hair, skin, and nails** and utilize the protein **keratin**, that is found in hair, skin, and nails, **as a nitrogen and energy source**. Infections are commonly referred to as **ringworm** or **tinea** infections and include:

- [tinea capitis](#) (infection of the skin of the scalp, eyebrows, and eyelashes)
- [tinea barbae](#) (infection of the bearded areas of the face and neck)
- [tinea faciei](#) (infection of the skin of the face)
- [tinea corporis](#) (infection of the skin regions other than the scalp, groin, palms, and soles)
- tinea cruris (infection of the groin; jock itch)
- [tinea unguium](#) (onychomycosis; infection of the fingernails and toenails)
- [tinea pedis](#) (athlete's foot; infection of the soles of the feet and between the toes).

The three common dermatophytes are ***Microsporum***, ***Trichophyton***, and ***Epidermophyton***. These organisms grow well at 25°C. They may produce large leaf or club-shaped asexual spores called macroconidia ([see Fig. 10B](#)) as well as small spherical asexual spores called microconidia, both from vegetative hyphae ([see Fig. 10A](#)).

- Scanning electron micrograph of the macroconidia of [Epidermophyton](#); courtesy of [Dennis Kunkel's Microscopy](#).

[Microsporum](#) commonly infects the skin and hair, [Epidermophyton](#), the skin and nails, and [Trichophyton](#), the hair, skin, and nails. Dermatophytic infections are acquired by contact with fungal spores from infected humans, animals, or objects. On the skin, the dermatophytes typically cause reddening, itching, edema, and necrosis of tissue. This is a result of fungal growth and a hypersensitivity of the host to the fungus and its products. Frequently there is secondary bacterial or *Candida* invasion of the traumatized tissue.

To diagnose dermatophytic infections, **tissue scrapings** can be digested with 10% potassium hydroxide (which causes lysis of the human cells but not the fungus) and examined microscopically for the presence of **fungal hyphae and spores**. To establish the specific cause of the infection, fungi from the affected tissue can be cultured on Dermatophyte Test Medium (DTM) and Saboraud Dextrose agar (SDA).

Dermatophyte Test Medium (DTM) has phenol red as a pH indicator with the medium yellow (acid) prior to inoculation. **As the dermatophytes utilize the keratin in the medium, they produce alkaline end products** that raise the pH, thus **turning the phenol red in the medium from yellow or acid to red or alkaline** ([see Fig. 17](#)).

The types of **macroconidia and microconidia** ([see Fig. 10B](#)) can be observed by growing the mold on **SDA** and observing under a microscope. In addition, many dermatophyte species produce yellow to red-pigmented colonies on SDA and the most common species of *Microsporum* fluoresce under ultraviolet light.

[Return to Menu for Lab 10](#)**C. DIMORPHIC FUNGI**

Dimorphic fungi may exhibit two different growth forms. **Outside the body they grow as a mold**, producing hyphae and asexual reproductive spores, but **inside the body they grow as a yeast-like form**. Dimorphic fungi may cause **systemic mycoses** which usually begin by **inhaling spores from the mold form**. After germination in the lungs, the fungus grows as a yeast. Factors such as body temperature, osmotic stress, oxidative stress, and certain human hormones activate a dimorphism-regulating histidine kinase enzyme in dimorphic molds, causing them to switch from their avirulent mold form to their more virulent yeast form.

The infection usually remains localized in the lungs and characteristic lesions called [granuloma](#) may be formed in order to wall-off and localize the organism. In rare cases, usually in an immunosuppressed host, the organism may disseminate to other areas of the body and be life threatening. Examples of dimorphic fungi include *Coccidioides immitis*, *Histoplasma capsulatum*, and *Blastomyces dermatitidis*.

1. ***Coccidioides immitis***

Coccidioides immitis ([see Fig. 11](#)) is a dimorphic fungus that causes **coccidioidomycosis**, a disease endemic to the southwestern United States. An estimated 100,000 infections occur annually in the United States, but one to two thirds of these cases are subclinical. The mold form of the fungus grows in arid soil and produces thick-walled, barrel-shaped asexual spores called **arthrospores** by a fragmentation of its vegetative hyphae ([see Fig. 13](#) and [Fig. 17](#)).

After inhalation, the arthrospores germinate and develop into **endosporulating spherules** in the terminal bronchioles of the lungs (see Fig. 14A and 14B). The spherules reproduce by a process called endosporulation, where the spherule produces numerous endospores (yeast-like particles), ruptures, and releases viable endospores that develop into new spherules.

Coccidioidomycosis can be diagnosed by culture, by a coccidioidin skin test, and by indirect serologic tests (discussed in Lab 18).

2. *Histoplasma capsulatum*

Histoplasma capsulatum (see Fig. 12) is a dimorphic fungus that causes **histoplasmosis**, a disease commonly found in the Great Lakes region and the Mississippi and Ohio River valleys. Approximately 250,000 people are thought to be infected annually in the US, but clinical symptoms of histoplasmosis occur in less than 5% of the population. Most individuals with histoplasmosis are asymptomatic. Those who develop clinical symptoms are typically either immunocompromised or are exposed to a large quantity of fungal spores.

The mold form of the fungus often grows in bird or bat droppings or soil contaminated with these droppings and produces large **tuberculate macroconidia** and small **microconidia** (see Fig. 15). Although birds cannot be infected by the fungus and do not transmit the disease, bird excretions contaminate the soil and enrich it for mycelial growth. Bats, however, can become infected and transmit histoplasmosis through their droppings. After inhalation of the fungal spores and their germination in the lungs, the fungus grows as a **budding, encapsulated yeast** (see Fig. 16).

Histoplasmosis can be diagnosed by culture, by a histoplasmin skin test, and by indirect serologic tests (discussed in Lab 18).

3. *Blastomyces dermatitidis*

Blastomycosis, caused by *Blastomyces dermatitidis*, is common around the Great Lakes region and the Mississippi and Ohio River valleys. Infection can range from an asymptomatic, self-healing pulmonary infection to widely disseminated and potentially fatal disease. Pulmonary infection may be asymptomatic in nearly 50% of patients. *Blastomyces dermatitidis* can also sometimes infect the skin.

Blastomyces dermatitidis produces a [mycelium with small conidiospores](#) and grows actively in bird droppings and contaminated soil. When spores are inhaled or enter breaks in the skin, they germinate and the fungus grows as a [yeast](#) having a characteristic thick cell wall. It is diagnosed by culture and by biopsy examination.

Concept map for Lab 10

For a description of **antifungal agents used to treat fungal infections**, see section [II E: Chemotherapeutic Control of Fungi](#) in your lecture E-text.

[Medscape](#) articles on infections associated with organisms mentioned in this lab exercise. Registration to access this website is free.

- [Aspergillosis](#)
- [Mucormycosis \(Rhizopus infections\)](#)
- [Tinea Capitis](#)
- [Tinea Barbae](#)
- [Tinea Faciei](#)
- [Tinea Corporis](#)
- [Tinea Cruris](#)
- [Tinea Unguium](#)
- [Tinea Pedis](#)
- [Coccidioidomycosis](#)
- [Histoplasmosis](#)
- [Blastomycosis](#)

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PROCEDURE (to be done individually)

A. COMMON MOLDS

1. Using a dissecting microscope, observe the SDA plate cultures of *Penicillium*, *Aspergillus*, and *Rhizopus*. Note the colony appearance and color and the type and form of the asexual spores produced.
2. Using your microscope, observe a prepared slides of *Penicillium*. **Focus first using the yellow-striped**

10X objective (100X magnification) and then rotate to the 40X objective (400X magnification). Note the type of asexual spores produced and on what they are borne. [Focusing instructions when using the 10X objective can be found in Lab 1.](#)

- Using your microscope and **using the yellow-striped 10X objective** (100X magnification), observe a prepared slides of [Aspergillus](#). Note the type of asexual spores produced and on what they are borne.
- Using your microscope and **using the yellow-striped 10X objective** (100X magnification), observe a prepared slides of [Rhizopus](#). Note the type of asexual spores produced and on what they are borne.
- Observe the prepared slide showing the [zygospore](#) of *Rhizopus* produced during sexual reproduction.

B. DERMATOPHYTES

- Observe the dermatophyte [Microsporum growing on DTM](#). Note the red color from production of alkaline end products indicating that it is breaking down the keratin in the agar. This indicates that the organism is a dermatophyte.
- Microscopically observe the SDA culture of *Microsporum*. Note the [macroconidia and microconidia](#).
- Observe the photographs of dermatophytic infections.

C. DIMORPHIC FUNGI



- Observe the prepared slide of *Coccidioides immitis* [arthrospores](#).
- Observe the pictures of *Coccidioides immitis* showing the mold form with arthrospores seen in the soil as well as the endosporulating spherule form seen in the lungs.
- Observe the pictures of *Histoplasma capsulatum* showing the mold form with tuberculate macroconidia seen in the soil as well as yeast form seen in the lungs.
- Observe the photographs of systemic fungal infections.



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RESULTS

A. COMMON MOLDS

Make drawings of the molds as they appear microscopically under high magnification and indicate the type of asexual spore they produce. Also note their color and appearance on SDA.

	
Penicillium	Aspergillus
Type of asexual spore:	Type of asexual spore:
Spores are borne on:	Spores are borne on:
Color on SDA =	Color on SDA =

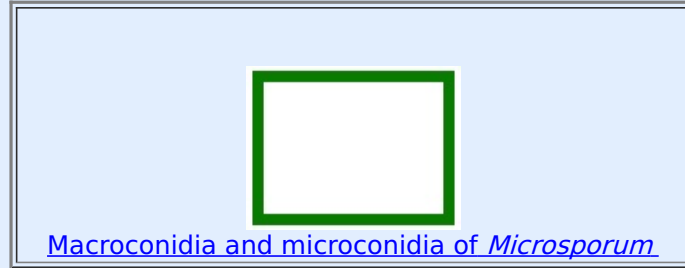
	
Rhizopus	Zygosporangium of Rhizopus
Type of asexual spore:	
Spores are found in:	
Color on SDA =	

B. DERMATOPHYTES

1. Describe the results of *Microsporium* growing on **Dermatophyte Test Medium (DTM)**:

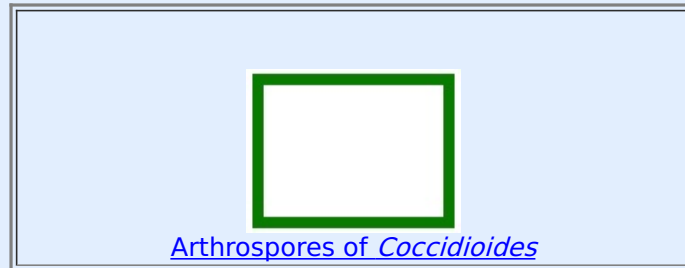
- [Original color of DTM](#) =
- [Color following growth of *Microsporium*](#) =
- Reason for Color Change =

2. Draw the **macroconidia and microconidia** seen on the SDA culture of *Microsporium*.

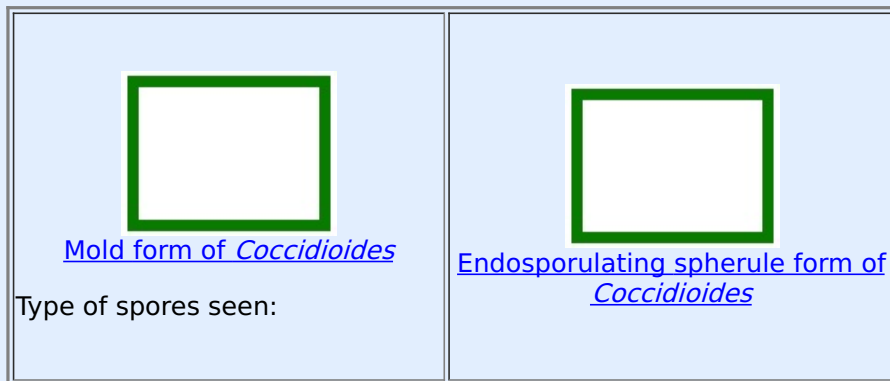


C. DIMORPHIC FUNGI

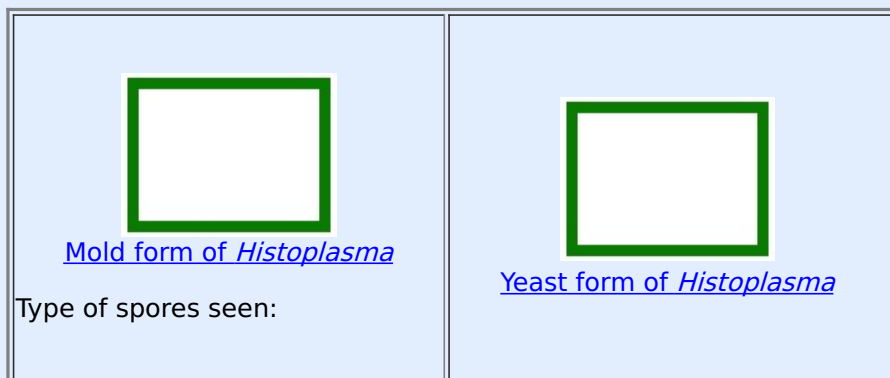
1. Draw the arthrospores of *Coccidioides immitis*.



2. Draw the mold form and endospore-forming spherule form of *Coccidioides immitis*.



3. Draw the mold form and yeast form of *Histoplasma capsulatum*.



PERFORMANCE OBJECTIVES FOR LAB 10

After completing this lab, the student will be able to perform the following objectives:

DISCUSSION

1. Define the following: hypha, mycelium, vegetative mycelium, and aerial mycelium.
2. Describe the principle way molds reproduce asexually.
3. State the main criteria used in identifying molds.

A. COMMON MOLDS

1. Describe conidiospores and sporangiospores and name a mold that produces each of these.
2. Recognize the following genera of molds when given an SDA plate culture and a dissecting microscope and state the type of asexual spore seen:
 - a. *Penicillium*
 - b. *Aspergillus*
 - c. *Rhizopus*
3. Recognize the following genera of molds when observing a prepared slide under high magnification and state the type of asexual spore seen:
 - a. *Penicillium*
 - b. *Aspergillus*
 - c. *Rhizopus*
4. Recognize *Rhizopus* zygospores.

B. DERMATOPHYTES

1. Define dermatophyte and list three common genera of dermatophytes.
2. Name four dermatophytic infections and state how they are contracted by humans.
3. Describe macroconidia and microconidia.
4. Describe how the following may be used to identify dermatophytes: potassium hydroxide preparations of tissue scrapings, DTM, and SDA.
5. Recognize a mold as a dermatophyte and state how you can tell when given the following:
 - a. a flask of DTM showing alkaline products
 - b. an SDA culture (under a microscope) or picture showing macroconidia.
6. Recognize macroconidia and microconidia.

C. DIMORPHIC FUNGI

1. Define dimorphic fungi and state how humans usually contract them.
2. Name three common dimorphic fungal infections found in the United States, state how they are transmitted to humans, and indicate where they are found geographically.
3. Describe the mold form and the yeast-like form of the following:
 - a. *Coccidioides immitis*
 - b. *Histoplasma capsulatum*
 - c. *Blastomyces dermatitidis*
4. Recognize *Coccidioides immitis* and its arthrospores when given a prepared slide and a microscope.

SELF-QUIZ

[Self-quiz](#)

[Answers](#)

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