EXPERIMENT 3

Identification of Fungi

INTRODUCTION

Although most of our work in this lab is done on bacteria, fungi are nonetheless an important aspect in microbiology. Besides being important food providers, fungi play central roles in recycling matter and production of various natural products with bioactivity, especially antibiotics.

Fungi are heterotrophic eukaryotic organisms belonging to the kingdom Fungi. The kingdom Fungi contains both multicellular organisms, or *molds*, and unicellular organisms known as *yeasts*. Although fungi may be plantlike and possess a cell wall, they do not contain chlorophyll nor do they photosynthesize. All fungi are considered saprophytes as they feed on dead and decaying matter.

Yeasts are unicellular, ovoid shaped organisms that are usually larger than bacteria. Yeasts possess a nucleus and reproduce by **budding,** or **binary fission**. A bud begins as a tiny protrusion of the parent cell that eventually enlarges in size and pinches off.

Molds grow as multicellular hair like filaments called **hyphae** into tangled masses called **mycelium**. If the hyphae contain internal cross walls (septa), they are considered *septate*, and those without the septa are considered *nonseptate,* or *aseptate* (Figure 1).

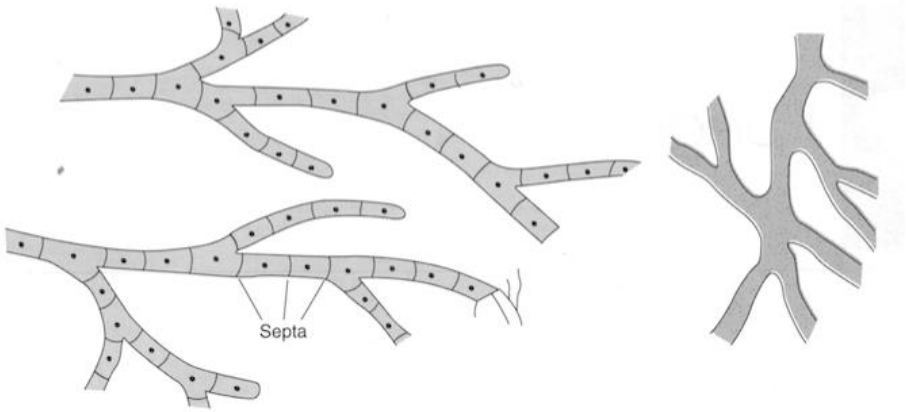

Figure 1. Septate and Aseptate fungi. Reference: Pollack et al. 2002

Molds reproduce by asexual or sexual production of spores. These spores become airborne to facilitate dispersal and reproduction. These spores are often the cause of allergies, infections, and laboratory contamination. It is therefore imperative to have an understanding of the types of molds and yeasts present in our daily environments.

Fungi can be classified by the type of sexual spores they produce. Based on the type of spore, fungi can be classified into four main divisions: Zygomycota, Ascomycota, Basidiomycota, and Deuteromycota. Zygomycota are characterized by asexual sporangiospores in sacs called **sporangia** (Figure 2a). These sacs, or sporangia, are produced on the tip of an aerial hyphae which extend from the mycelium mass. The sacs burst and release the spores through the air. Ascomycota are characterized by asexual conidiospores, or **conidia** that form externally on aerial hyphae (Figure 2b). Basidiomycota are characterized by sexual basidiospores that form within a mushroom body. Finally, Deuteromycota have an unknown reproductive cylcle. Figure 3 shows *Fusarium* sp. and an example of how fungi can be identified based on morphology and spore production. Figure 4 gives an insight into the array of fungus and yeast that can be captured in the air by exposing a petri dish. To aid in fungal identification, Figure 5 demonstrates the common fungal spore morphologies and characteristics found in nature. Additionally, you can use the Supplemental chapter at the end of the manual for help in identification.

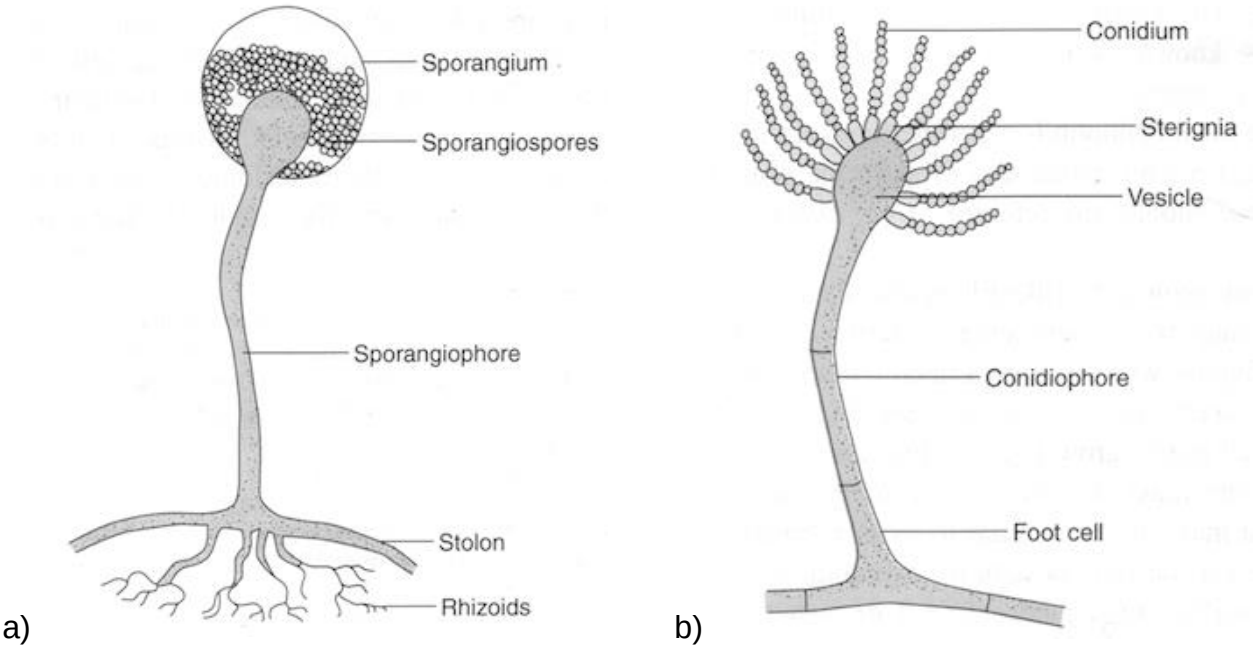

Figure 2. *Rhizopus* (a) and *Aspergillus* (b) showing the different morphologies of Zygomycota and Ascomycota, respectively. Reference: Pollack et al. 2002

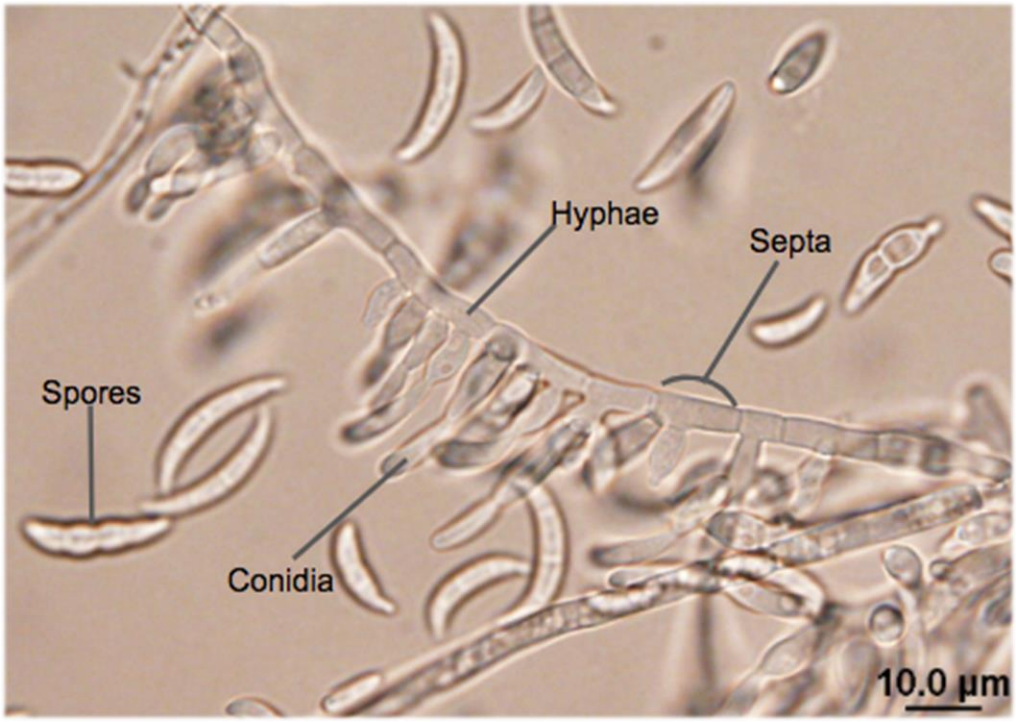

Figure 3. *Fusarium* sp., a common soil fungus. This fungus is septate, with curved fusiform conidiospores, or conidia, and belongs to the division Ascomycota.

Figure 4. An agar plate left open in order to capture fungal spores in the room. Notice the different colored shapes, sizes, and textures of the fungal colonies.

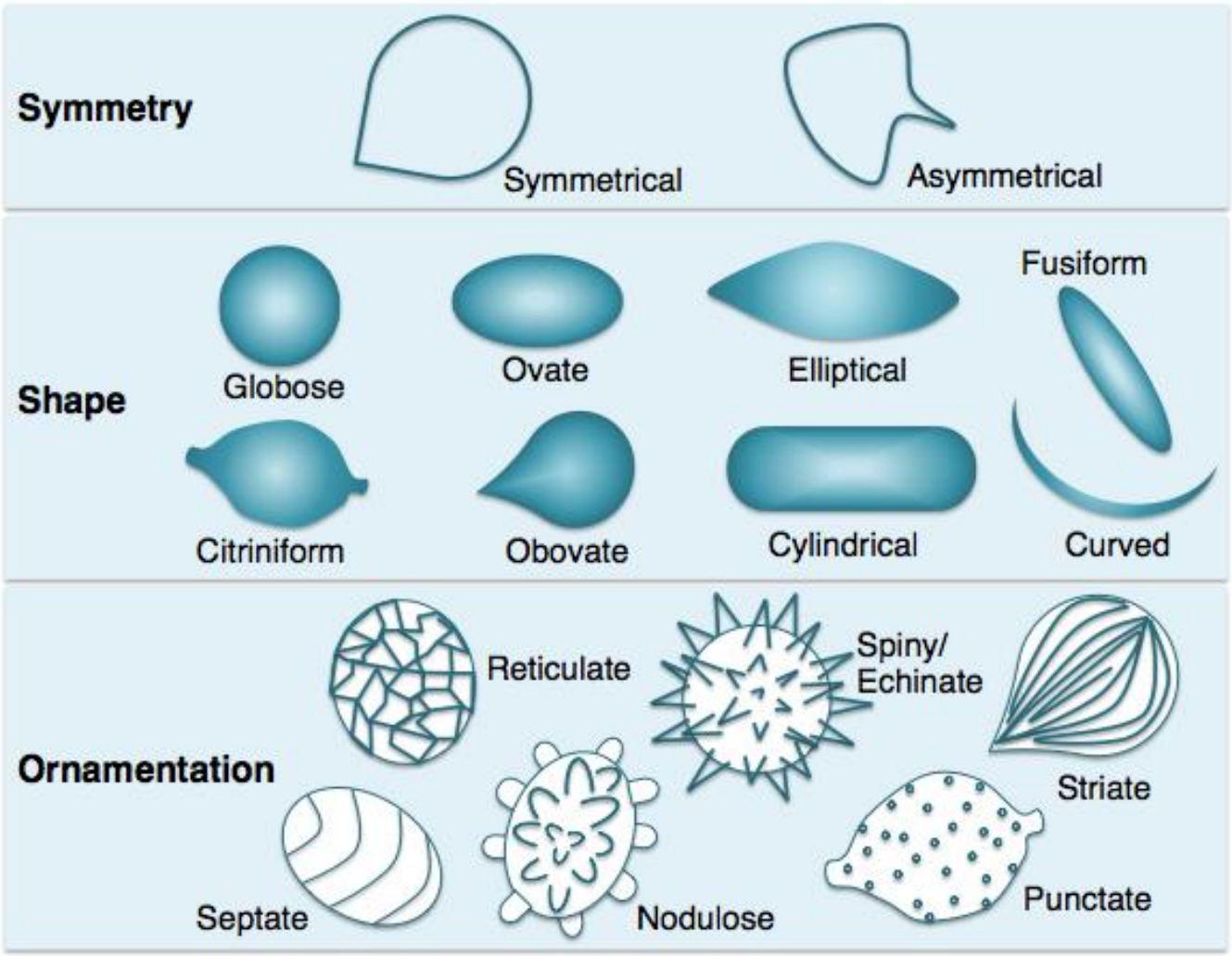

Figure 5. Diagram of fungal spore morphology including symmetry, shape, and ornamentation to aid in identification.

Materials:

1. **Sabouraud dextrose agar plates from home exposed to outside and inside**
2. **(1) Sterile Sabouraud Agar plate per group (Optional lab#13)**
3. **Loop**
4. **Slides and coverslips**
5. **Lactophenol mounting medium**
6. **Parafilm (Optional Lab #13)**

Procedures:

Part I. Fungal Enumeration

1. Examine the two Sabouraud dextrose gar plates. Count the total individual fungal colonies from both plates, the plates exposed to outside and inside.

2. Count the number of different types of colonies found on each plate.

3. Make sure not to open the petri plates until you will use it. The spores are easily airborne and can cause allergy, infection, or laboratory contamination.

Part II. Fungal Identification

1. From the Sabouraud dextrose agar plates, pick **THREE** different mycelia colonies and circle and label with a marker on the back of the dish.

2. Place a drop of **lactophenol** as a mounting medium onto three different slides, each labeled with the colony from the plates.

3. With a sterile loop, rub the top of the mycelium and smear onto the mouting medium.

4. Quickly sterilize loop, allow to cool, and continue to place the other colonies onto the remaining slides. Place a cover slip over the preparation.

5. Observe each fungi under the microscope and sketch the hyphae and spores and continue onto identification.

Procedures for future Lab (Optional)

1. From the indoor or outdoor Sabouraud agar plates, choose one fungal colony and inoculate onto a sterile Sabouraud agar plate. Cover with parafilm.

2. Incubate at room temperature for 48 hours.

Part I Fungal Enumeration (1 point)
Directions: Complete the chart by counting the total number of colonies and the number of different colony types (shape, texture, color, margin) on both plates.

Agar Plate	Number of colonies	Number of Colony Types
Indoors		
Outdoors		

Part II Colonial Characteristics (2 points)
Directions: Complete the chart below with details of your fungal colony observations on the agar plates.

Colony Characteristics	Colony A	Colony B	Colony C
Color			
Diameter (mm)			
Texture/ Margin			
Soluble Pigments in agar (y/n)			

Part III. Reproductive Structures (2 points)
Directions: Include detailed drawings of the hyphae with reproductive structures and asexual spores.

Fungal Colony	Hyphae with Reproductive Structures	Asexual Spores
A		
B		
C		

Part IV Questions

Directions: Answer the following questions with full sentences. Conduct web or library searches where necessary. (**5 points)**

1. In what way can we distinguish fungi from: **(1 point)**
    - Algae
    - Bacteria
2. When enumerating fungal colonies, were there more fungi inside or outside the tested facility? **(0.5 point)**
3. When enumerating fungal types, was there more variety inside or outside the tested facility? **(0.5 point)**
4. What are the possible consequences of having more molds inside than outside? Vise versa? **(1 point)**
5. Conduct a web/library search through fungal ID keys with the data attained from each fungus you inspected. Below include the presumed genus of each of your fungal isolates. **(1 point)**

a.

b.

c.

6. Based on your web searches, were there any important human applications for each of the fungal isolates? (i.e. Medical, pathological, Food, Agricultural). **(1 point)**

a.

b.

c.