

E X E R C I S E

4

Observing Fungi and Yeast

OBJECTIVES

At the conclusion of the exercise, you should...

1. understand where the fungi are placed in the classification of microorganisms.
2. be able to recognize the characteristics of fungi to identify certain species of fungi macroscopically and microscopically.
3. observe the differences between bacteria and fungi growing on agar plates.
4. know the types of media that are used for growing fungi.
5. observe wet mounts of yeast.
6. be introduced to the concept of **compromised host**.
7. know the difference between a **pathogen** and an **opportunistic pathogen**.

INTRODUCTION

Fungi (molds) are a diverse group of single-celled or multicellular organisms that obtain food by direct absorption of nutrients. Fungi are classified with the eucaryotic microorganisms. They are identified by their reproductive spores from the asexual and sexual stages of their life cycles. They play a very beneficial role in the food chain by decomposing dead plant matter, providing food (mushrooms), and aiding in the production of foods (bread) and drugs (alcohol). Also, over the last 10 years, there has been an increase in fungal infections in people with compromised immune systems. Thus, identification of a fungus is often necessary for the treatment of a disease. The study of fungi is called Mycology. In this laboratory, you will observe asexual and sexual structures of different species of fungi, and learn how these characteristics aid in identification.

MATERIALS

Cultures: plates and prepared slides

Penicillium species (phialospores/conidiospores)–PDA (potato dextrose agar) or SAB (Sabouraud's agar) plates & slides

Aspergillus species (phialospores/conidiospores)–PDA or SAB plates & slides

Rhizopus species (sporangiospores) PDA or SAB plates & slides

Rhizopus species (zygospores)– slides only

Fusarium species (macroconidia)–PDA or SAB plates & slides

Saccharomyces cerevisiae (yeast)–SAB plates and broths (for wet mounts) & slides

Candida albicans (yeast)–SAB plates and broths (for wet mounts) & slides

Plates of bacteria for comparison

Supplies:

Prepared microscope slides of the fungi and yeast listed

PROCEDURES

Technical Background

The **thallus** is the body of a fungus and it consists of long filaments of cells joined together, called **hyphae** (singular: *hypha*). Hyphae can contain cross walls called **septa** (singular: *septum*) and are called **septate** hyphae. In a few classes of fungi, the hyphae will not contain septa, and are called **non-septate hyphae** or **coenocytic hyphae**. These filamentous fungi can reproduce both sexually and asexually by the formation of **spores**. Two kinds of asexual spores are produced: conidia or **conidiospores**—formed on specialized hyphae called **conidiophores**—and **sporangia** or **sporangiospores**, which are formed within a **sporangium** (sac) at the end of an aerial hypha called a **sporangiophore**. There are several types of conidiospores. **Phialides** are vase-shaped cells that produce chains of conidia called **phialospores**.

Sexual spores result from sexual reproduction where (+) and (-) nuclei fuse to form sexual spores. Three types of sexual spores are produced in fungi, **zygospores** (inside a zygote), **ascospores** (inside an **ascus**), and **basidiospores** (inside a **basidium**).

Fungi are classified into four categories : **Zygomycota**, **Ascomycota**, **Basidiomycota**, and **Deuteromycota**.

- **Zygomycota** have nonseptate hyphae, and produce asexual sporangiospores. An example is *Rhizopus*, a common bread mold.
- **Ascomycota** produce conidiospores. Examples are *Aspergillus* and *Penicillium*.
- **Basidiomycota** have septate hyphae, and produce conidiospores. Mushrooms are in this division.
- **Deuteromycota** is a “holding place” for fungi if they have not yet been found to produce sexual spores. It is sometimes referred to as the “imperfect fungi.” It is a large group, containing over 15,000 species. Many of the pathogenic fungi are in this division.

Yeasts are classified as unicellular fungi that are non-filamentous. Yeast reproduce by either **fission** (divide evenly to produce two new cells) or **budding** (parent cells form a bud that eventually breaks off). When buds are produced and fail to detach, they form a chain of cells called a **pseudohypha**. Two examples of yeast are *Saccharomyces cerevisiae* (bread-making yeast) and *Candida albicans* (opportunistic pathogen).

Candida albicans is a **dimorphic** yeast, meaning that it takes on two forms. Most of the time it exists as single, oval-shaped yeast cells, which reproduce by budding. Under the right conditions, *Candida albicans* can develop **pseudohyphae**, which are composed of a chain of cells and grow as irregular filaments. Room temperature (25°C) yields the single and budding cells. Body temperature (37°C) and body pH, along with the presence of serum, allows *C. albicans* to be capable of producing pseudohyphae. A rapid identification test to identify whether a yeast colony is *C. albi-*

C. albicans is a “Germ Tube” test. Human serum is inoculated and incubated at 37°C for 2-3 hours. The presence of pseudohyphae along with no pseudohyphae at room temperature gives a presumptive identification of *C. albicans*. Further biochemical tests can be performed. *C. albicans* can be found in normal humans in the mouth, gut, and vagina. Infections occur when a patient has become immunocompromised. Infections caused by *C. albicans* are called candidiasis or “thrush.” *Candida* can also cause pneumonia, septicemia, and endocarditis.

Saccharomyces cerevisiae are single-celled fungi that multiply by budding or by division (fission). *S. cerevisiae* is known as the “bread-making” yeast because growth in the dough produces CO₂, causing the dough to rise. It is also used for wine and beer production. It is a member of the Ascomycota (ascus-forming) fungi. *Penicillium* and *Aspergillus* also produce these types of spores.

Culture Media: There many different types of culture media used for growing fungi. Potato Dextrose Agar (**PDA**), or broth, and Sabouraud’s Agar (**SAB**), or broth, are two of the most commonly used. SAB culture media contains an antibiotic to inhibit bacterial growth. PDA consists of a potato infusion and dextrose, and will encourage the growth of fungi and yeast.

Compromised host: a person whose immune system is suppressed and, therefore, can be susceptible to invasion by microorganisms. A suppressed immune system can result from various diseases, therapies, or burns.

Opportunistic pathogen: microorganisms that invade the body when the body’s immune defense is suppressed or compromised.

Pathogen: any microorganism that can cause disease in a healthy person.

Viewing Fungi and Yeast Samples

1. Examine the Petri plates of the fungi provided (please do not open the plates).
2. Describe the color of the thallus and hyphae.
3. Describe and draw the colony characteristics for the front and back of the colony.
4. Compare the cultures to the drawings and photos provided.
5. Examine the prepared slides of the fungi provided.
6. Describe the type of hyphae and spores seen.
7. Make wet mounts of the yeast provided and note the budding or presence of pseudohyphae.
8. Use the Evaluation of Results section to make drawings and write descriptions.
9. Observe plates of yeast and compare to plates of bacteria.

Examples of Fungi

Examples of Fungi

EVALUATION OF RESULTS

(EXERCISE 4: OBSERVING FUNGI AND YEAST)

Purpose

Data

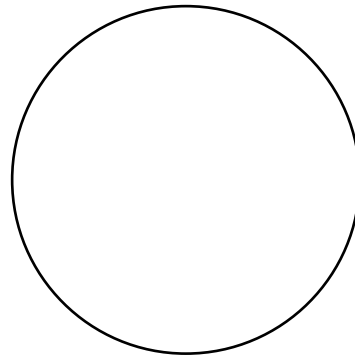
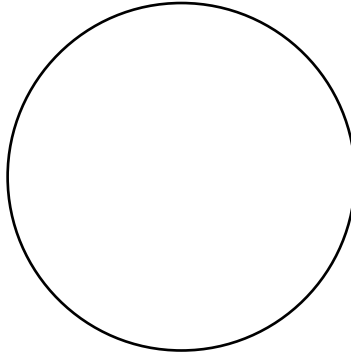
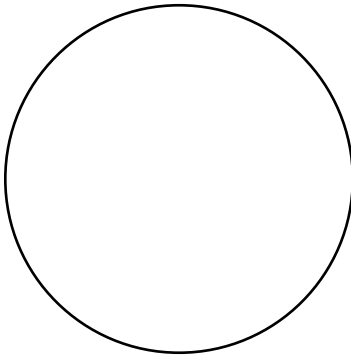
Describe in your own words the fungal colonies growing on agar medium. Label the drawings to include color, type of growth (wrinkled, smooth, etc.). Then examine the prepared slides of each fungus. Draw the arrangement of hyphae and spores for each fungus.

Top view

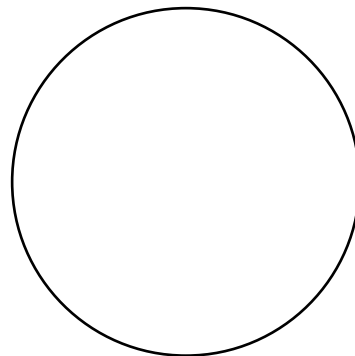
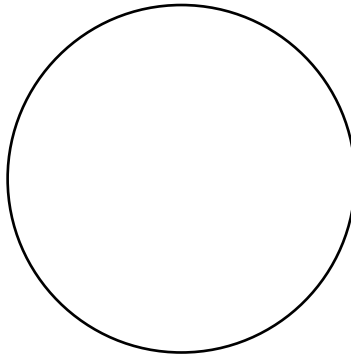
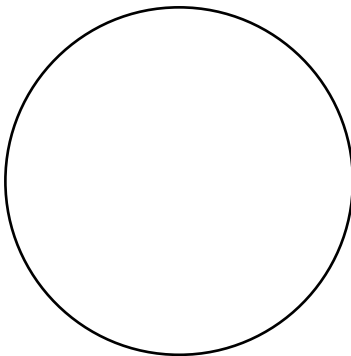
Bottom view

Microscopic observations of
prepared slide at 400X

Penicillium



Rhizopus

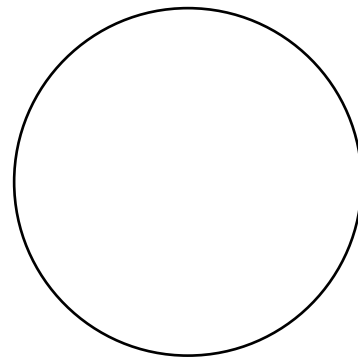
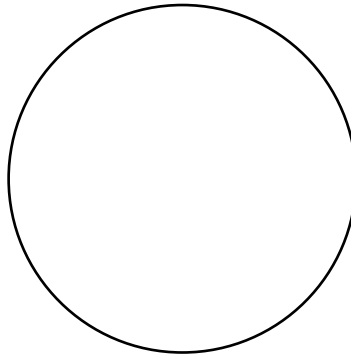
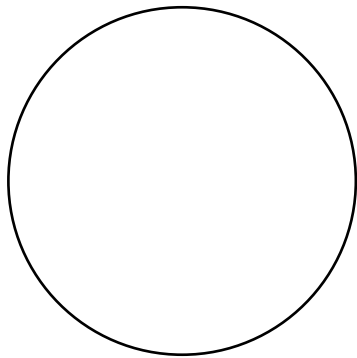


Top view

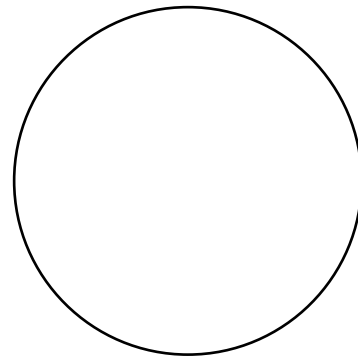
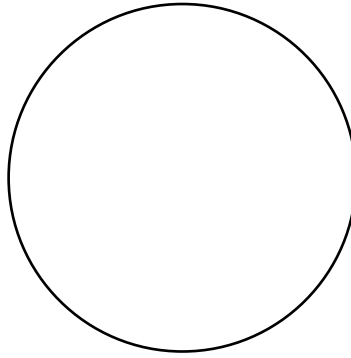
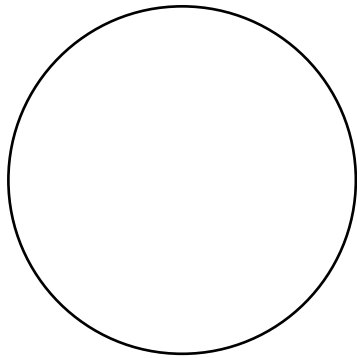
Bottom view

Microscopic observations of prepared slide at 400X

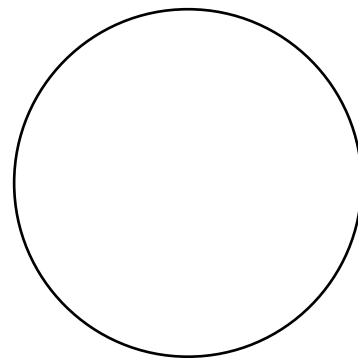
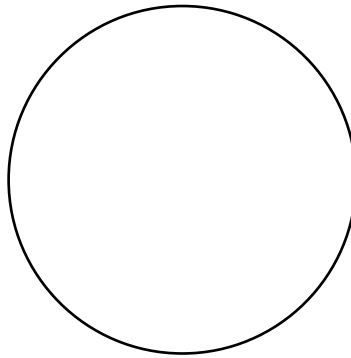
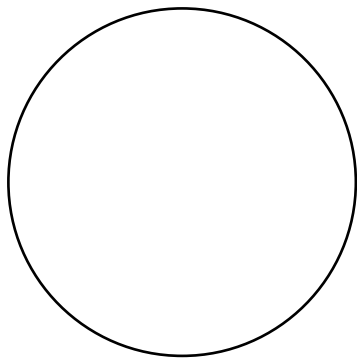
Aspergillus



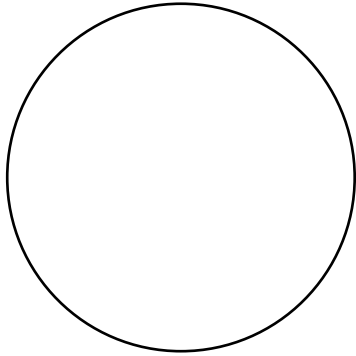
Fusarium



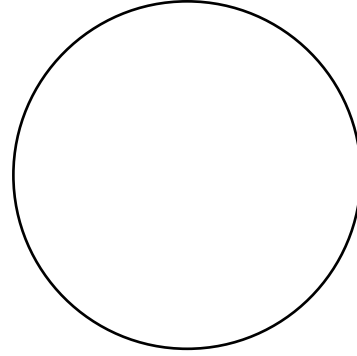
Candida



Wet mount of yeast *Saccharomyces cerevisiae*
cerevisiae (400X)



Stained slide of yeast *Saccharomyces*



CONCLUSIONS, DISCUSSIONS, AND QUESTIONS

1. Which slide preparation of yeast, the wet mount or stained slide, is easier to view? Why?

2. Match the following:

Eucaryote

a. science that studies fungi

Mycelium

b. type of cell fungi have

Pseudohyphae

c. chain of yeast blastopores

Mycology

d. mass of mold filaments visible without a microscope

Dimorphic

e. type of hyphae seen in *Penicillium*

Macroconidia

f. fungi with yeast and mold stages

Septate hyphae

g. type of hyphae seen in *Rhizopus*

Non-septate hyphae

h. asexual spores of *Fusarium*

