Fungal Fighters Lab Procedures

Fungal Fighters - A Fungal Competition Lab Module for Budding Microbiologists

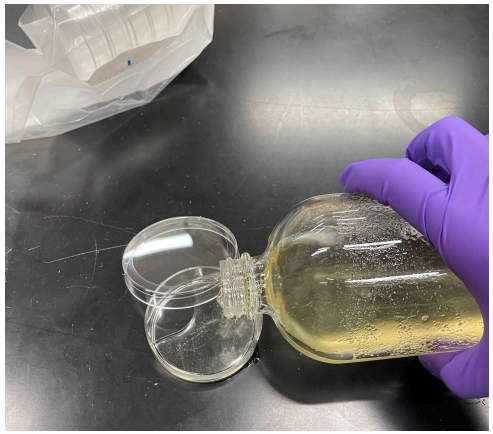
Lai, E.Y., Mejia, E., Parker, I.M., & Gilbert, G.S. (2024)

Fungal Fighters Lab Procedures

Make agar plates

Materials	Hazards/safety tips
Media: Malt extract powder, agar powder, DI water, glass bottle Other: Sterile plates, Parafilm	Liquid media will be hot, allow ample time to let the bottle cool before pouring. The container should feel like body temperature.

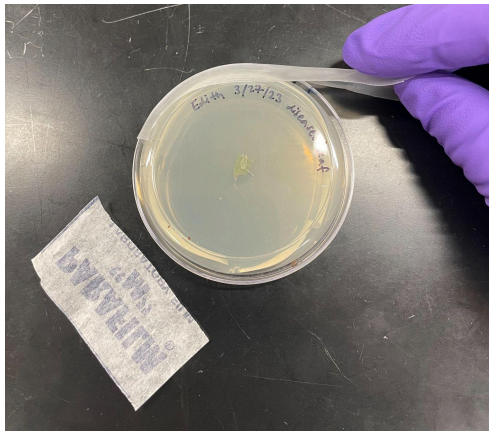
Media recipe (prepare before lab)



1. Prepare one 200mL bottle per group of 4 students
2. 4g Malt Extract powder, 3g Agar powder, 200mL DI water
3. Keep media bottles in a hot water bath, microwave again if necessary

Pouring media into plates

1. Prep Petri plates
    - ☐ Open the plastic sleeve to unpack plates 4-5 plates per student on the lab bench
    - ☐ Keep the lids closed since the plates come sterilized in the package
    - ☐ Make sure to keep the bag for storage
2. Pour media into plates
    - ☐ Hold the bottle in your dominant hand and lift the lid in your other hand
    - ☐ Slowly pour media into the plate until the liquid forms a complete layer, being careful not to splash or spill. Pour just enough liquid to cover the bottom of the plate, do not fill up the petri dish any higher
    - ☐ Immediately place the lid back onto the plate to cool until solid
3. Recognizing and understanding contamination
    - ☐ As a group, leave one plate open while pouring the other plates to collect contaminating spores from the air
    - ☐ Students may also poke the agar gel to feel the solidified texture
    - ☐ Label the plate as “Contaminated plate [group number, date]”
4. Practice using Parafilm on the contamination plate (repeat using the same plate)



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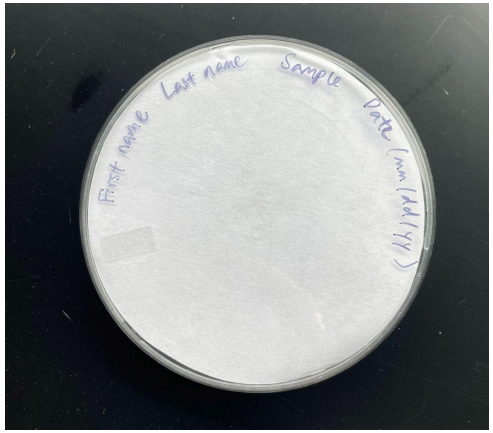
- ☐ Cut a strip of Parafilm from the class roll, and remove the paper backing
- ☐ Stick one end of the parafilm to where the lid and plate meet
- ☐ Hold the plate closed with your non-dominant hand
- ☐ Slowly stretch the parafilm completely around the plate
- ☐ Unpeel the parafilm and let your other lab mates practice using the same plate

5. Stack your finished plates and place them back into the sleeve for storage
   - ☐ Check that plates had enough time to fully cool and solidify
   - ☐ Those plates must remain closed and sterile until we use them on Thursday
   - ☐ Bag the plates and seal the bag with tape



Fungal Fighters Lab Procedures

Fungal isolation

Materials	Hazards/safety tips
Leaf samples Poured agar plates Parafilm Tools: Tweezers, scissors Sterilization: Tea strainers, beakers, 70% ethanol, 10% bleach	10% bleach is corrosive, keep your gloves on and be careful not to splash the liquid. If there's a spill, notify your teacher.

Observing Contaminated Plates

1. Check in on the contaminated plates
2. Contrast how contaminated and non-contaminated plates look
3. Discuss how sterile technique reduces the chance for unwanted growth

Plate a leaf sample

1. Make sure your group has enough diseased leaves for everyone
    - ☐ Each student should use a different leaf sample
2. Put on PPE (personal protective equipment)
3. Sterilize your workspace and tools
    - ☐ Wipe down surfaces with 70% ethanol
    - ☐ Pour out a labeled beaker of 70% ethanol and a beaker of 10% bleach
    - ☐ Place scissors and tweezers in the beaker of 70% ethanol
    - ☐ Leave tools in ethanol between every use to prevent cross-contamination between samples
4. Prep your leaf sample
    - ☐ Hold your leaf sample in one hand using tweezers

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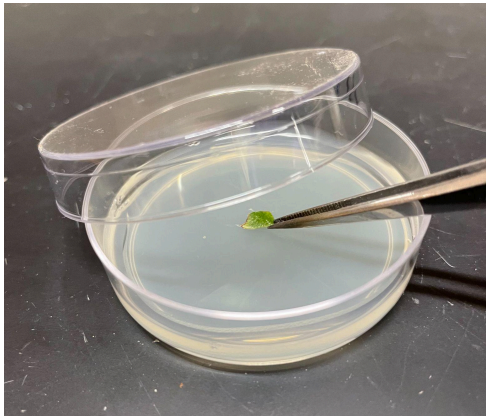
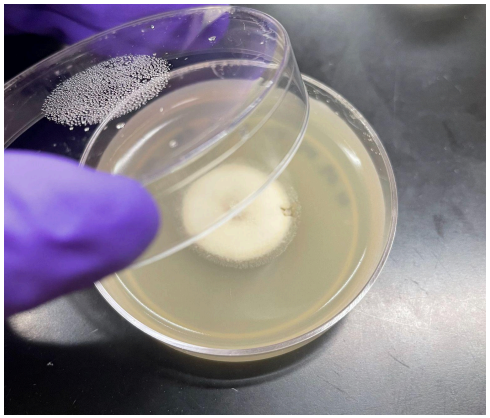
- [ ] Cut a 5mm x 5mm square of diseased spots using the scissors
- [ ] Place the diseased leaf piece into the tea strainer

5. Surface sterilize your leaf sample
   Sterilizing the outside of the leaf ensures that we only isolate the fungi from inside tissue
   - [ ] Hold the leaf piece in the tea strainer for the next 2 steps
   - [ ] Submerge and hold in 70% ethanol for 1 min with a timer, drip dry
   - [ ] Sumberge and hold in 10% bleach for 1 min with a timer, drip dry
6. Plate your sample
   - [ ] Open the tea strainer and pick up the leaf piece with sterile tweezers
   - [ ] Put one agar plate on the lab bench in front of you
   - [ ] Carefully open the petri dish and place the sample in the middle of the agar media
   - [ ] Open and close the dish quickly to minimize exposure to airborne microbes
7. Store the sample
   - [ ] Flip the plate over so that the agar side faces upwards
   - [ ] Label with your name, table number, and date
8. Seal the petri dish with a strip of Parafilm
   - [ ] Cut a small strip of Parafilm
   - [ ] Peel off the backing paper and stretch the material around the dish
   - [ ] Stack all plates allow to sit for approximately 5 days undisturbed





Isolate a pure culture

After 5 days, there should be blobs of mystery fungi growing, some plates may actually be growing more than one species.

1. Put on PPE and sterilize your workspace and tools as before
   - [ ] Wipe down surfaces with 70% ethanol
   - [ ] Place scissors and tweezers in a beaker of 70% ethanol

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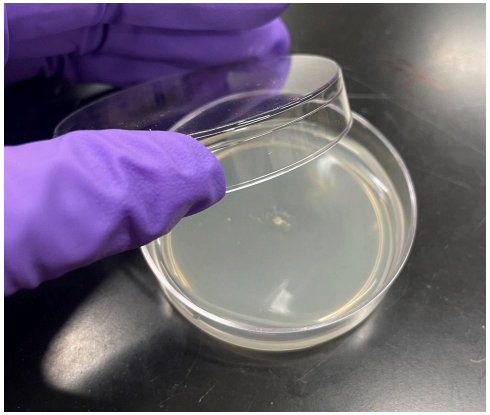
- ☐ Leave tools in ethanol between every use to prevent cross-contamination between samples

2. Place your petri dish on the table with the lid facing up
   Keep the plate closed as much as possible between steps to reduce contamination
   - ☐ Unwrap the parafilm and open the petri dish
3. Transfer some of your fungal samples to a new plate
   - ☐ Cut a 1mm x 1mm piece of fungi using sterile scissors and place it in the center of a new plate. Size does not have to be exact
   - ☐ Label with your name, “isolated culture,” group number, and date
   - ☐ Parafilm to seal the new petri dish
   - ☐ Store plated sample for 5 days to allow for growth
4. Make a second plate for fungal growth rate
   - ☐ Cut another 1mm x 1mm piece of fungi using sterile scissors
   - ☐ Instead of placing the inoculum in the center, place the sample at the edge of the plate so that the fungi will grow toward the middle
   - ☐ Label with your name, “growth rate,” group number, and date
   - ☐ Parafilm to seal the new petri dish



Fungal Fighters Lab Procedures

Fungal growth tests

Materials	Hazards/safety tips
Petri plate with your culture Extra agar plates Parafilm Tools: Tweezers, scissors, ruler Sterilization: Tea strainers, beakers, 70% ethanol	Students do not need to use PPE while the plates remain sealed throughout the growth rate activity

Growth rate

1. Grab your fungal growth plate from last class: recall you made one for isolation and one for fungal growth
2. Measure growth after 3 days
    - ☐ Trace the outline of your fungal culture (the mycelium) directly on the plate bottom
    - ☐ Using a ruler, measure the diameter (cm) of the sample from edge to edge
    - ☐ Record this length on your worksheet
    - ☐ Start fungal fights on this same day (procedure below)
3. Measure growth after 7 days
    - ☐ Trace the outline of your fungal culture directly on the plate
    - ☐ Using a ruler, measure the diameter of the sample from edge to edge
    - ☐ Record this length on your worksheet
4. Complete the growth rate worksheet
    - ☐ Calculate the rate of growth
    - ☐ Graph the growth distance in relation to days growing
    - ☐ Compare with the rest of the class

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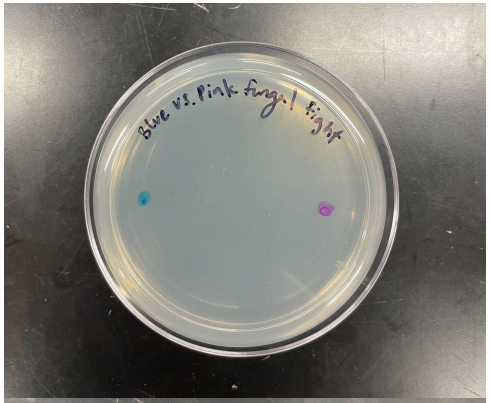
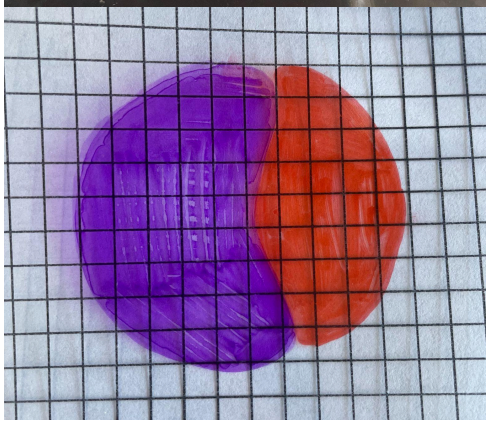
Fungal fights

1. Put on PPE and sterilize your workspace and tools
    - ☐ Wipe down surfaces with 70% ethanol
    - ☐ Place scissors and tweezers in a beaker of 70% ethanol
    - ☐ Leave tools in ethanol between every use to prevent cross-contamination between samples
2. Place your petri dish on the table with the lid facing up
    - ☐ Unwrap the Parafilm and open the petri dish
    - ☐ Keep the plate closed as much as possible to reduce contamination
3. Set up fight matchups in your lab group
    - ☐ Assign a color (or shape if there aren't enough Sharpies) to each person, and record a key on the fungal fights worksheet
    - ☐ Make predictions on who will win each matchup
    - ☐ Make sure to label plates clearly with the color of each side of the matchup
4. Transfer both opponents' fungal samples to a new petri dish
    - ☐ Place one transfer on one side of the plate and the other transfer at the other side (refer to image setup)
    - ☐ Label each person's fungal sample with their color Sharpie
    - ☐ Parafilm to seal the new petri dish
    - ☐ Store plated sample to allow for growth
5. Measure growth after 7 days
    - ☐ Place a sheet of clear acetate printed with a grid on top of the closed petri dish
    - ☐ Trace the area of both fungal cultures onto the sheet
    - ☐ Calculate the area by counting the number of squares filled and multiplying by the area of each grid square
    - ☐ Record the results on the fungal fight worksheet
    - ☐ Determine group winner
6. Report back to the class to set up new matchups between group winners





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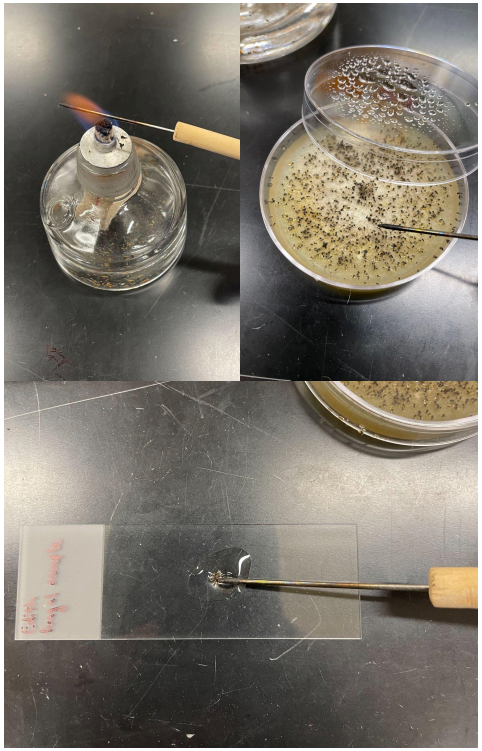
Viewing your fungi

Materials	Hazards/safety tips
Petri plate with your culture Parafilm Tools: Metal probe, eyedropper Sterilization: Flame burner Microscopy: Slides, coverslips, water	Be careful with sleeves when reaching around an open flame, make sure to turn it off after the sterilization step Microscope slides are made of glass and can break easily. Let your teacher know if there is broken glass that needs to be cleaned up. Don't pick up broken glass with your bare hands.

Make microscope slides

1. Collect your slide materials: microscope slide, cover slip, water, and eyedropper
2. Place 1 drop of water on the center of the microscope slide
3. Grab your fungal culture and unwrap Parafilm from the petri dish
4. Flame sterilize the metal probe
    - ☐ Set up the flame burner with teacher supervision
    - ☐ Wave the metal probe swiftly across the open flame
    - ☐ Hold the metal probe in the air to prevent contamination of your tool
5. Collect a small sample of fungi with the probe
    - ☐ Open and close quickly to minimize exposure to airborne microbes
    - ☐ Scoop a small sample of fungi with the probe without digging into the agar
    - ☐ Place this sample on the slide, mixing it into the drop of water. Using two probes (one in each hand) may be helpful to break up clumps
6. Seal in the sample
    - ☐ Slowly place the coverslip on top of the droplet of water
    - ☐ Lightly tap out air bubbles with your pinky



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Microscopy

1. Set up microscope
    - ☐ When moving microscopes, always use two hands: one to hold the bottom and the other to hold the neck
    - ☐ Plug in the microscope and turn on the light
    - ☐ Lower the stage platform to the lowest setting
    - ☐ Turn the magnification lens to 4x
    - ☐ Place the slide on the stage platform
2. Focus the view using the coarse and fine adjustment knobs
3. View your slide
    - ☐ Record observations on the microscopy worksheet
    - ☐ As a class, look for identifying structures such as spores