

Mold—A Zygomycete

10.11.17

Zygomycetes (600 species) include common bread molds and derive their name from characteristic resting spores called zygospores. Most zygomycetes are saprophytic (they decompose dead material), and their vegetative hyphae lack septa (i. e., they are aseptate).

If you leave a piece of bread or other bakery product covered and at room temperature for a while, a fuzzy gray or black mold will appear. This fungus, *Rhizopus stolonifer*, a common bread mold, is representative of the zygomycetes, members of the division (phylum) Zygomycota. This group of fungi is characterized by the formation of zygospores--special resting structures composed of a zygote surrounded by a thick protective wall. The zygospores germinate to form a fruiting structure, the sporangium, which produces spores by meiosis. Accumulations of these dark-colored sporangia give *Rhizopus* its gray-black color.

In this activity, you will observe the structure of this species to see many general fungi characteristics. Fungi are made up of threadlike individual filaments, called hyphae, which are organized into the body of the fungus, called the mycelium. This filamentous mass secretes enzymes into the substrate and digests food that will then be absorbed into its cells. Cells of fungi have cell walls made of chitin combined with other complex carbohydrates, including cellulose. You may recall that chitin is the main component of insect exoskeletons.

Most zygomycetes obtain carbon from decomposing dead material. The term that describes these types of organisms is: _____

Tell what it means when hyphae lack septa: _____

Give the scientific name for black bread mold: _____

Give the division name for the zygomycetes: _____ (Note: Division and phylum are the same for purposes of taxonomy. The term division has historically been used for plant-like organisms and phylum for animal-like organisms.)

Name two places in nature chitin is found: 1) _____ 2) _____

Culture preparation: Obtain a slice of bread and a non-sterile petri dish for each student in your group. Use the BOTTOM of the petri dish as a cookie-cutter to make a bread cookie. Remove the cookie from the petri dish, dispose of the extra bread.

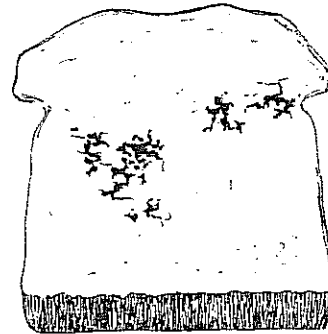
Find a “dusty spot” and use your bread cookie as a sponge to wipe the area. You don’t need to go overboard on this since any contact with the dusty environment will guarantee an ample supply of mold spores.

Return you bread cookie, contaminated side UP, to the petri dish. Sprinkle a SMALL amount of water on the surface of the bread and close the dish. Label the dish with your name, today’s date, and the substrate (bread).

Store the petri dish cover side UP as directed by your instructor. Plan to wait several days before seeing any results.

Locate a mass of **Black Bread Mold** on your slice of bread—use only the white hyphae and the black sporangia for your observaions....(Don't use blue, yellow, etc.)

Examine the mold with your dissecting microscope. Note the whitish mass of filaments growing over the surface of the bread. Each filament is called a **hypha** (plural **hyphae**). The total mass of hyphae is a **mycelium**. Some hyphae grow upward and form small, black, globelike structures called **sporangia**. Inside the sporangia are cells called spores. They are released when the sporangia open.

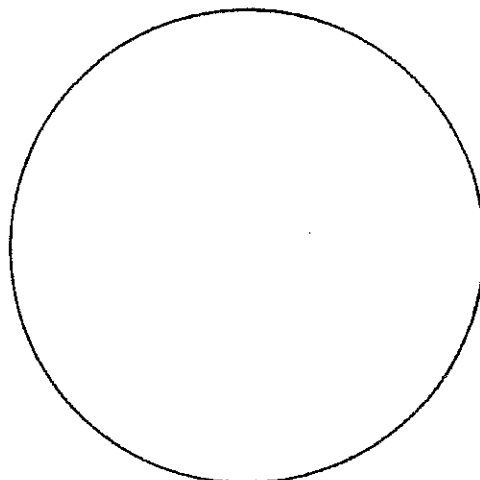
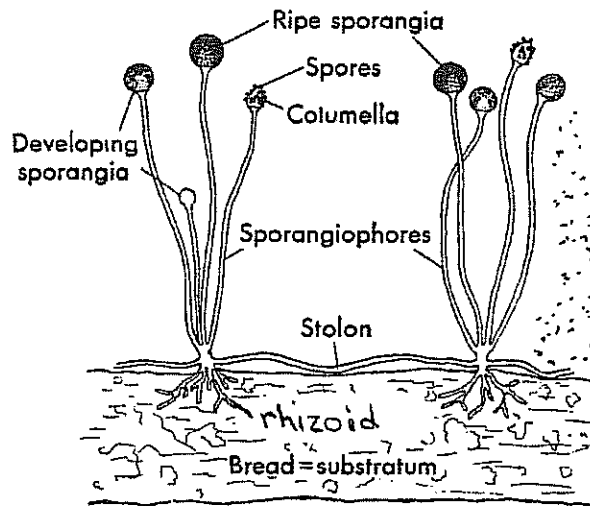
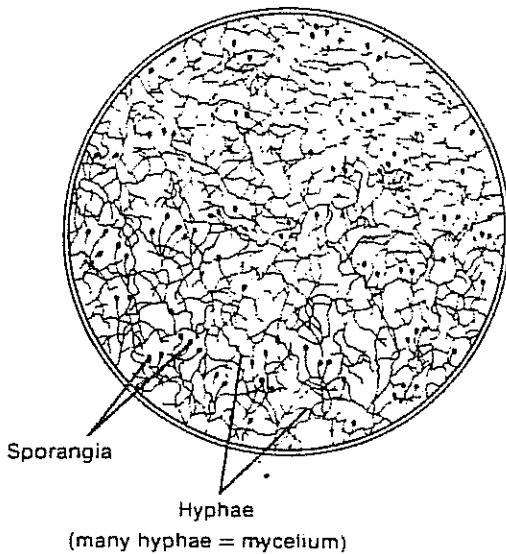


What is the function of the spores? _____

Other specialized hyphae penetrate the agar. Turn the dish over, and focus downward through the bread to locate small, rootlike hyphae called **rhizoids** growing into the bread.

Suggest a function for the rhizoids: _____

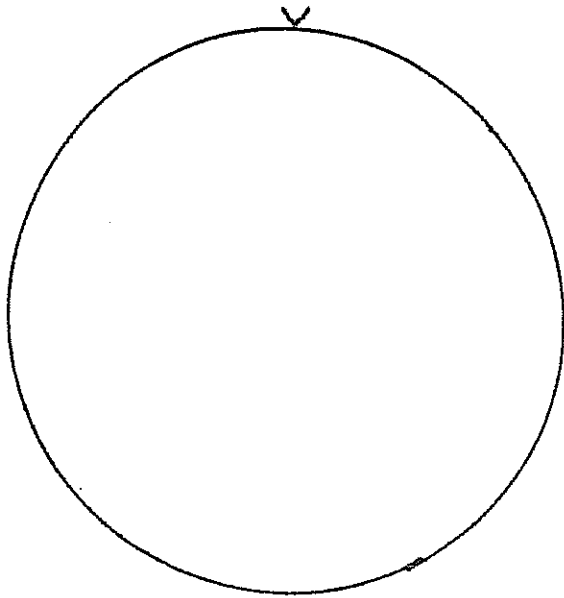
Examine the specimen with the stereoscope (dissecting microscope). In the space below draw what you see. Label the sporangia, spores, sporangiophores, stolons, and rhizoids.



Magnification _____

Verified _____

Observations: Make an index mark on the side (mark the bottom, not the top) of your petri dish. Observe growth daily as directed by your instructor. Draw the succession of organisms. Use color as needed. Use the key to identify some of the more common species of mold. Remember, not everything on your bread is *Rhizopus*.



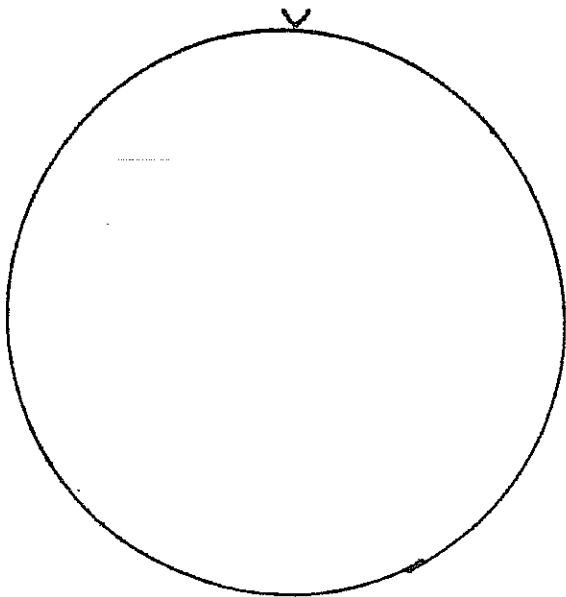
Culture Age _____

1 Growth mainly black, white, and/or gray	2
Hyphae yellow or blue-green to gray	4

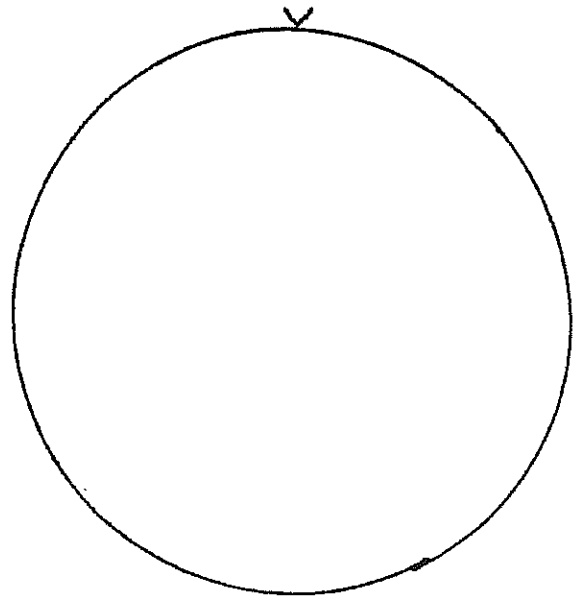
2 Numerous black sporangia; hyphae not readily apparent	Aspergillus flavus
Hyphae white to gray	3

3 Hyphae beige-white; sporangia inconspicuous	Mucor sp.
Hyphae gray to white with conspicuous sporangia	Rhizopus sp.

4 Hyphae yellow; numerous dark-colored sporangia	Aspergillus niger
Hyphae blue-green to gray; compact; few if any sporangia	Penicillium sp.



Culture Age _____

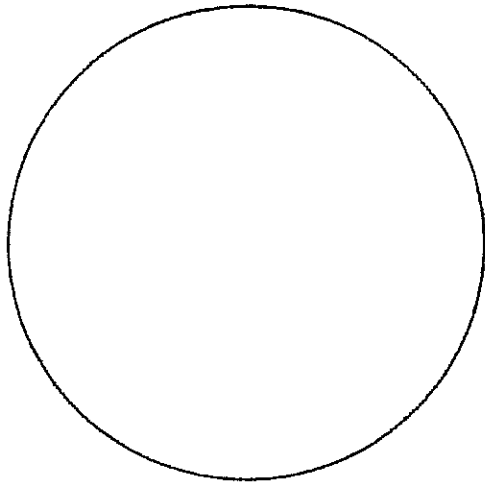


Culture Age _____

Smudge a SMALL amount of detergent (wetting agent) on a slide. The best way to do this is to touch your finger to the end of a soap dispenser and make a light smear on the slide. Add a drop of water. (The spores are so light they float on the surface of pure water and do not get wet. Wetting agents break the surface tension of the water enabling the spores to sink beneath the surface.)

Remove a few of the sporangia (go for the black areas of the dish). Place them in the drop of wetting agent. Add a cover slip and view under the compound microscope.

Observe the spores under high power. Draw several spores. Estimate their size and color.



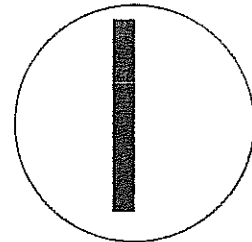
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Size _____ (Show your calculations in the space to the right.)

Verified _____



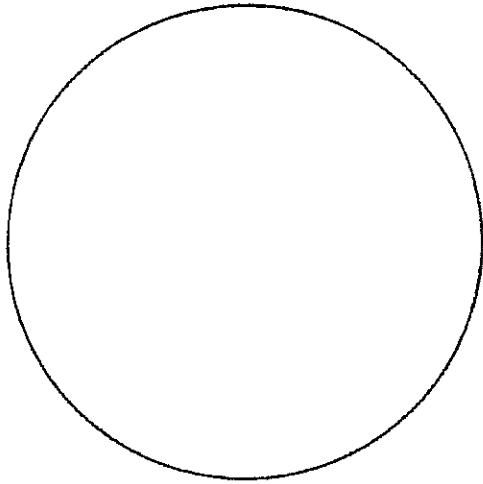
Obtain a Petri dish of potato-dextrose agar and a cotton-tipped applicator. Dip the applicator in water and “sling it” to remove any excess—the applicator should be damp. Once again, find an area of your bread-mold plate with *Rhizopus* (look for a black area) and dip the tip of the applicator in the culture. Open the PDA plate and make a single streak over the plate as if you want to draw a line that divides the dish into halves—make only ONE pass and rub lightly so you won’t gouge the agar.



INVERT the dish and place it in the incubator overnight.

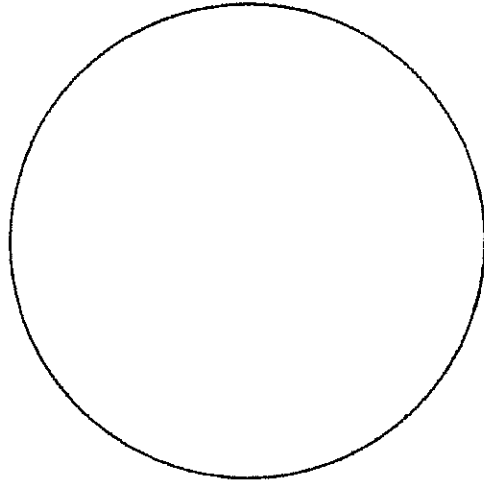
Examine the PDA with a stereoscope over a multi-day period. Draw what you see each day on the next page. You will make TWO drawings each day—one of the Petri dish (a gross observation of the total amount of growth) and one of the magnified image observed through the stereoscope. Label your drawings appropriately.

Gross Observations

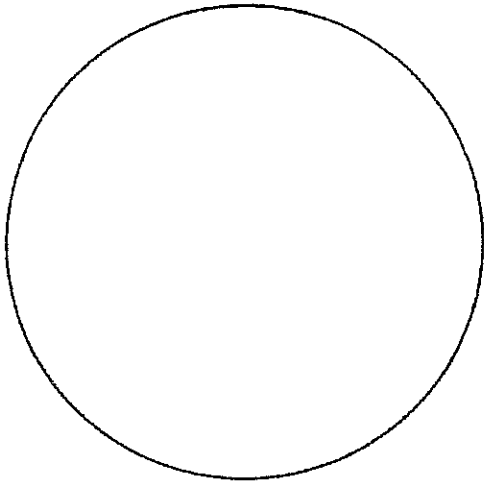


Date _____

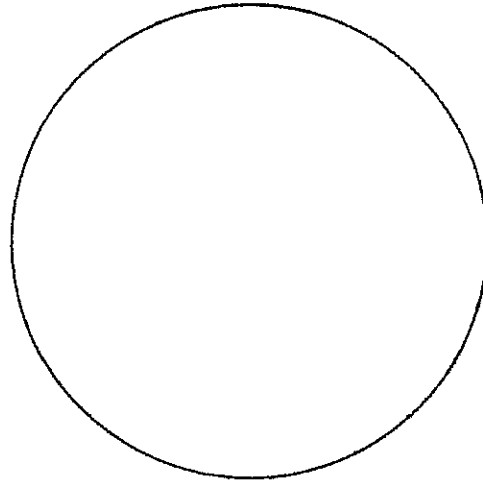
Stereoscope View



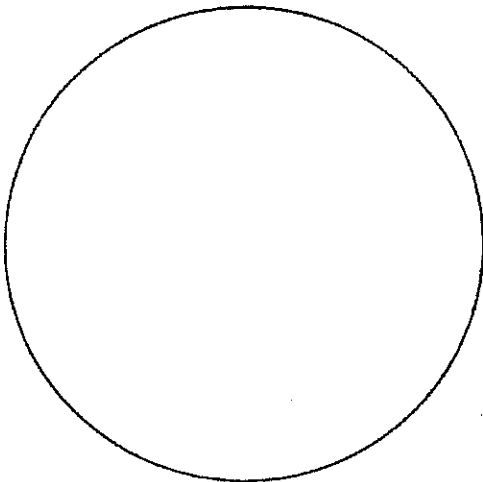
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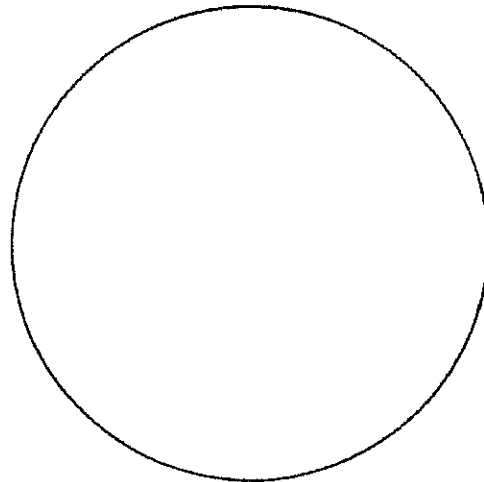
Date _____



Magnification _____



Date _____



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Environmental effects and mold growth....

Obtain one slice of bread and four “zip-loc” bags for your group.

Label the bags with your initials, today’s date, and one each of the treatments below (use the numbers 1-4 for your labels).

“Contaminate” the slice of bread with mold spores by gently rubbing it on the floor—yes, there are plenty of mold spores available in the room by now!

Use a pair of scissors to quarter the slice of bread—make each “corner” as nearly equal in size as you can. Place a quarter slice in the four sandwich bags.

You will apply one of the following treatments to each bag. (Unless directed, leave enough air in the bag so the specimen will not be crushed.) When ready, place your zip-locs in the designated trays.

1. Bright light, room temperature.
2. No light, room temperature.
3. No air—Leave a corner of the bag unsealed. Squeeze out all the air. Use your mouth to draw out the remaining air and seal the bag.
4. Warmer temperature than room temperature.

After a given amount of time for incubation....

Without opening the closed bags, examine the slices of bread. To determine the effects of certain environmental conditions on the growth of the bread mold, each piece of bread was treated in a different manner. In the chart that follows, indicate the four conditions and state the results. Also rank the treatments from most to least growth.

Condition tested...	Description of growth...	Rank
1.		
2.		
3.		
4.		

Based on your data, what conditions favor the growth of mold? _____
