Frugal Fun With Fungal Cultures

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Abstract: A home kitchen can serve as a stock room to provide the supplies and equipment needed to culture fungi for classroom use. Some alternative media and cultural techniques are provided along with two alternative classroom investigations that can be employed by classes from elementary through college levels.

Keywords: fungi, growth rate, mycology, sterile culture, mold

Introduction
FUNgi are FUN to work with and can be an inexpensive alternative to other organisms in studying a variety of life processes. The emphasis of this paper is to demonstrate a number of inexpensive alternatives to the usual laboratory procedures used to culture various molds. Although the protocols used in mycological research can be quite complex and the costs involved may be prohibitive for use in most K-12 classrooms, there are many simple investigations that can be fun, informative and inexpensive.

Alternative Materials and Media for Fungi.
Sterile technique and basic microbiological procedures are generally employed when working with living cultures of plants and fungi. However, certain of the procedures may be eliminated and substitutes may be used in place of expensive media. For instance, the usual method of sterilization is to autoclave liquid media for 15 min. at 15 lb. pressure. One alternative to this is to use a kitchen pressure cooker, but even this are becoming rare in many households. A more commonly available alternative is the microwave oven; two minutes on high should be sufficient to "sterilize" media for routine use. When using the microwave, it is important to use a container of more than twice the volume being treated. For instance, to sterilize 250 ml of media, I use at least a 1 liter flask or beaker.

Although typically one covers the end of the container with a cap or foil cover while it is being sterilized, this is not necessary in a microwave if you are able to immediately pour your plates. If a cover is necessary, invert a glass beaker over the neck of the flask or cover it loosely with plastic wrap; do not seal it as the gas inside will expand greatly and cause sealed plastic wrap to burst.

In research labs, inoculations and transfers are performed in a transfer cabinet or laminar flow hood ($400.00-$5,000.00+). However, for routine classroom use, you can do such procedures on an open bench top, providing air movement in the room is kept to a minimum. Caution students not to move around in the room as classmates are working. They should also avoid all sudden movements around their work area while they are making sterile transfers. Alternatively, you can construct a homemade transfer cabinet from a cardboard box, aluminum foil and clear plastic wrap. Use foil to provide a washable lining that can be sprayed with a 10% commercial bleach solution for disinfection. Clear plastic wrap, e.g., Saran Wrap™, is essential sterile as it is pulled off the roll; use it to provide a sterile "window." You can also use Saran Wrap™ in place of Parafilm™ to seal petri dishes and other containers.
The most commonly used media for culturing fungi include: Potato Dextrose Agar ($60.00/500g), Corn Meal Agar ($140.00/500g) and Rabbit Dung Agar ($144.50/100 plates). The cost of these products derives in large part from the cost of Agar itself ($88.00/500g). Agar has two main advantages over its main alternative -- gelatin. First, most microbes do not digest agar and second, agar is more heat stable than gelatin and thus remains solid in incubators above room temperature. However, for general use in growing fungi, these characteristics are relatively unimportant. Thus, commercial gelatin can be substituted for agar in most media for classroom use. The following are grocery store formulae for fungal growth media:

**Potato Medium**

Peel and dice about 100g of white potatoes and boil for 1 hr in 350 ml of distilled water. Strain the supernatant through cheesecloth (or through a coffee filter) and bring the fluid back to 350 ml with additional distilled water. Add 10 g gelatin to solidify. You may also want to add 1 g sugar as a substitute for potato dextrose agar. Straining is necessary only to produce a clear medium. A simpler technique is to blend the potatoes and water to produce a liquid slurry. As an alternative to using whole potatoes, you may want to try instant potato flakes. The "active nutrient" in the potatoes is starch, you may also substitute soluble starch that is processed potato starch and works well. You also can use commercial cornstarch. Add sugar or not. Sterilize by pressure cooking or microwaving.

**Cornmeal Medium**

This recipe is similar to the oatmeal medium. Blend 50 g of cornmeal in 800 ml distilled water and let set overnight in the refrigerator. Simmer for an hour, bring to 1 liter, add agar (gelatin) and sterilize (microwave). Again, filtering may be done to produce a clearer medium.

**Rabbit Dung Medium**

Presterilize rabbit pellets, then blend equal volumes of pellets and distilled water. Add enough agar (gelatin) to make a 1.5% solution and sterilize.

**Sterile Technique**

In order to maintain pure cultures of organisms, and to prevent contamination by microbes such as bacteria or other fungi, it is necessary to use special techniques and procedures, collectively called sterile technique. When using sterile technique, assume that all surfaces are contaminated, so if any of your sterile instruments or a piece of tissue touches a surface, you should assume that it was contaminated and must be resterilized or discarded. Similarly, sterilize the work space before beginning any procedure. Sterilization is the process of destroying all organisms. In the laboratory, sterilization is accomplished through the use of heat, chemicals, or both. Instruments, such as scalpels, forceps, needles, etc., are usually treated by passing them through a flame. Larger equipment may be sterilized by heating in an oven at 165-170 C (325-340 F) for 2-3 hours. Instruments, and especially prepared growth media, are usually sterilized by exposure to steam under 15 lb. pressure (over 100 C) for at least 15 minutes. Bench tops, work surfaces, instruments and the surface of living tissue are usually sterilized by exposure to a 10% solution of household bleach in water for 5 - 10 minutes. The outer cells of the tissue will be killed by such a treatment and must be removed with a sterile blade before trying to culture cells or organs sterilized in this way. Bench tops are usually sterilized by spraying bleach solution on them and then wiping with a paper towel to spread the bleach (not dry the table -- leave it wet). Similarly, transfer chambers can be sterilized by spraying all inner surfaces with bleach or alcohol solutions. If a flame will be used to heat sterilize instruments, surface sterilize your area with bleach, not alcohol, to reduce the potential for fire. Of course, bleach has the potential to spot dyed clothing, so students should work carefully and preferably wear some kind of smock or lab coat.

The air space above a sterile work surface is also considered sterile. As a result, anytime something passes over the sterile field, assume the work surface became contaminated. Never pass your hand over the work area and be sure to heat sterilize any portion of an instrument that will be passed over the sterile field. Also, keep hand movements and air currents to a
minimum to reduce the potential for airborne contamination.

Before beginning work, wash your hands and arms, leaving them moist so that any potential contaminants will stick to your skin, rather than falling off and contaminating your sterile field. If you have long hair, tie it back or wear a hat or net to keep it out of the way. Avoid unnecessary talking to minimize air movement around your work area.

Some Alternatives to the "Bread-in-a-Bag" Types of Investigations -- The Great Fungus Race

The challenge is for student groups to produce the fastest growing fungal culture in the class. Students can use any medium they like, usually based on adaptations of one of the media listed above. They may grow their fungi under any environmental conditions they choose. Typically different temperature and light regimes are used in addition to different culture media. However, if gelatin is substituted for agar in any of the formulae, the medium will become soluble at higher temperatures, so incubating at above room temperature may turn what was a solid medium into a liquid one. There is not necessarily anything wrong with this as far as growing fungi, but it may cause some surprises for the students and it has the potential to become messy if students are not careful in handling the liquified cultures!

The first challenge is to obtain a pure fungal culture. Fungi may be isolated from the environment in a number of ways. The simplest method is to add bits of soil, dung, decaying material or debris to the surface of a prepared medium. One technique is to push the inoculant along the surface of the plate. Within a day or two, filamentous mycelia should be observed growing out from several locations. At this point, choose a likely looking mold candidate, and use a sterile blade to cut off some of the tip of the growing hyphae, including the chunk of medium it is growing on, and transfer it to a fresh plate of medium. You should now have a pure culture of the chosen fungus.

If spores are already present on a piece of potential inoculant (e.g., some moldy food rescued from the home refrigerator), individual pure cultures can be started by the spore touch method. To do this, cut out a tiny chunk of sterile medium with a needle or blade tip and fix the chunk of medium on the tip by simply jabbing it. Now touch this chunk against the spores of your inoculant, then transfer it back to the sterile plate. The spores are the powdery, colored particles that form on the surface of growing molds. If you touched only a single type of spore, you will produce a pure culture of that species. Pure cultures of mushrooms and mushroom-like fungi can be started by sticking a small piece of fruiting body to the lid of a dish containing medium. Vaseline or a similar sticky paste can be used to fix the small piece of mushroom to the lid. After several minutes to several hours (depending on the species and the state of the specimen) spores will be shed onto the surface of the medium below.

Once students have chosen the fungus they want to race and isolated it in pure culture, they are ready to begin timing their competitor. To do this, they should inoculate the center of a plate of medium and record the time this was done. Once or twice a day for the next several days, measure the distance the fungal mycelium has grown from the original center of inoculation. The colony will be circular, so measure and average several radii to determine the average growth at that time. Record the time of measurement and the calculated average. When the culture nears the edge of its plate, make the final measurements and calculate the average rate in mm/hr. The best way is to calculate the least squares regression line for the data of distance and time. The slope of the line is the rate of growth. A crude approximation can be made from the final distance divided by the total elapsed time. The student group whose fungus had the fastest time (greatest rate) wins the contest.

The type of fungal isolate and any of the environmental variables can affect the growth rate obtained, sometimes in unexpected ways. For instance, incubating at high temperature, such as 37°C as typically used for culturing bacteria, will produce slower growth in most fungi (remember that if your
students are using a gelatin based medium, it will be a liquid at that temperature!)

**Nutrient Effects on Colony Morphology**

In addition to growth rate, environmental conditions, especially available nutrients, can induce considerable variations in colony morphology. This includes not only the texture, but also the color, size and shape of the colony. A challenge along these lines is to have a contest to see which student group can produce the largest number of different looking colonies from the same inoculant. A key analysis question here is how can they determine that two very different looking cultures at the end of their experiment are actually of the same fungus, rather than of the initial isolate and some kind of contaminant?

**Some Important Rules for Safety.**

Although the vast majority of molds that students capture will be innocuous, there is always the potential that a pathogenic organism will be isolated. Always assume that anything in culture is a potential pathogen. Be sure to use sterile technique and disinfect the table tops you are working on both before and after the experiment. Similarly, students should wash their hands before and after working. Initial cultures should be sterilized and disposed of as soon as subcultures have been made and pure cultures should be sealed closed once they are inoculated and not be reopened. Be sure that students sterilize and disposed of all cultures when they are finished.

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**Annotated List of Additional Sources of Information**

The American Type Culture Collection, 14th Ed. (1980). *Catalogue of Strains*. Rockville, MD. Not only does this contain the catalog of microbes available from the collection, including the suggested media for culturing for various protocols, but it also contains media recipes.


Koch, William J. (1973). *Plants in the Laboratory: a manual and text for studies of the culture, development, reproduction, cytology, genetics, collection, and identification of the major plant groups*. Macmillan. New York. Chapter 3 deals with methods in isolation and culture of fungi. Chapters 16 and 17 present special cultural methods for molds and mushrooms, respectively, and chapters 21-27 survey the various groups of fungi from slime molds to lichens.

Morholt, Evelyn, Paul F. Brandwine and Alexander Joseph. (1966). *A Sourcebook for the Biological Sciences, 2nd ed*. Harcourt, Brace & World, Inc. New York. Chapter 13, Growing Plants Useful in the Classroom, includes sections on algae, slime molds and fungi, mosses and ferns. In addition, fungi are used as model organisms in reproduction and growth (Chapters 8 and 9 respectively) and ecology (Chapter 11).

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