

FUNGAL REPRODUCTION LAB SEXUAL REPRODUCTION IN A ZYGOMYCETE

Introduction

The Fungal kingdom contains the simplest multicellular organisms. The body of a fungus is characteristically made up of threadlike filaments that show little specialization and are similar in many species. An individual fungal filament is called a hypha; the entire mass of hyphae of a single fungus is called a mycelium. The hyphae of a fungus may be septate, because they form cross walls (septa) between nuclei, or they may be coenocytic and lack such cross walls. Fungi exist predominantly in the haploid state and do not produce multicellular diploid forms.

Most fungi reproduce asexually and sexually; both processes usually involve the production of hyphae that form specialized structures. Sexually reproducing fungi produce specialized hyphae that are of two different mating strains. Because the two strains cannot be distinguished visually, they are designated simply as either "+" or "-." Differences in the patterns and structures involved in sexual reproduction are the main criteria used to separate the fungi into four Divisions.

The genus *Phycomyces* is characteristic of the Division Zygomycota. It reproduces asexually through the formation of sporangia born on top of sporangiophores. Disintegration of a sporangium results in the release of spores, which can then germinate and form new mycelia. The hyphae typically do not form septae. *Phycomyces* reproduces sexually through the production of "+" and "-" strains of hyphae. Septae are formed near the tips of the hyphae, and the cells formed by these cross walls are called gametangia. When hyphae of two different mating strains meet, their gametangia fuse; "+" and "-" nuclei then fuse to form zygotes. The now single, fused cell containing numerous zygotes enlarges and forms a hard, irregular wall around itself. This structure is called the zygosporangium and it gives the division its name. Following a period of dormancy, the zygotes in the germinating zygosporangium undergo meiosis. The sporangium that germinates releases both "+" and "-" haploid spores into the air. Each of these can then form new mycelia.

Objectives

- A. To observe sexual reproduction in a fungus.
- B. To observe nonreproductive fungal hyphae.
- C. To learn sterile techniques for culturing organisms in the laboratory.

Principles

Cultured "+" and "-" strains of *Phycomyces blakesleeanus* will be provided; these will have been grown on potato dextrose agar. You will place samples of both strains near each other in a fresh plate of the same kind of agar, using sterile technique. You will examine your plate one week later, at which time you will identify and examine zygosporangia as well as nonreproductive hyphae.

Procedures

A. Before you begin this exercise, make certain that you have all of the following materials readily available:

Culture plates of "+" and "-" strains of *Phycomyces blakesleeanus*, grown on potato dextrose agar
A culture plate containing potato dextrose agar
Small forceps
Sterile chamber, containing:
 Gas burner
 Striker
 Ethanol (70%), in a wash bottle
Black Sharpie pen
Dissecting microscope

B. Prepare a culture plate of *Phycomyces blakesleeanus* containing different mating strains

1. Turn on the lights in the sterile chamber. Wash your hands thoroughly. Squirt some ethanol on the work surface of the sterile chamber; wipe with a paper towel. Discard the paper towel.
2. Obtain a fresh culture plate containing potato dextrose agar. Using the Sharpie pen, write your name, lab number and time, date, and "*Phycomyces* '+' and '-' on the lid of the plate. Place the culture plates of "+" and "-" strains of *Phycomyces blakesleeanus* and the fresh culture plate in the sterile chamber.
3. Flame sterilize the forceps. Remove the lid from the *Phycomyces* "+" plate and, keeping the lid in one hand, remove a piece of the hyphae. Replace the lid on the *Phycomyces* "+" plate.
4. Remove the lid from the fresh culture plate and keep the lid in one hand. Transfer the hyphae to the fresh culture plate. Replace the lid on the plate.
5. Repeat steps #3 and 4 above, removing a small piece of the hyphae from the *Phycomyces* "-" plate. The two pieces of hyphae should be about 2 cm apart.

6. Remove your culture plate from the sterile chamber and place it on a shelf designated by your lab instructor/assistant instructor for culture of the fungi.

B. Make observations of culture plate one week later

1. At your next laboratory meeting, obtain your culture plate of *Phycomyces blakesleeanus* "+" and "-" strains.
2. Remove the lid from the plate and place the plate under a dissecting microscope. Observe the fungal structures present on your plate at different magnifications. Refer to the illustration at the end of this exercise, depicting structures of the Zygomycete *Rhizopus stolonifer*, to help you identify the structures you see.
3. Make drawings of the structures you observe on your plate and label your drawings. Be sure to include a drawing of nonreproductive hyphae, including some growing tips.
4. Return your culture plate to the culture shelf or discard it, as instructed by your teaching assistant / assistant instructor.

Results and analysis

1. Your labeled drawings are to be turned in to your teaching assistant / assistant instructor as part of the laboratory report for this exercise.
2. Fungi, like animals, are heterotrophic. Yet, like plants, they are nonmotile. How do fungi obtain food and water and supply all the cells of their bodies with these substances?
3. Under what kinds of conditions would you predict that a fungus would begin to produce specialized hyphae and undergo sexual reproduction? How might sexual reproduction provide an advantage over asexual reproduction for the survival of a species?

Text reference pages (*Biology*, fifth edition, 1989, by Curtis and Barnes)

Chapter 23: The Fungi (pp. 479-492)

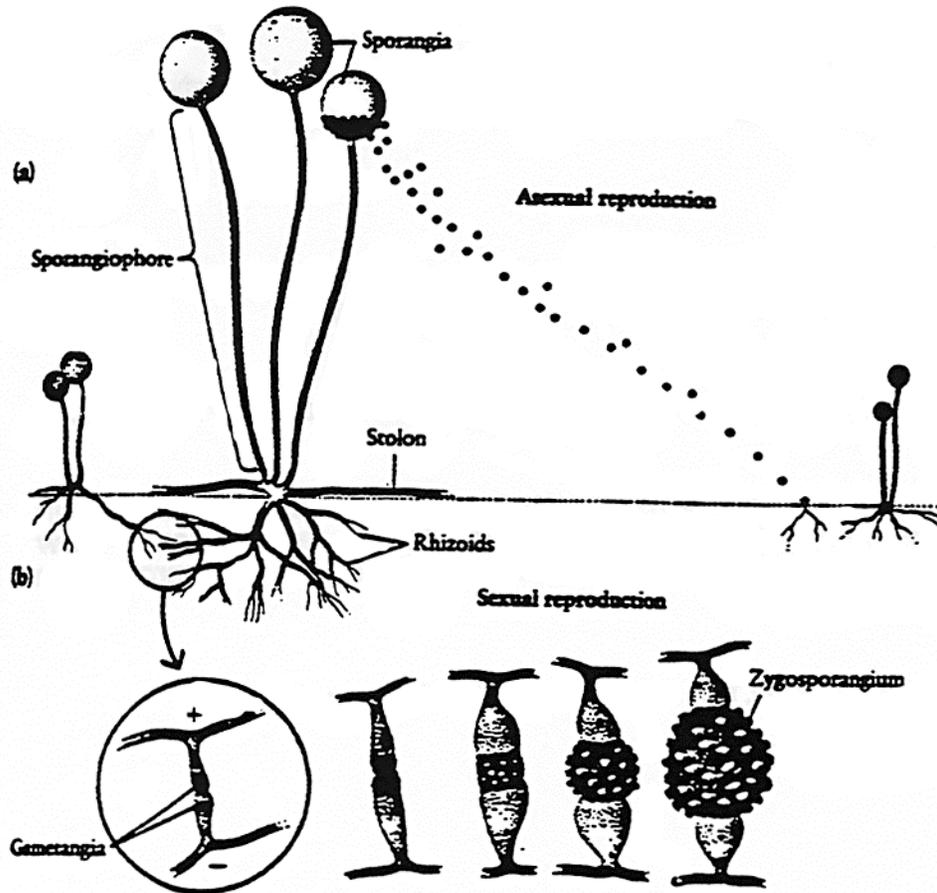


Figure 1. Reproductive structures of the Zygomycete *Rhizopus stolonifer*

Asexual and sexual reproduction in bead mold, *Rhizopus stolonifer*, illustrated in figure 1 is similar to that in *Phycomyces*. Most easily seen are the branched hyphae that make up the mycelium. This consists of rhizoids that grow into the media and anchor the fungus and stolons that are runners across the media. Sporangiophores rise above the media and terminate in round structures called sporangia. When mature, the walls of the sporangium breaks down and releases haploid spores. The spores are dispersed in the air currents to new habitats. Spores that land on a suitable habitat that is warm and moist germinate into a new population of hyphae.

When hyphae of two different strains meet, sexual reproduction may occur. Meeting hyphae come together and form gametangia. The gametangia fuse and develop into a diploid structure, zygosporangium that is resistant to adverse environmental conditions. The zygosporangium contains zygotes. Although most of the structures of *Phycomyces* resemble those of *Rhizopus*, the spiky zygosporangium of *Phycomyces* looks very different from that of *Rhizopus*. The zygosporangium remains dormant for a while and then the zygotes undergo meiosis, germinate a new haploid sporangium, and new haploid spores are released.