



## Lab Manual

### Introductory Biology (Version 2.4)



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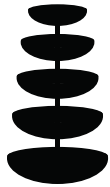


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## Common Labware found in ESL Kits

A—Beakers  
B—Measuring Cup  
C—Measuring Spoon  
D—Mortar and Pestle  
E—Wash Bottle

F—Canning Jar  
G—Graduated Cylinder  
H—Standing Test Tube  
I—Funnel  
J—Micropipettes

K—Weigh Boats  
L—Pipettes  
M—Glass Test Tube  
N—Plastic Test Tube (17 x 100mm)  
O—Plastic Test Tube (13 x 100mm)

P—Petri Dish  
Q—Syringe  
R—Thermometer  
S—Spray Lid





## Lab Safety

Always follow the instructions in your laboratory manual and these general rules:

eScience Labs, Inc. designs every kit with safety as our top priority. Nonetheless, these are science kits and contain items which must be handled with care. **Safety in the laboratory always comes first!**

### Lab preparation

- **Please thoroughly read the lab exercise before starting!**
- If you have any doubt as to what you are supposed to be doing and how to do it safely, please STOP and then:
  - ✓ Double-check the manual instructions.
  - ✓ Check [www.esciencelabs.com](http://www.esciencelabs.com) for updates and tips.
  - ✓ Contact us for technical support by phone at 1-888-ESL-Kits (1-888-375-5487) or by email at [Help@esciencelabs.com](mailto:Help@esciencelabs.com).
- Read and understand all labels on chemicals.
  - ✓ If you have any questions or concerns, refer to the Material Safety Data Sheets (MSDS) available at [www.esciencelabs.com](http://www.esciencelabs.com). The MSDS lists the dangers, storage requirements, exposure treatment and disposal instructions for each chemical.
- Consult your physician if you are pregnant, allergic to chemicals, or have other medical conditions that may require additional protective measures.

### Proper lab attire

- Remove all loose clothing (jackets, sweatshirts, etc.) and always wear closed-toe shoes.
- Long hair should be pulled back and secured and all jewelry (rings, watches, necklaces, earrings, bracelets, etc.), should be removed.
- Safety glasses or goggles should be worn at all times. In addition, wearing soft contact lenses while conducting experiments is discouraged, as they can absorb potentially harmful chemicals.
- When handling chemicals, always wear the protective goggles, gloves, and apron provided.



### Performing the experiment

- Do not eat, drink, chew gum, apply cosmetics or smoke while conducting an experiment.
- Work in a well ventilated area and monitor experiments at all times, unless instructed otherwise.
- When working with chemicals:
  - ✓ Never return unused chemicals to their original container or place chemicals in an unmarked container.
  - ✓ Always put lids back onto chemicals immediately after use.
  - ✓ Never ingest chemicals. If this occurs, seek immediate help.

**Call 911 or "Poison Control" 1-800-222-1222**

- Never pipette anything by mouth.
- Never leave a heat source unattended.
  - ✓ If there is a fire, evacuate the room immediately and dial 911.

### Lab Clean-up and Disposal

- If a spill occurs, consult the MSDS to determine how to clean it up.
- Never pick up broken glassware with your hands. Use a broom and a dustpan and discard in a safe area.
- Do not use any part of the lab kit as a container for food.
- Safely dispose of chemicals. If there are any special requirements for disposal, it will be noted in the lab manual.
- When finished, wash hands and lab equipment thoroughly with soap and water.

**Above all, USE COMMON SENSE!**

# Kingdoms



Lab 7

Fungi





## Concepts to explore:

- Phylums
- Decomposers
- Saprophytes
- Hyphae
- Mycelium
- Reproduction
- Spores
- Dikaryon
- Commercial uses

## Introduction

Fungi are immobile, multicellular organisms (yeast is unicellular) that have some unique characteristics and play a profound role in ecology and human health. Fungi fall into four phylums:

- *Zygomycota* (bread molds)
- *Ascomycota* (sac- yeast and molds)
- *Basidiomycota* (club mushrooms, rusts)
- *Deuteromycota* (no identifiable sexual stage)

Fungi are decomposers that secrete enzymes to break down material into soluble organic compounds, which they can then absorb. They are saprophytes that digest dead and decaying material (e.g. ground, rotting wood, dung) and are critical in decomposing and recycling nutrients for the environment.

Multicellular fungi are primarily filamentous, with long, thin, somatic cells called hyphae. Unlike most plant and animal cells, hyphae can have more than one nucleus. Mycelium, a network of hyphae that lack distinct cells, are the structures that invade and digest material in soil, wood and other matter. Chitin is a light but strong substance, found in the cell wall (in hard shells of insects and crustaceans) that adds rigidity and strength.

Since the cytoplasm of hyphae are linked within the mycelium, a unique form of mitosis occurs in fungi. Instead of dividing nuclear material *and* the cytoplasm, mitosis occurs in the nucleus, which then divides (the nuclear envelope does not dissolve).

Spores are the basic reproductive structure of fungi. Fungi reproduce either asexually or sexually, depending on their environment. During the asexual phase, microscopic haploid spores are created by mitosis (the spores are identical). When they land in the right conditions, they germinate and grow new hyphae. There are five types of asexually produced spores:

1. *Arthrospores*, produced in fungi that have divided hyphae, are individual segment of the hyphae that break off into spores.



Figure 1

2. *Chlamydospores*, a segment of the mycelium, become thick-walled and breaks away as the spore.



Figure 2

3. *Sporangiospores* are produced in a sac-like structure called the sporangium.

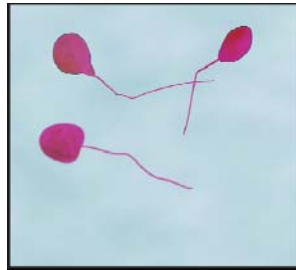


Figure 3

4. *Conidiospores* are not enclosed, but are produced in the conidiophores.



Figure 4

5. *Blastospores* are produced by a budding process (mitosis with unequal division of the cytoplasm).

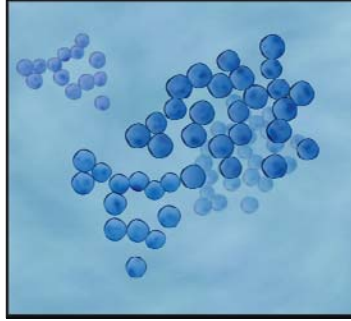


Figure 5

Fungi form gametes through mitosis (unlike other multicelled organisms) and spend the majority of their life cycle in a haploid state ( $1n$ ).

During sexual reproduction, hyphae of two different specimens, from the same species, meet and fuse together to form a dikaryotic cell ( $1n+1n$ ). Once the nuclei have fused into a zygote, it goes through meiosis producing that mature into a new haploid organism.

Fungi produce three types of spores:

1. The fusion of two nuclei produces a thick-walled zygosporangium which manufactures “+” and “-” haploid zygosporoes

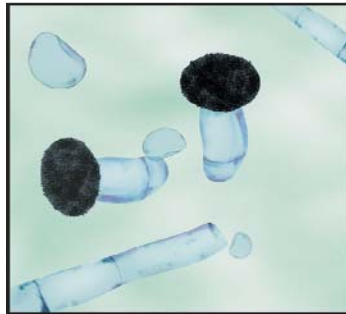


Figure 6

2. Two fungi touch exchange nuclei that fuse together in a sac-like structure called the ascus. Meiosis then produces four nuclei which subsequently undergo mitosis, producing eight ascospores.

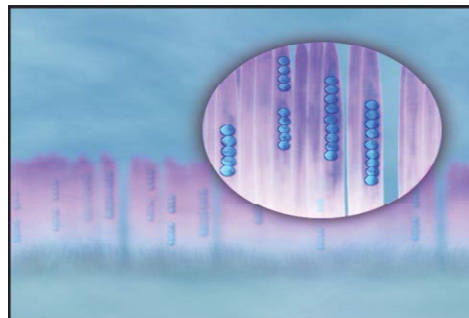


Figure 7



3. Mycelia fuse together to form a secondary structure with two nuclei. They maintain this dikaryotic state until fruiting occurs and the “gills” of the mushroom develop. Meiosis then produces four nuclei which migrate into the gills forming basidiospores.

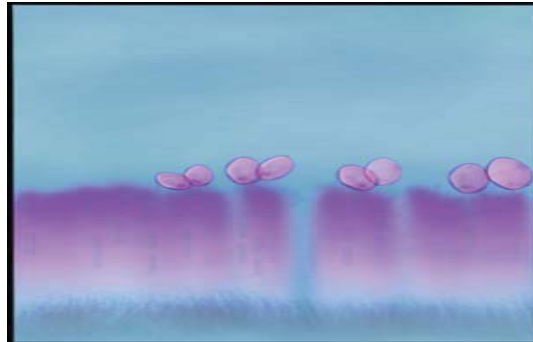


Figure 8

Many fungi are harvested commercially for food, bread leavening, alcohol production, antibiotic production and other uses. Many symbiotic relationships have evolved between fungi and other plants and animals.

Fungi can produce chemicals that make food unpleasant, carcinogenic, or poisonous. They can cause athlete’s foot, ringworm and can kill plants (e.g. potatoes, elms and oak trees). Some are parasitic, feeding off living cells, absorbing their nutrients and killing their host.

### Mature Mushroom

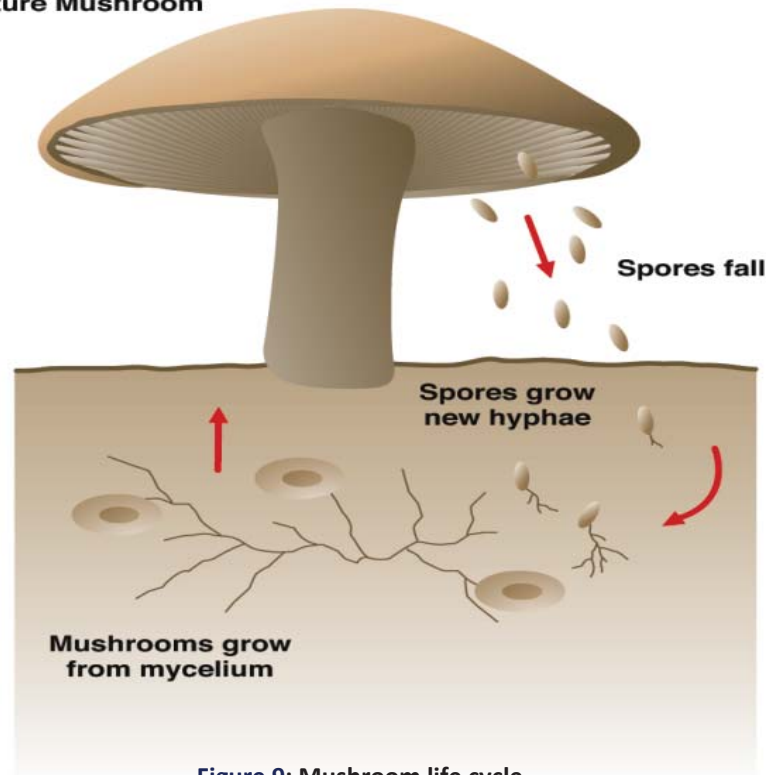


Figure 9: Mushroom life cycle



## Definitions:

**Chitin:** a resilient and tough polysaccharide found in the cell wall of fungi, as well as the exoskeletons of insects and crustaceans

**Dikaryon:** two cells that merge into one without fusing nuclei; a cell with two haploid nuclei

**Hyphae:** the branching, filamentous cells of fungi

**Saprophyte:** an organism that feeds on non-living organic matter

## Experiment 1: Growing and Observing Zygomycota (Bread Mold)

Bread mold will generally ruin a day when you are looking forward to a sandwich, but will make it much easier to observe a live fungus. In this lab you will be cultivating the common bread mold. Any type of bread can be used, but fresh bakery bread will likely grow more colorful species since it does not contain the preservatives that many processed varieties do.

### Materials

Piece of white bread\*  
Water  
Spray bottle lid and empty bottle  
Ziploc bag  
Digital slide picture of *Rhizopus*

\* You must provide

### Procedure

1. Take a slice of bread and, spray it with water until moist. Avoid soaking the bread since this will lead to bacterial growth.
2. Place the moistened bread in a Ziploc bag, and seal.
3. **Incubate the bag for 3-7 days** (depending on how fresh the bread is) in a dark, warm spot.
4. Examine the bread once mold starts growing.



5. Examine the digital slide pictures of *Rhizopus* (Figure 10).

A. Identify the structures.

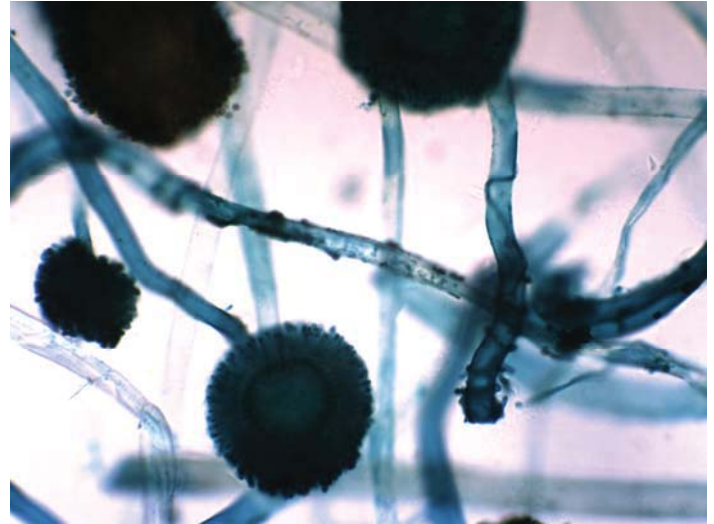
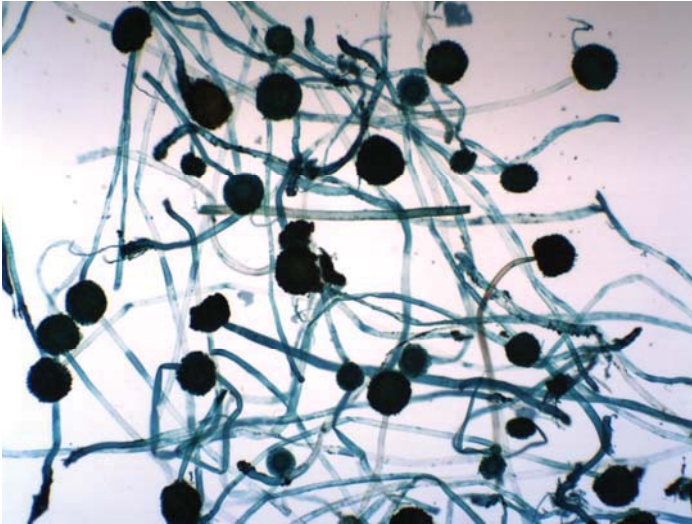
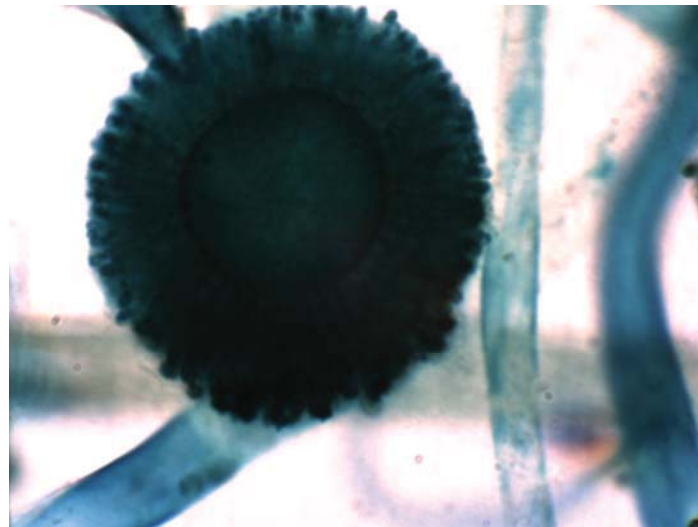


Figure 10: *Rhizopus* digital slide pictures; 100X (above left), 400X (above right), 1000X (below)



### Questions

1. What structures did you see in the bread mold?



2. Why was it important to moisten the bread before sealing it in the Ziploc bag?
  
3. Is the bread mold reproducing sexually or asexually? Can you tell?
  
4. What is the mold doing to the bread?
  
5. What would you expect to find if you left the bread for 6 months?
  
6. Were multi-nucleated hyphae prevalent when looking at the Rhizopus slide?

### Experiment 2: Observing Ascomycota (Yeast)

Yeast is a commercially important member of the Fungi Kingdom. It leavens bread and produces beer and wine. Yeast is unique because it is unicellular and reproduces by mitosis and budding.

#### Materials

Digital slide pictures of yeast  
Digital slide pictures of *Peziza*

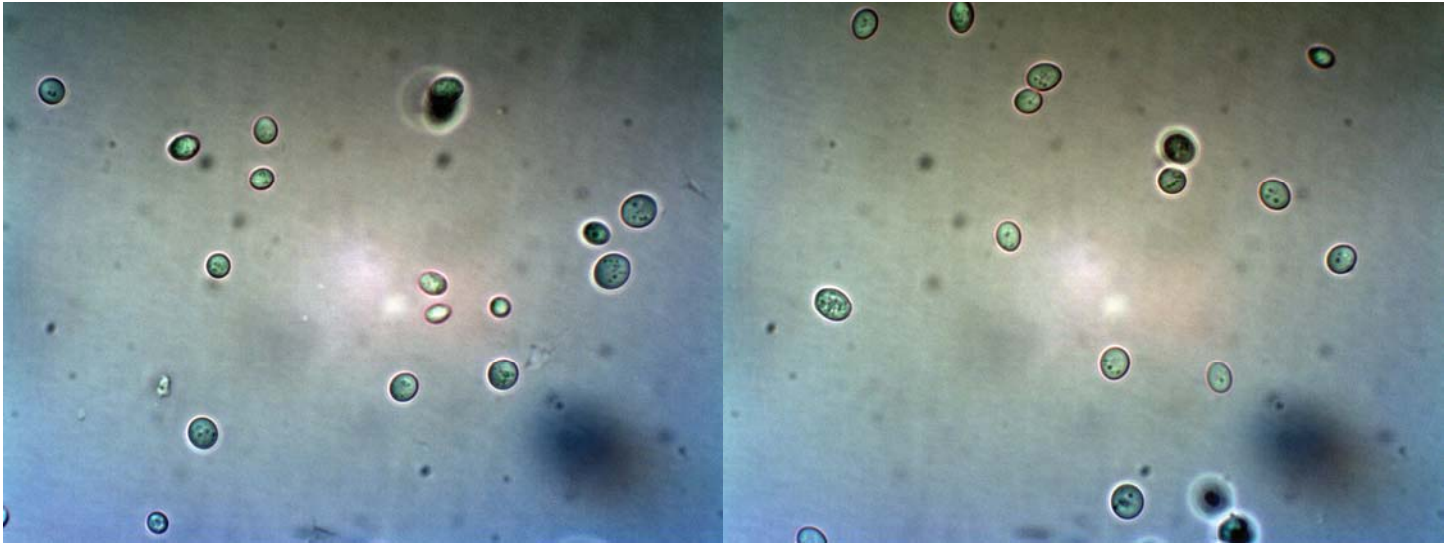


Figure 11: Yeast 1000X; field 1 (left) and field 2 (right)

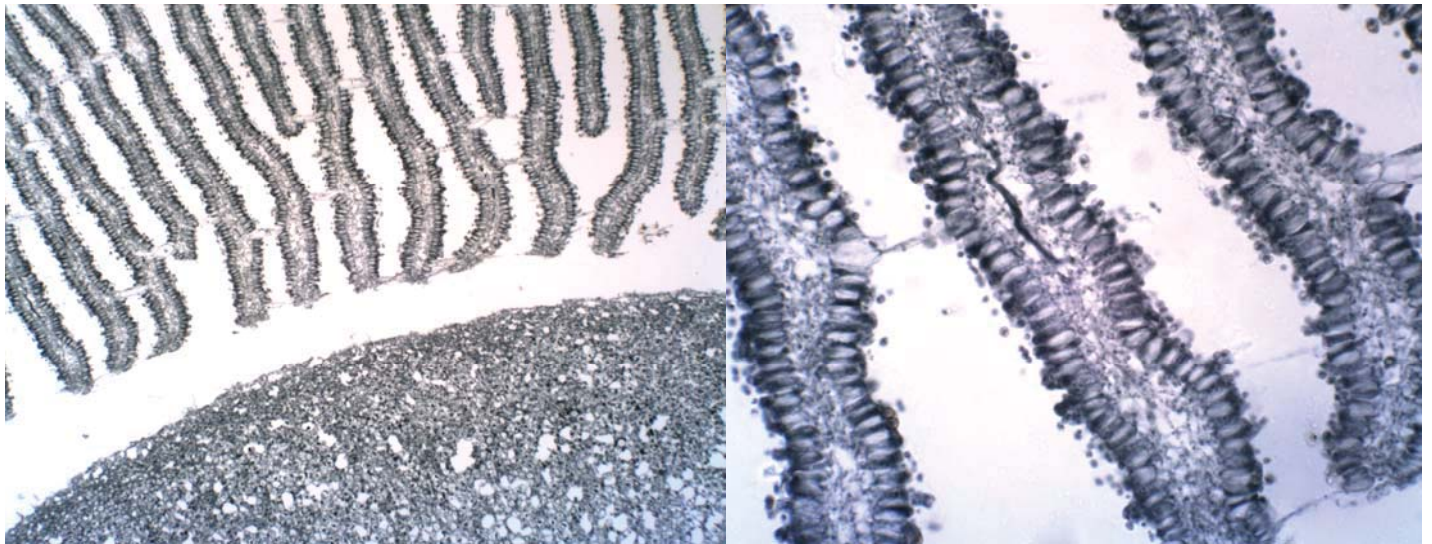
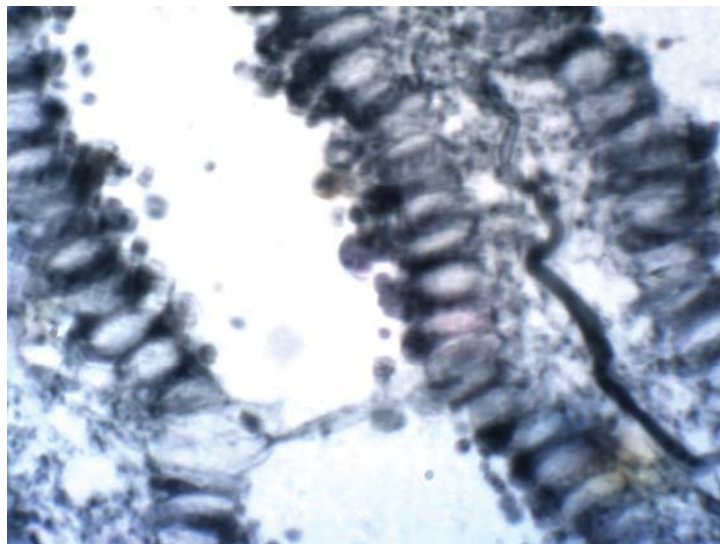


Figure 12: Digital slide pictures of peziza; 100X (above left), 400X (above right); 1000X (below)





## Procedure

1. View the digital slide picture of yeast (Figure 11). Locate the budding cells and label what you see in the space below. Using the slide as a reference, draw a budding yeast cell and include important structures (ascus, ascospore) on your picture.
2. View the digital slide pictures of *Peziza* (Figure 12). Identify the asocarp, asci, ascospores, and sterile hyphae.

## Questions

1. Compare yeast to the *Peziza* slide.
2. How many ascospores are in each ascus of the yeast?
3. Are the ascospores of the *Peziza* inside or outside of the asci?





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