

**Background Information**

The Kingdom Fungi includes some of the most important organisms, both in terms of their ecological and economic roles. By breaking down dead organic material, they continue the cycle of nutrients through ecosystems. In addition, most vascular plants could not grow without the symbiotic fungi, or mycorrhizae, that inhabit their roots and supply essential nutrients. Other fungi provide numerous drugs (such as penicillin and other antibiotics), foods like mushrooms, truffles and morels, and the bubbles in bread, champagne, and beer.

Fungi also cause a number of plant and animal diseases: in humans, ringworm, athlete's foot, and several more serious diseases are caused by fungi. Because fungi are more chemically and genetically similar to animals than other organisms, this makes fungal diseases very difficult to treat. Plant diseases caused by fungi include rusts, smuts, and leaf, root, and stem rots, and may cause severe damage to crops. However, a number of fungi, in particular the yeasts, are important "model organisms" for studying problems in genetics and molecular biology.

Fungi may reproduce sexually or asexually. In asexual reproduction, the offspring are genetically identical to the parent organism (they are clones). During sexual reproduction, a mixing of genetic material occurs so that the offspring exhibit traits of both parents. Many species can use both strategies at different times, while others are strictly asexual. Sexual reproduction has not been observed in some fungi. These are commonly referred to as Fungi imperfecti and are classified in the phylum Deuteromycota.

Yeasts and other unicellular fungi can reproduce simply by budding, or pinching off a new cell. Many multicellular species produce a variety of different asexual spores that are easily dispersed and resistant to harsh environmental conditions. When the conditions are right, these spores will germinate and colonize new habitats.

Sexual reproduction in fungi is somewhat different from that of animals or plants, and each fungal division reproduces using different strategies. Fungi that are known to reproduce sexually all have a haploid stage and a diploid stage in their life cycles. Ascomycetes and basidiomycetes also go through a dikaryotic stage, in which the nuclei inherited by the two parents do not fuse right away, but remain separate in the hyphal cells.

**Materials**

water  
24 hour culture of yeast in glucose  
2 week growth of bread mold  
Sordaria culture  
basidia (prepared slide)

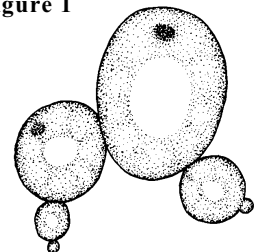
glass stir rod  
microscopes (both types)  
sporangia (prepared slide)  
ascus (prepared slide)  
Petri dish

slides  
medicine dropper  
conjugation (prepared slide)  
mushrooms  
glass marking pencil

**Part A Yeast - Unicellular Fungus**

Yeast is a single celled eukaryotic organism. It is technically a fungus and is one of the lower forms of eukaryotic life. However, that does not mean that they are not sophisticated organisms. They can undertake some incredible chemistry that our cells cannot do. For example, yeast can turn a small amount of sugar and water into ethanol and carbon dioxide, something the baking and brewing industries depend on for their existence.

In many simple organisms, reproduction is not a very complicated thing. It generally involves only one organism. The resulting offspring often have the exact same genetic information as the parent. This type of reproduction in which one parent is involved in the production of an identical offspring is called asexual reproduction. One type of asexual reproduction found in the kingdom Fungi is called budding. When conditions are favorable, yeast cells can reproduce through budding. Budding involves one parent dividing its nucleus equally, but cytoplasm unequally. Figure 1 shows an example of a yeast producing buds.

**Figure 1**

The onset of the cellular events is accompanied by the nuclear events of mitosis. The initial events of budding can be seen as the development of a ring of chitin around the point where the bud is about to appear. This reinforces and stabilizes the cell wall. Enzymatic activity and turgor pressure then act to weaken and extrude the cell wall. New cell wall material is incorporated during this phase.

Cell contents are forced into the progeny cell, and as the final phase of mitosis ends with the formation of a cell plate, the point at which a new cell wall will grow inward. Separation of the bud from the parent leaves a scar. When chains of yeast cells do not fully separate it can create a pseudomycelium.

### Procedure

1. Make a wet mount slide with 1 or 2 drops of the yeast culture.
2. Examine the yeast cells under high power magnification. Look for chains of cells of varying size. These are most likely budding yeast cells. Try to identify the *cell wall*, *cytoplasm*, *nucleus*, and *starch granules*.
3. In Observations, make a drawing of budding yeast cells. Use the above terms to label your yeast cell drawing.
4. Before cleaning up your wet mount slide, measure a single yeast cell's diameter. Record your measurement here. \_\_\_\_\_  $\mu\text{m}$
5. Answer questions 1 and 2 in Observations about yeast.

### Part B Bread Mold

Fungi provide us with food and return important nutrients to the environment for future use. But when fungi appear on our food as mold, they are definitely not welcome!

One of the most commonly purchased food items in a grocery store is bread. Bread has a relatively short shelf-life (the time it takes for food to spoil). And bread is often spoiled by the presence of mold. Food producers have developed chemical additives that help to extend the shelf-life of many foods. For example, to extend the shelf-life of bread, producers often add calcium propionate to bread to prevent the growth of mold.

### Procedure

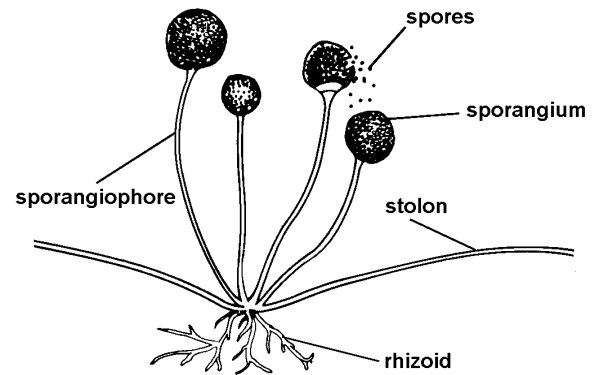
1. Obtain a plastic Petri dish from your instructor and place your name and period number on the lid.
2. Gently wipe a 1/4 slice of bread over the table top or floor so that it picks up some dust. Do not crush the bread. Place the bread in the plastic Petri dish. What is the brand name of the bread that you are using for this experiment?
2. Add a little water to the bread to keep it from drying out. Try not to overdo this process for too much water may be harmful to the bread mold.
3. Close the Petri dish and place it in a warm location like the top of your refrigerator. Observe the bread daily for two weeks. Record your observations in Data Table 1. At the end of the two weeks bring your mold growth to class and report your observations. Complete Data Table 2 and answer Questions 3 - 5 in Observations.

One well-known member of the kingdom Fungi is the common bread mold *Rhizopus nigricans*. *Rhizopus* is a heterotrophic organism made up of many multinucleated, branching filaments called hyphae. The bread mold digests and absorbs carbohydrates from bread by growing hyphae into the bread. The digestive enzymes secreted by the hyphae produce the characteristic "moldy" odor.

The hyphae of the mold form a tangled mat called a mycelium. The hyphae that extend along the surface of the food are called stolons. The rhizoids are hyphae that grow down into the food. They give off the digestive enzymes and then absorb the digested materials.

Other hyphae, called sporangiophores, grow up into the air from the mycelium. The sporangiophores bear the fruiting bodies, the sporangia, on the top of the stalks. Spores are produced in the sporangia and released into the air. See Figure 2. Each spore can produce new hyphae under the proper conditions.

**Figure 2**



## Procedure

1. With a dissecting microscope, examine some bread mold. Locate the stolons extending across the surface of the bread.
2. With two dissecting needles, tease the bread apart a bit. Then examine it with the dissecting microscope at various powers. Note the rhizoids extending down into the bread.
3. *Optional.* With a forceps, transfer some of the mycelium, including some sporangiophores, to a clean slide. Make a wet mount slide by adding a drop of water and then placing a cover slip over the specimen.
4. Examine a prepared slide under low power. Sketch some hyphae and a few sporangia in different stages of development. Make your drawing in Observations entitled *Asexual Reproduction*.

In bread mold, the haploid hyphae of two opposite genetic strains designated as a + and - will undergo a process called conjugation. The two hyphae will form gametangia. The two gametangia will fuse, forming a zygosporangium, which becomes a resistant zygospore. When this zygosporangium germinates, it quickly undergoes meiosis, generating new haploid hyphae and asexual sporangiospores. These sporangiospores may then be distributed and germinate into new genetically identical individuals, each producing their own haploid hyphae.

5. Obtain a prepared slide showing conjugation in bread mold. Examine it under low power. Look for different stages of sexual reproduction. Make two drawings - one should represent the early stage of conjugation and the other should represent the mature zygosporangium. Place your drawings under the area called Observations.

## Part C Basidiomycota - Introduction to the Common Mushroom

The basidiomycetes have a septate mycelium which often bears specialized structures. The spores are borne externally on a specialized cell, the basidium (basidia, plural). Each basidiospore is produced at the tip of a delicate projection of the basidium, the sterigma (sterigmata, plural). Typically, four basidiospores are borne on each basidium. The common mushroom of the grocery store is a basidiomycete, and numerous other members of this group are edible. Some mushrooms are extremely poisonous. Unknown or doubtful forms of these mushrooms should not be eaten. Many destructive plant diseases are included in this group of fungi. Some of these are corn smut, wheat rust, wood rots, and potato scab. There are many interesting basidiomycetes, but we will only study a few of them.

## Procedure

1. Examine basidiocarp of the common cultivated mushroom. Look at the umbrella-shaped **cap** bearing on its lower surface the **gills**. The whole structure is raised above the ground by a stalk, or **stipe**. Note the ring, or **annulus**, on the stipe. Label the appropriate diagram of a common mushroom in Observations. Use the above bold terms for your labels.
2. Very carefully cut a mushroom lengthwise into equal halves, so as to split the stipe in the exact center, or use a specimen that has already been cut for you by your instructor. In Observations, label the sketch of the longitudinal section showing the characteristics of the typical mushroom. Shade the gills lightly with a pencil.
3. Study Figure 3 which shows the basic make-up of a basidiocarp (mushroom). Note the central tissue of the gill. On each side of this tissue will be the cells that produce the basidia and basidiospores. In Observations, label the drawing showing basidia in various stages of development. Use the following terms for labeling the diagram: **basidium**, **sterigma**, and **basidiospore**.

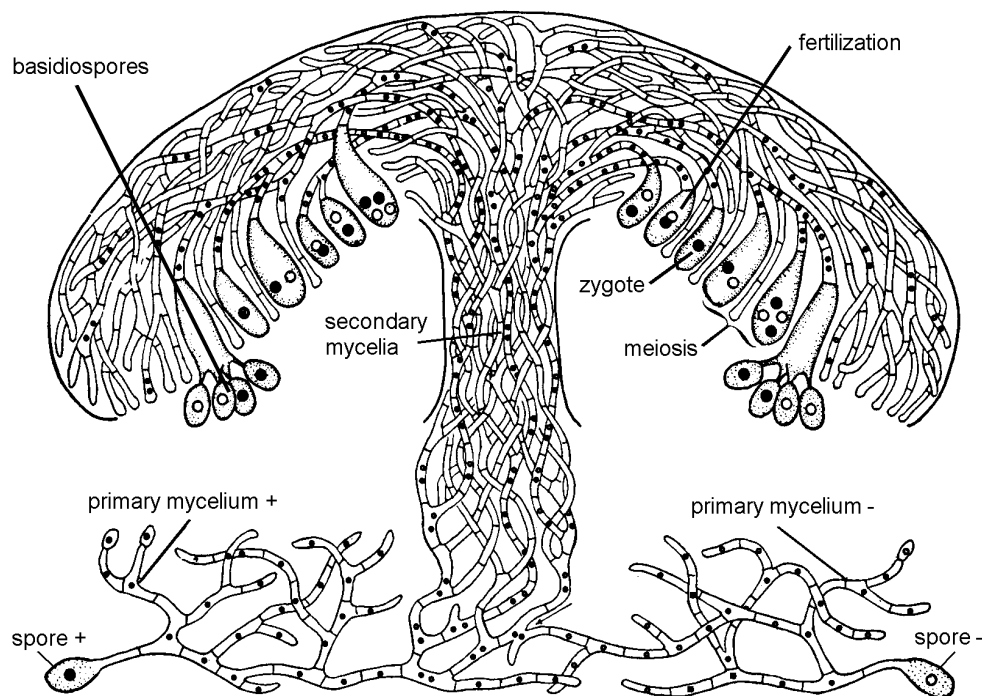
Study the diagram below to better understand the composition of the hyphae that form the basidiocarp. Shown in the diagram are the fusion of monokaryotic hyphal strands (primary mycelium) to form dikaryotic hyphae (secondary mycelium), fertilization, meiosis, and the formation of basidiospores. See Figure 3.

On the left of the diagram (+) nuclei of spore and hyphae are represented by black dots (•). On the right side minus (-) nuclei and hyphae are represented by small circles (o). Early stages of development are shown in the lower portion of the diagram; later stages in the upper portion.

The mycelium resulting from the development of either a (-) or a (+) spore is called a primary mycelium. When a monokaryotic (+) hypha fuses with a minus (-) hypha, the resulting dikaryotic hyphal mass is called secondary mycelium. Cells of the secondary mycelium contain paired but not fused (+) and (-) nuclei. The entire fruiting body (basidiocarp) of a mushroom is composed entirely of secondary mycelium.

At the surface of the mushroom gills, many of the hyphal tips (containing paired (+) and (-) nuclei) enlarge to become basidia. The (+) and (-) nuclei in a young basidium fuse to form a zygote (2n). This 2n nucleus divides meiotically, resulting in four haploid (n) nuclei; two containing (+) nuclei and two containing (-) nuclei. Each nucleus migrates into one of the four small protuberances, the sterigmata, developed on the basidium tip where they develop into basidiospores. Basidiospores fall from the basidia when mature.

**Figure 3**



4. Answer Questions 6 - 9 in Observations.

## Part D Ascomycota - Introduction to the Sac Fungus

The ascomycetes are characterized by a septate mycelium and the formation of spores (ascospores) in a specialized cell called the ascus (asci, plural). The ascus is ordinarily a sac-like structure, usually containing eight ascospores. Yeast is an ascomycete important in baking and in the production of alcohol. Numerous plant diseases are caused by ascomycetes, including fruit rots, mildews, cankers, leaf spots, blights, and Dutch elm disease. Ergot of rye is the source of useful and powerful drugs. We will take a closer look at some ascomycetes called *Peziza* and or *Sordaria* to demonstrate certain typical ascomycete characteristics.

### Procedure

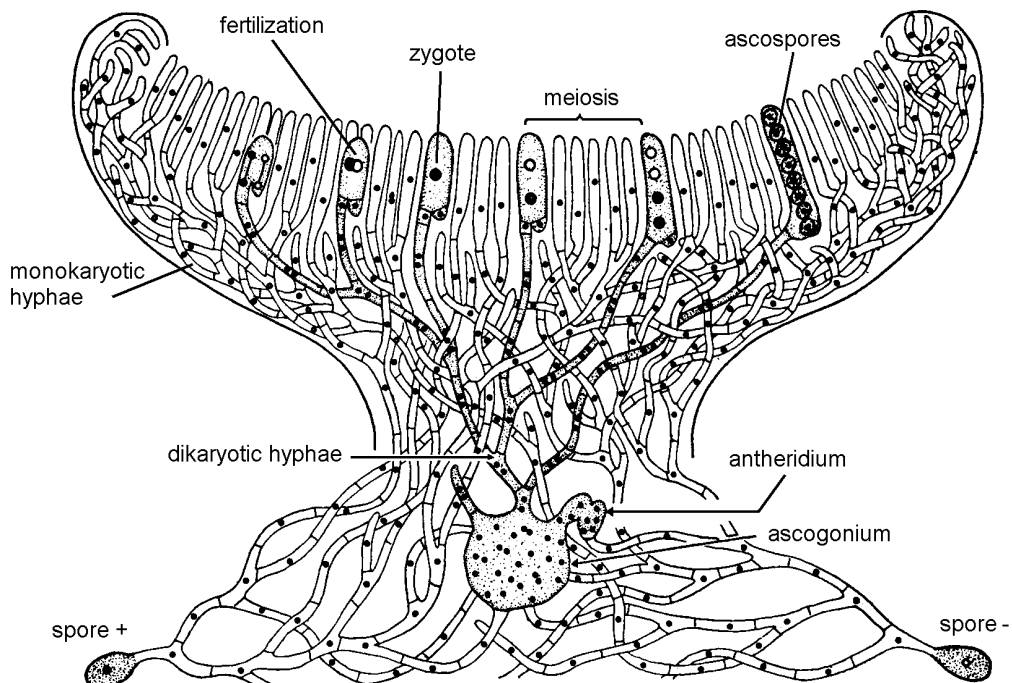
1. Examine the fruiting body of a cup fungus. It is called an **ascocarp** and resembles the appearance of cup or dish with the edges of the ascocarp slightly turned upward. The reproductive sacs, or **asci**, are located on the surface of the cup.
2. Add labels to the sketch of *Peziza* fruiting structures in Observations. Use the bold terms above for your labels.
3. In Observations, label the longitudinal section of an ascocarp. Use the same terms as above.
4. In Observations, label the drawing of the asci. Use the following terms: **ascus**, **ascospore**, and **hyphae**.

Refer to Figure 4 below to better understand the composition of the hyphae that form the ascocarp. Shown in the diagram are the sexual gametangia (ascogonium and antheridium) dikaryotic cells, fertilization, meiosis, and ascospore formation.

On the left side of the diagram plus (+) nuclei in spore and hyphae are represented by black dots (•). On the right side minus (-) nuclei of the spore and hyphae are represented by small circles (o). Early stages of development are shown in the lower portion of the diagram; later stages in the upper portion.

A hypha from the (-) spore (male) forms an antheridium containing (-) nuclei. A hypha from the (+) spore (female) forms an ascogonium containing (+) nuclei. The smaller antheridium becomes attached to the larger ascogonium and a number of (-) male nuclei pass from it into the ascogonium. In the resulting mixture (+) and (-) nuclei become paired but do not fuse in the ascogonium. Dikaryotic hyphae contain paired (+) and (-) nuclei in their cells grow upward. Further events within the hyphal tip result in fusion of a pair of (+) and (-) nuclei to produce a zygote. This  $2n$  nucleus of the zygote divides meiotically, resulting in four haploid ( $n$ ) nuclei in an elongated ascus sac. The four nuclei then divide mitotically forming eight ascospores enclosed in a mature ascus. When an ascus ruptures the ascospores are released. A mature ascocarp is composed of both dikaryotic hyphae entering into ascospore formation and monokaryotic hyphae containing either (+) or (-) nuclei, but not both.

Figure 4



## Observations

Yeast - Budding

1. Did you find individual yeast cells to be more common than budding yeast cells?
2. When you observed yeast cells, did you reduce the amount of light by using the iris diaphragm?
3. Calcium propionate is an additive put in bread to prevent mold growth. Did your bread contain a chemical for maintaining “freshness”?
4. Bread mold can appear in many colors depending on the type of species of mold. What color of mold seems to be the most common?
5. Why were the Petri dishes placed in a warm location?
6. Why is a mushroom called a gill fungus?
7. Why are some mushrooms called toadstools?
8. What is the texture of the inside of the stipe?
9. What type of hyphae form the basidiocarp? (septate or coenocytic) (monokaryotic or dikaryotic)

**Data Table 1 - Your Bread Mold**

Date	Observation of Growth
Day 1 -	
Day 7 -	
Day 14 -	

**Data Table 2 - Class Data**

Brand	Date When Mold Appeared	Natural or Preservatives

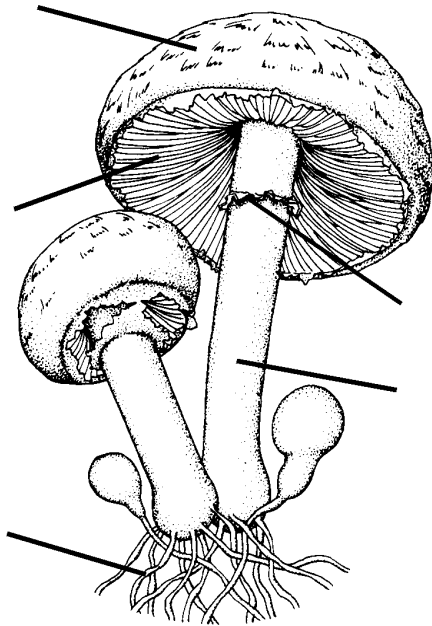
Asexual Reproduction - Sporangia in Bread Mold

Early Conjugation in Bread Mold

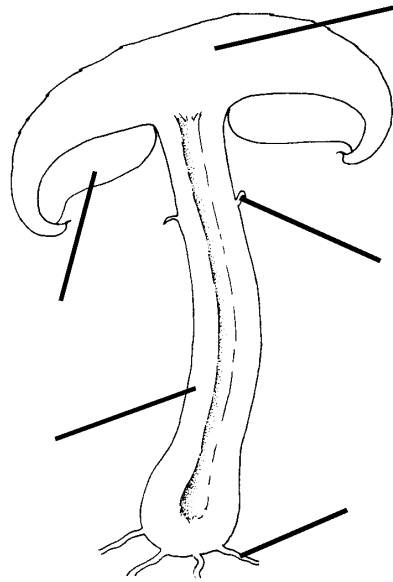
Mature Zygosporangium in Bread Mold



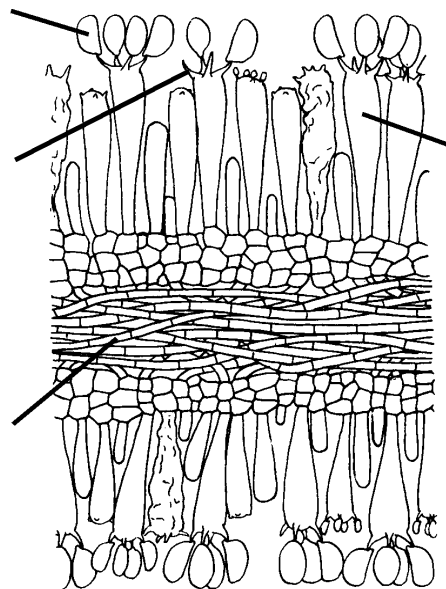
Common Mushroom



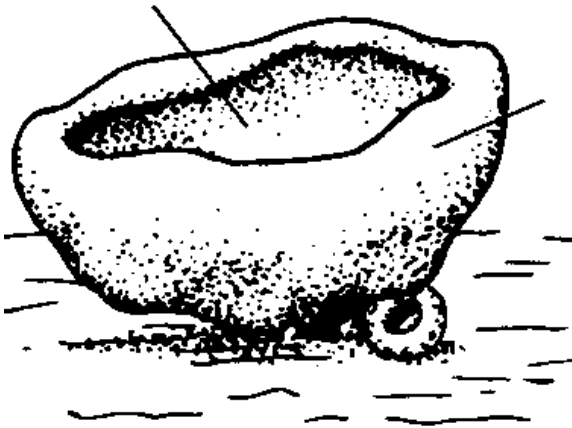
Common Mushroom - Longitudinal Section



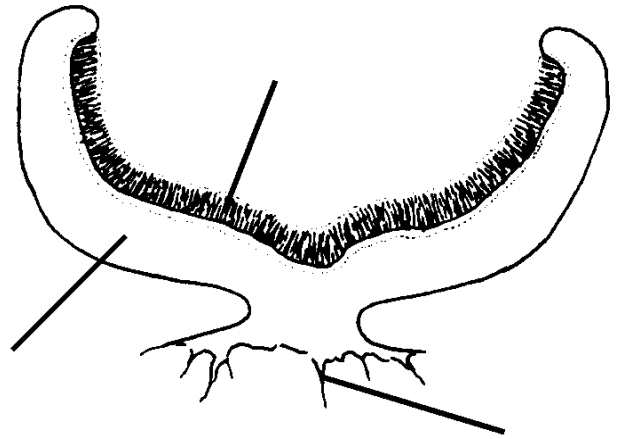
Basidia of a Mushroom



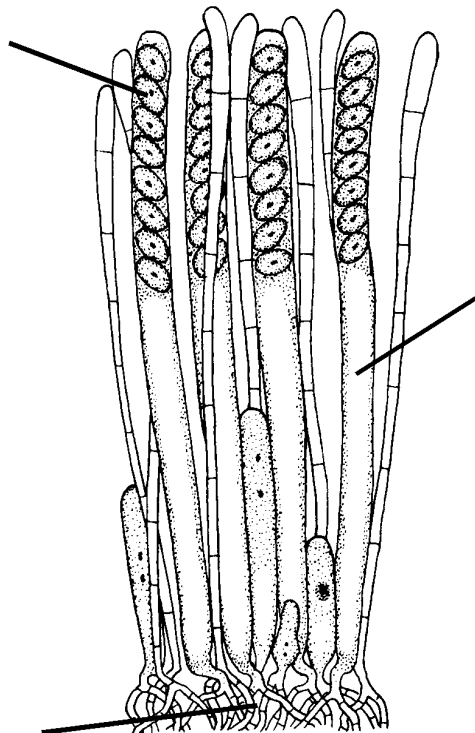
Peziza Fruiting Structure



Ascocarp Longitudinal Section



Asci of a Cup Fungus



## **Analysis and Conclusions**

1. What indication of metabolic activity did you observe in the yeast culture ?
2. How is budding similar to binary fission?
3. Name the two products that yeast produce when they enter into a metabolic pathway called fermentation.
4. What conditions favor the growth of mold?
5. Would you expect homemade breads or commercially produced breads to have a longer shelf-life? Explain your answer.
6. What is the difference between dikaryotic and diploid cells?
7. Compare the number of spores in a basidium to that of an ascus.
8. How do the conidiospores, sporangiospores, ascospores, and basidiospores differ? How are they similar?

## **Critical Thinking and Application**

1. Many dehydrated foods do not contain chemical additives. Would you expect mold to grow on dehydrated foods? Explain your answer.
2. Some molds that grow on bread are green. Can these green molds produce their own food through photosynthesis? Explain your answer.