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Introduction to *Physarum* Kit

TEACHER'S MANUAL WITH STUDENT GUIDE

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Introduction to *Physarum*

Teacher's Manual

Overview 3
Materials 3
Methods and Procedures 3
Preparation 6
Information About Cytoplasmic Streaming and Motility 6
References 7

Photocopy Masters

Student Guide S-1
Background S-1
Introduction S-2
Procedures S-2
Observations and Investigations S-4
Suggestions for Further Investigations S-6

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Introduction to *Physarum*

Overview

Physarum polycephalum, a plasmodial slime mold, is useful for the instruction of students from introductory to advanced levels. Because plasmodia are easy to culture and handle, students can focus their attention on posing questions and designing studies and experiments to answer these questions. Using the instructions presented here, students will learn how to culture and handle *Physarum* and perform observations of cytoplasmic streaming, locomotion, and plasmodial fusion. They will also undertake two experimental questions: 1) What factors influence plasmodial growth, and 2) What factors influence whether the plasmodium forms the resistant structures called sclerotia. The reproducible Student Guide also includes suggestions for further inquiry. *Physarum* plasmodia offer the opportunity for students to connect their own work to active investigations being done all over the world.

Materials

Included in the kit:

- *Physarum* plasmodium plate
- petri dishes
- non-nutrient agar
- semi-defined medium
- oatmeal flakes
- filter paper
- aluminum foil

Needed, but not supplied:

- scalpels or spatulas
- stereomicroscope
- autoclave or pressure cooker
- forceps
- Parafilm® or tape
- distilled water
- permanent marker

Methods and Procedures

Culturing on Non-nutrient Agar w/ Oatmeal Flakes

Although students can culture their own plasmodia, you may instead choose to prepare plasmodia for your class. If so, each student should have a culture to work from to set up experiments and do observations. The best method is to subculture yellow, glistening, fresh plasmodia pieces on non-nutrient agar w/ oatmeal flakes four days before use. To subculture from a growing plasmodium, use a sterile scalpel or spatula to cut a block of agar on which a piece of plasmodium is present. The block should measure approximately 1 cm^2. Place the agar block, plasmodium side down, on the non-nutrient agar plus oatmeal flakes. To culture from a sclerotium, use sterile forceps to transfer a piece of filter paper containing this resting stage to a dish containing non-nutrient agar w/ oatmeal flakes. Wet the filter paper with a drop of sterile water. Once the cultures are set up, seal the edge of each petri dish with Parafilm®, plastic, or electrical tape, and wrap the dishes in aluminum foil to keep out light. Incubate the cultures at room temperature (20–22°C).

It is good practice to subculture your stocks every 3 or 4 days when your class is actively studying *Physarum*. You can store plasmodia in the refrigerator for weeks and then subculture from these plates if you wish. For long-term storage, it is best to make sclerotia. If you start cultures from sclerotia, you will need an extra day or two before growth commences.

Culturing on Oatmeal Agar

Growth on oatmeal agar is slower than on non-nutrient agar w/ oatmeal flakes. Consequently, subculturing does not have to be done as often. Culturing on oatmeal agar is appropriate if you want slow growth of plasmodia at room temperature.

Culturing on Semi-defined Medium

Growth on semi-defined medium is vigorous and observations of plasmodia are easily made when they are cultured on this medium. It is better not to subculture from semi-defined medium because the nutrient richness of this substrate increases the risk of microbial contamination.

Culturing on Semi-defined Medium and Filter

Plasmodia can be grown on top of sterile filters that have been placed on hardened semi-defined medium. This technique allows you to transfer intact growing plasmodia by simply picking up the filter paper and moving it to another medium or environmental condition. It is important to use aseptic technique when culturing with this method.

How to Make Sclerotia

To make sclerotia for long-term storage (up to a year), culture a plasmodium on non-nutrient agar w/ oatmeal flakes. After 3–4 days, transfer pieces of the actively growing plasmodium onto sterile filter papers placed on top of nutrient agar. Incubate the plasmodia in the dark. After 24 hr, remove the filter paper with the migrating plasmodia and place it into a sterile petri dish. Incubate in the dark at room temperature. Once the filter has dried, you will see that the plasmodium has formed dry, crusty sclerotia. Cut the filter paper, with attached sclerotia, into approximately 1-cm squares and store them in a dark, dry place. Alternatively, transfer pieces of actively growing plasmodia to non-nutrient agar. Incubate in the dark. After 72–96 hours, the plasmodium will retract its veins, cease cytoplasmic streaming, and gather into a yellow blob. Cut out agar pieces bearing these blobs and transfer them to empty, sterile petri dishes. Incubate in the dark. These blobs will dry out and form sclerotia. Store the sclerotia in a dark, dry place.

Starving Plasmodia

For some experiments, it will be helpful to have plasmodia that are starving rather than actually feeding. To do this, transfer an agar block with a piece of plasmodium on it to a petri dish containing non-nutrient agar. Seal the edge of the dish and cover it with aluminum foil. Incubate at room temperature for at least 15 hours (but not more than 48 hours) before use. If you wish to starve plasmodia for a longer time period, incubate in the light during starvation. Incubation in the dark for longer than 48 hours will cause plasmodia to stop migration.

How to Clean Contaminated Cultures

Physarum cultures maintained on non-nutrient agar w/ oatmeal flakes usually do not exhibit growth of microbial contaminants. Because of its nutrient richness, microbial contamination can be more of a problem on semi-defined medium. If any of your cultures do become contaminated, try to subculture a piece of plasmodium from a "clean" area onto a new dish. Alternatively, place a piece of plasmodium in a petri dish containing sterile non-nutrient agar. Position the plasmodium at the edge of the dish. Cover the dish with aluminum foil and poke a hole in the foil on the side of the dish adjacent to the position of the plasmodium. The plasmodium will migrate away from the light but its contaminants will not. Usually this will clear contamination after one journey away from the light, but if not, transplant the plasmodium to another dish of non-nutrient agar and let it migrate again. After this second round of migration, transfer the plasmodium to the growth medium of your choice.

Culture Media

Non-nutrient Agar

Add 20 g of agar to 1 L of distilled water in a flask. Plug the top of the flask with cotton or a foam plug and cover the top with aluminum foil. Autoclave for 20 min using slow exhaust (liquid cycle). Pour petri dishes.

Sterile Oatmeal Flakes

Place oatmeal flakes (not instant) into a beaker. Cover the top with aluminum foil and autoclave 20 min, slow or fast exhaust.

Non-nutrient Agar w/ Oatmeal Flakes

Sprinkle 25 to 30 sterile oatmeal flakes onto the surface of non-nutrient agar in petri dishes.

Oatmeal Agar

Add 15 g of agar and 30 g of oatmeal flakes (not instant) to 1 L of distilled water in a flask. Plug the top of the flask with cotton or a foam plug and cover the top with aluminum foil. Autoclave for 20 min using slow exhaust (liquid cycle). Pour petri dishes.

Semi-defined Medium

Add the following to 1 L of distilled water in a flask:

10 g glucose
10 g peptone
1.5 g yeast extract
3.5 g citric acid
2 g KH_2PO_4
0.9 g $CaCl_2$-$6H_2O$
0.6 g $MgSO_4$-$7H_2O$
0.034 g $ZnSO_4$-$7H_2O$
0.06 g $FeCl_2$-$4H_2O$
15 g agar

Plug the top of the flask with a cotton plug or a foam plug. Cover the top with aluminum foil. Autoclave for 20 min using slow exhaust (liquid cycle). After autoclaving, add 10 mL of sterile hemin stock solution per liter of medium. To make this stock solution, dissolve 0.5 mg hemin per mL of 1% NaOH. Autoclave the stock solution for 20 min using slow exhaust. Pour petri dishes.

Store the hemin stock solution and the semi-defined medium (with or without the hemin added) in the refrigerator.

Sterile Filters

Cut filter papers to fit petri dishes, wrap them in aluminum foil, and autoclave 20 min, slow or fast exhaust.

Preparation

Photocopy the Student Guide at the back of this manual for each student (or group, according to your lesson plan). Unless students will start their own cultures as part of the lab work, prepare a culture of *Physarum* for each student or group. Cultures on non-nutrient agar w/ oatmeal flakes are recommended.

Information About Cytoplasmic Streaming and Motility

If students are patient in their observations of cytoplasmic streaming and motility, they will be able to report a great deal. To help you guide them, here is some interesting information about these behaviors:

- Cytoplasmic streaming, which is also called shuttle streaming, depends upon the proteins actin and myosin. Although not identical to the actin and myosin of skeletal muscle, they operate similarly to the muscle proteins to produce contraction.
- Cytoplasmic streaming, in a given vein, goes in one direction, stops, and reverses flow. These reversals of flow occur at regular intervals, every 1–3 minutes.
- The reversals of cytoplasmic flow don't necessarily occur in adjacent channels at the same time.
- The advancing edge of the migrating plasmodium is composed of a continuous sheet of cytoplasm interspersed with small veins. Behind this leading edge is a network of larger veins. There are usually spaces between these veins.
- Motile plasmodia do not have a permanent morphology. The pattern of veins in a given area changes.
- Starving plasmodia will migrate for days, especially if incubated in the light. They leave a slime track when they migrate. Consequently, the path(s) taken by plasmodia can be easily observed.
- A close look at the veins reveals ectoplasm, the outer, clearer, more viscous cytoplasm, and endoplasm, the inner, flowing, grainy-looking cytoplasm. Within the endoplasm are nuclei, mitochondria, ribosomes, vacuoles, and pigment granules.
- The mechanism of plasmodial motility has similarities to amoeboid motion in that both involve endoplasm to ectoplasm interconversions, and ectoplasmic contractions.