Beginner's Grow Guide

Chapter 2. Working with Agar

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WORKING WITH AGAR

- ## What is Agar?

  - **Agar is a simple mix of different ingredients that are heated and sterilized. While still in a liquid form are poured into clean plastic dishes of different sizes. Agar Agar being one of the main ingredients and is a jelly like substance harvested from algae. The dishes are then cooled down to harden and used immediately or stored for later use.**

Pictured above: A rainbow colored agar plate poured in front of a custom flowhood on a stainless-steel table, cooling down.

- **What is the difference between agar plates and agar slants?**
    - Agar plates and slants are a two-dimensional growth media; meaning what you see is a flat plane of growth.
    - **Agar Plates**
        - The most common and ranging in different sizes, 60mm to 150mm plates
        - 100mm are the best available size and is about the size of a hockey puck for reference*
        - Pre-poured plates that vendors sell online are great options for anyone who does not have a pressure cooker and would like to get to work with agar more immediately. Amazon and Etsy have good options with a range in mixes and food for mycelium to thrive on.
        - Learning how to pour agar is more ideal in my opinion. The best way to learn about how agar works is to simply dive in and try it. Google and other books on growing mushrooms have many great recipes. Be sure to find a recipe specifically for *P. Cubensis*.
        - The use of colors in agar is not important and using a good gel food coloring grade is ideal. In the many colors I've used for agar, avoid red. Red is a terrible pigment and can turn my mycelium a shade of

pink. This observation comes from doing a few rainbow plates and putting different genetics on every color.

- Activated charcoal agar plates. By now you've may have come across these plates and they're pretty rad. When the mycelium grows it is easy to see. The benefits of using these plates are many, the properties of activated charcoal and its antibacterial are one of many reasons that people seek to use these instead of gentamicin (powdered antibiotic).

- Agar Slants
  - These are becoming more and more popular; they are great for long term storage and keeping mycelium in a hibernation state.
  - Think of these as a mini time capsules.
  - Uncommonly, you can pour a top layer on top of the growing mycelium thus sealing or enclosing the living culture for later use. I read about this in a book, and I rarely come across anyone else using this method. I personally work very little with slants.
  - Agar slants are good to learn how to navigate, only a few vendors offer these for sale.

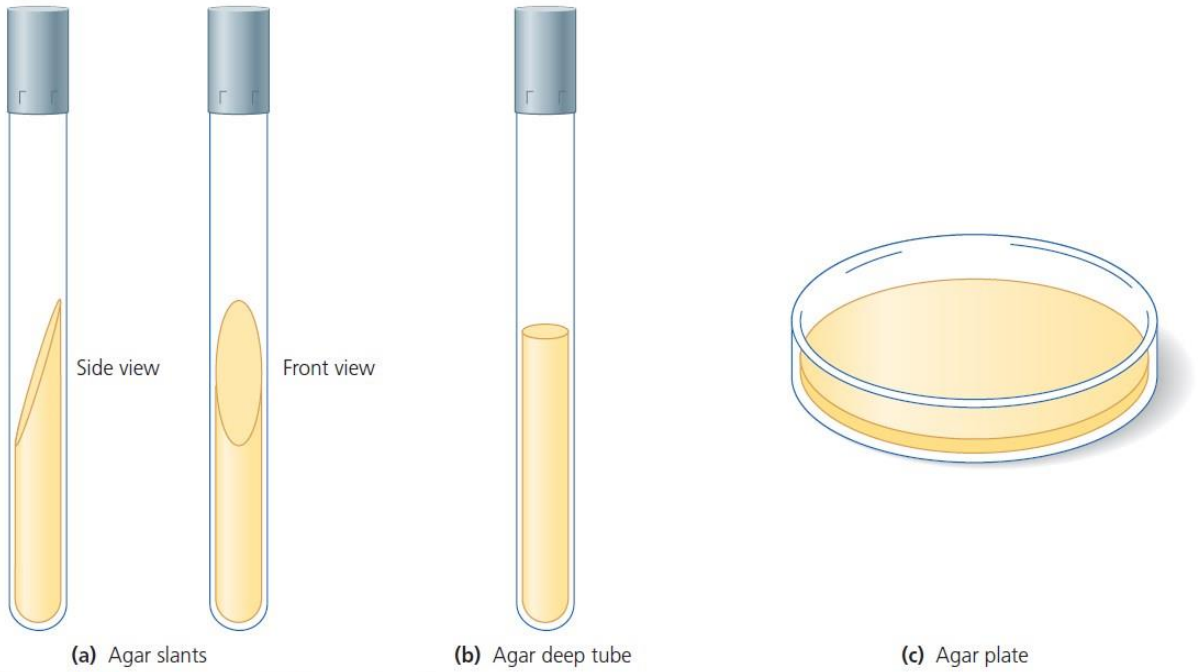


Figure P1.2 Forms of solid (agar) media

G. Cappuccino, James, and Natalie Sherman. MICROBIOLOGY a Laboratory Manual. 10th ed., Boston Columbus, 2008.

- **"Anything" Agar Recipe from Psilocybin Mushroom Handbook by L.G Nicholas and Kerry Ogamé**
    - **20 grams of anything (Malt Agar Extract is my preferred medium)**
    - **22 grams of Agar Agar**
    - **1 liter or 1000ml of water**
    - **The book says to add 3% H2O2 (added after sterilization) but this step is not necessary, and I have never done this.**
    - **Add peptone or yeast to this recipe**

- **Agar pouring tips**
    - **Wait till the agar is 123 degrees before pouring, you should be able to touch the bottles.**
    - **Mason jars do work for this process, so you do not need to get any special bottles. If you want to step up your agar game the scientific media glass bottles are what we use.**
    - **All American pressure cooker or Presto to sterilize.**

Pictured above: 1000ml glass media bottle
https://www.horticulturesource.com/

- **Getting to know agar has several benefits**
    - **Breeding**
        - **Cloning a mushroom or taking tissue from the mushroom and being able to watch a tissue form into a new mycelium network of growth helps you clone and keep genetics.**
    - **Spore germination**
        - **Another popular use for agar. Mycologists love to hunt new genetics. Working with something like the reproductive bodies of the mushrooms via their spores is a huge step forward into breeding and understanding mushroom identification.**
    - **Identification**
        - **Mushrooms are categorized by spore colors and different spore shapes.**
    - **Contamination hunting**
        - **Agar plates are used for another purpose and a lot of home labs are utilizing this technique. Basically, as a test for common contaminations you can take a clean agar plate and leave on a tabletop for about 5 mins and then put into an**

incubation unit (not necessary) and it can provide a very broad sense of the load of contamination in the air.

- A neat trick to see if your flow hood needs maintenance is opening a clean agar plate and having it face the first air from the flow hood. Count to 30, wrap and wait and see.

- Testing
    - Contaminations in syringes, spore swabs and spore prints are present. A common use for agar plates is to place a few drops of any liquid from a spore syringe or liquid culture syringe and allow it to grow to identify any contaminations present in the syringe.
    - A few wild or lab grade spores sprinkled on an agar plate will also help identify contaminants, take only the clean cuttings of healthy growth if this is what you're doing to clean up spores.
    - Ideally you want to use this method along with a microscope and reagents to further identify any microorganisms that cannot be seen with the naked eye.

- There's contamination in my plate, what do I do?
    - Different contaminations will arise and being familiar with them is important.

- **Some contaminations will only be present on a small portion of a plate and others will likely take over the entire dish.**
- **I suggest, throwing away fully contaminated plates to avoid spreading further in the clean environment you have.**
- **With contaminations that are minute, consider holding small experiments such as '*Will the mycelium win or the trichoderma*?'**

• Things to also consider with a contaminated plate

- **"Gentamicin" or an antibiotic added to agar is a common practice people use to combat bacterial outbreaks. Shroomsupply.com sells gentamicin by the metric gram.**
- **Mites or other small insects are easier to see on plates and can wreak havoc if left untreated.**
- **Trichoderma is a sticky contamination, meaning if you touch it or if it floats into the air, it will attach itself to a new surface immediately. Handle with caution.**
- **Look above and under the plate. I have been fooled by a clean looking plate but when I looked underneath dots of contamination**

were present that mycelium simply continued to grow over.

Pictured above: Agar plate with mycelium that has the presence of mites. Notice tiny holes have formed and the mycelium no longer is touching.

- ## My agar is fully colonized and healthy, now what?

    - **You have a healthy agar plate and now would like to see what it produces! Great start by prepping any sterilized jars, grain bags or even more agar plates or slants, or liquid culture jars.**

        - **Prepare your workspace to work with the plate here's examples of materials needed:**

            - **Clean scalpel**

- Alcohol to clean with
- Torch to heat scalpel during different cuts
- PPE (facemask, gloves)
- Flowhood, Flow box or Still Air Box (SAB)
- Parafilm or other plate wrapping materials

- If I plan to take transfers (duplicating or taking a small square of healthy agar to another new plate of agar) where do I cut from?
  - A very common question, on an agar plate there is a few growth signs that you will see.
  - The outer range on the plate where the mycelium is newest is a favorite to take a cut from.
  - The most vigorous or toughest looking mycelium on the plate is another good cloneable piece.
  - Anything that looks healthy.
  - Tomentose vs rhizomorphic growth are two words you'll hear often. Basically, fluffy fuzzy, or strong and rope looking are the best way to describe these terms.
  - Both types of growth are important to know because some genetics only grow on a plate looking like fluffy clouds.
  - Some of the biggest genetics I've worked with looked horrible and not so happy on agar plates.
- Set up so you only open a plate one time

        - What I mean by this is, say you are making liquid culture and you have a wonderful clean plate. Take those small cuttings and then with the left-over bits on the plate put them onto a grain jar or grain bag right away. Now you've made two items, a jar of liquid culture and agar inoculated a sterilized grain jar.
        - Plan out those inoculations and growth cycles so that you are wasting little and utilizing your time and space best.
    - Cold stored items
        - My rule of thumb is to let anything in cold storage come back up to room temperature before using.
        - Plates and slants up to a year old are still good to use.
        - Don't be afraid of condensation
        - Use Ziplocs or other storage to separate your cultures from anything else in the refrigerator or cold fridges. Wine coolers are another good cold storage utility.
- Finished my transfers
        - Plates are going to do well in temperatures between 60-80 degrees. If you have an incubator, use that but not needed.
        - Play around with these temperatures for different genetics. Example: My Yeti and Apes love 65 degrees, but my warm weather Hawaiians and Brazilians love 77-80 degrees. You

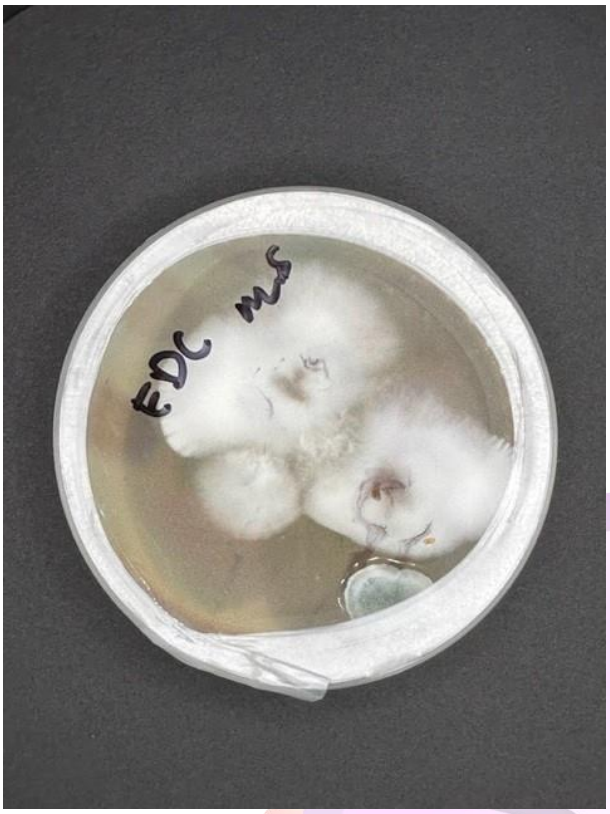
can watch the mycelium every day and see these changes too.

- Avoid heat and other hazard areas. Don't place them in places with blowing air or huge fluctuations in temperatures.
- Wrap plates completely and quickly. Don't let agar plates sit out too long before securing them. Get them into their storage spaces quickly. They do dry out.
- Handle with clean hands.
- Label everything!

Lastly be confident, enjoy what you are doing and don't be afraid to try new things!

Pictured above: Healthy rhizomorphic growth, two cuts of healthy agar clones growing into each other on an agar plate. No visible signs of contaminations.

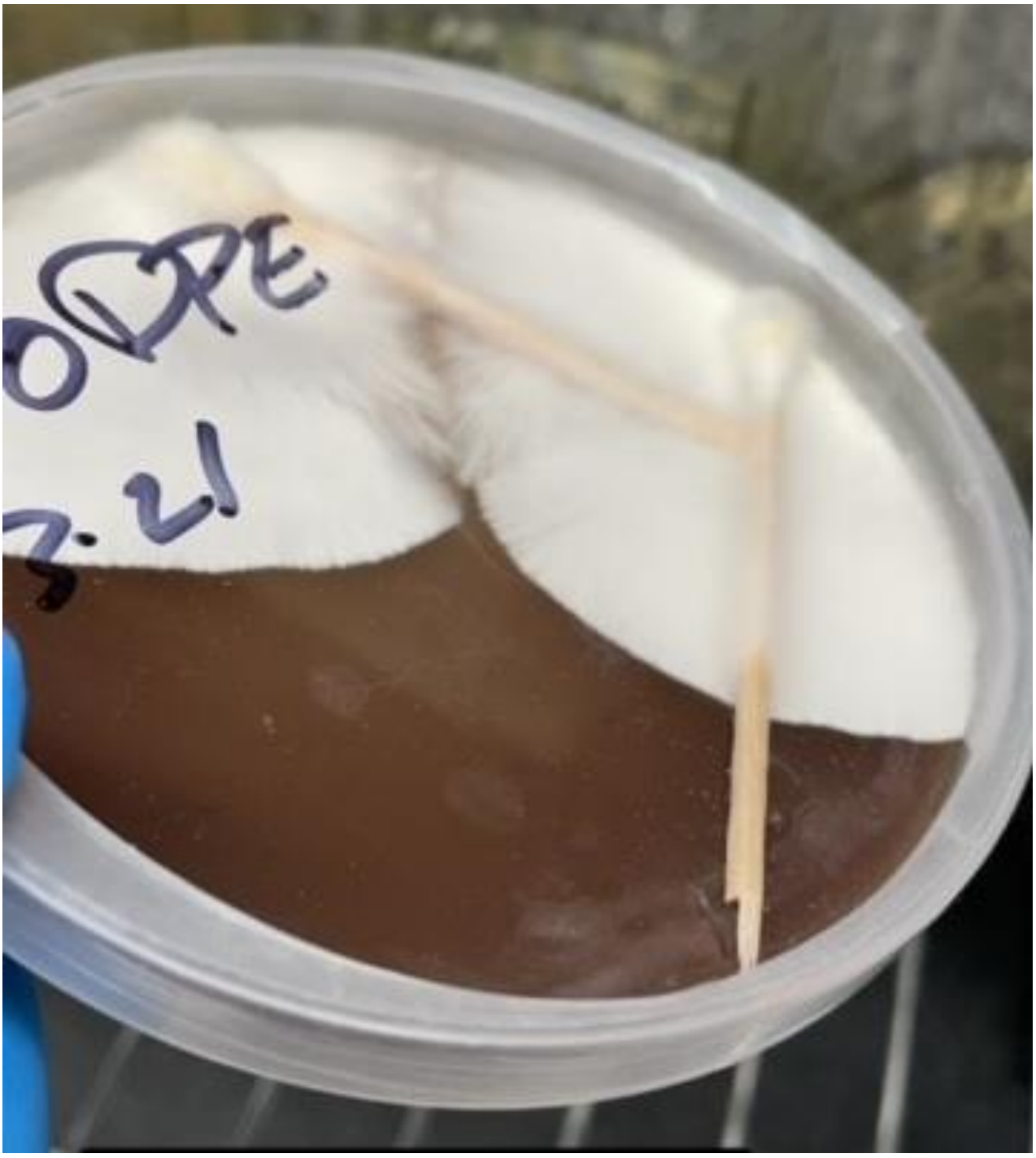
Pictured above: Ten fully colonized plate of popcorn enigma showing healthy and contamination free growth from a single transfer or cut from the original plate.



Pictured left: Elephant Dung Cambodian mushroom gills sections on a plate after five days of growth. Trichoderma contamination at the bottom observed by its green and white colors.

Pictured right: Photo of the same plate as above. The underside of the plate showing pieces of mushroom gill and the mycelium growth. Also present is the backside of the Trichoderma

Pictured above: 10ml agar plate with a tissue sample of PE7 after seven days of growth. Visible is the rhizomorphic (rope looking growth moving downward on the plate) and tomentose (fluffy growth moving upward on the plate) mycelium types. Both are healthy signs of growth.



Pictured above: Two spore swabs on an MEA (malt extract agar) plate. Good and clean growth, no contaminations present. Healthy white mycelium. This is a successful multi spore swab plate.

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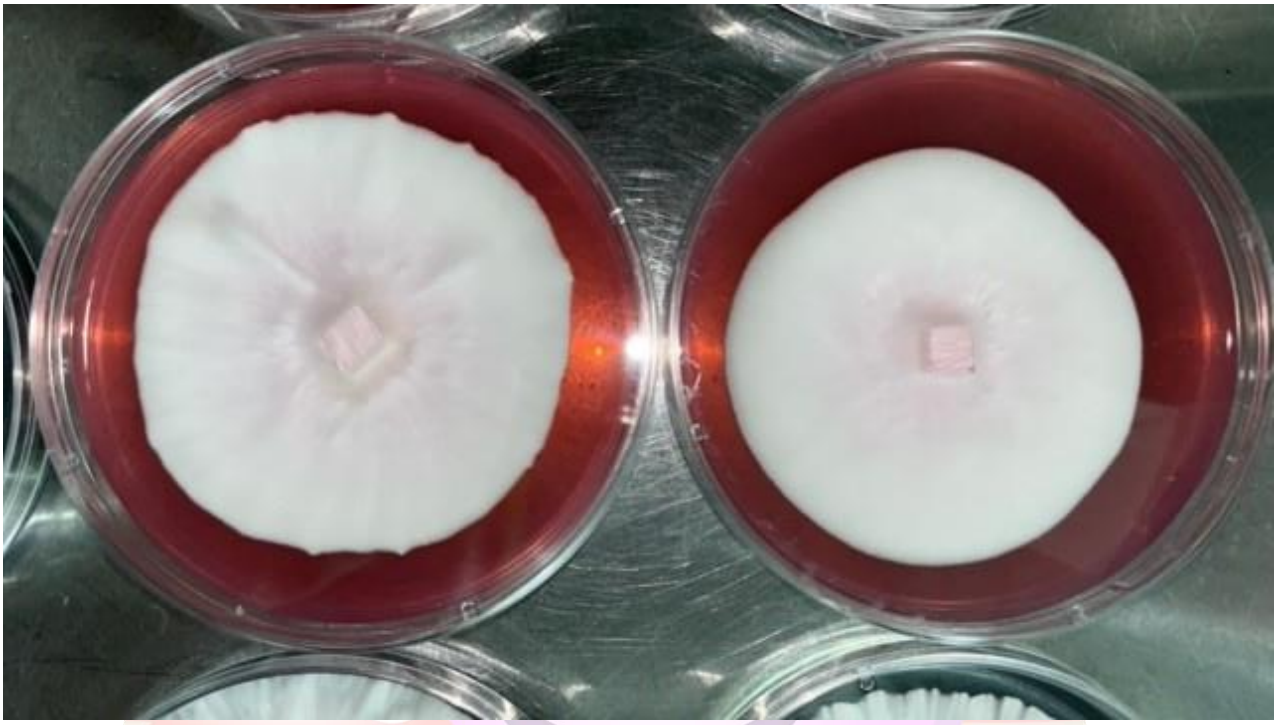
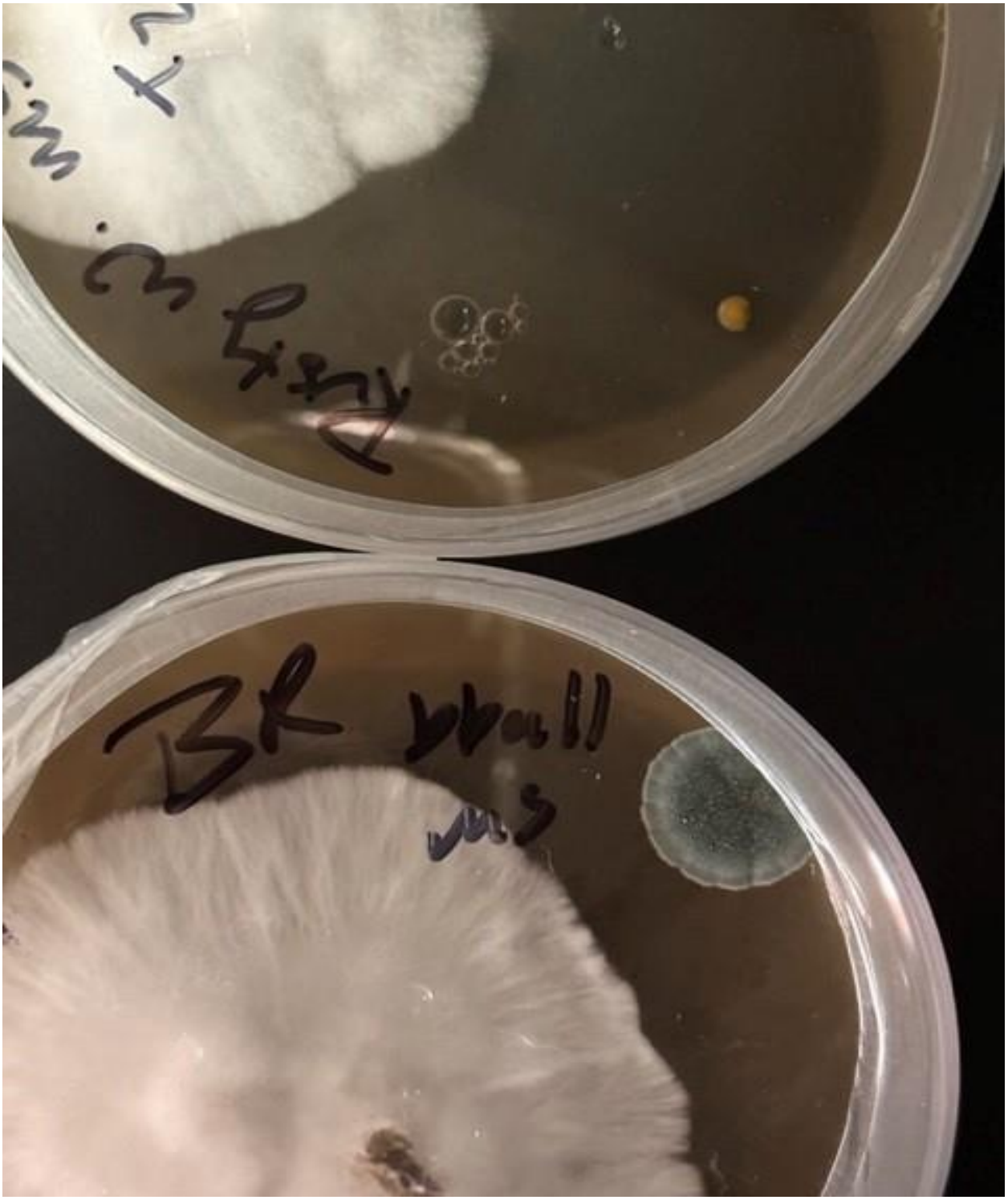
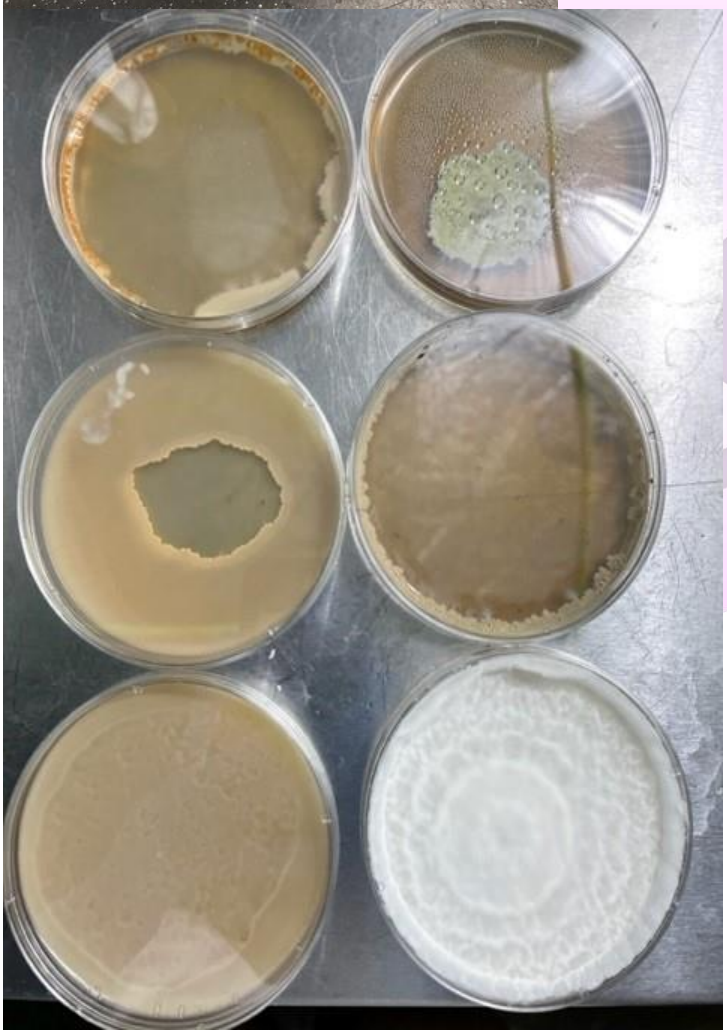


Pictured above: Two healthy tomentose plate grown on a dyed red agar plate. After using red dyes and discussing it with other mycologists, I do not suggest using it anymore. The pigment red has not been an ideal candidate to work with.



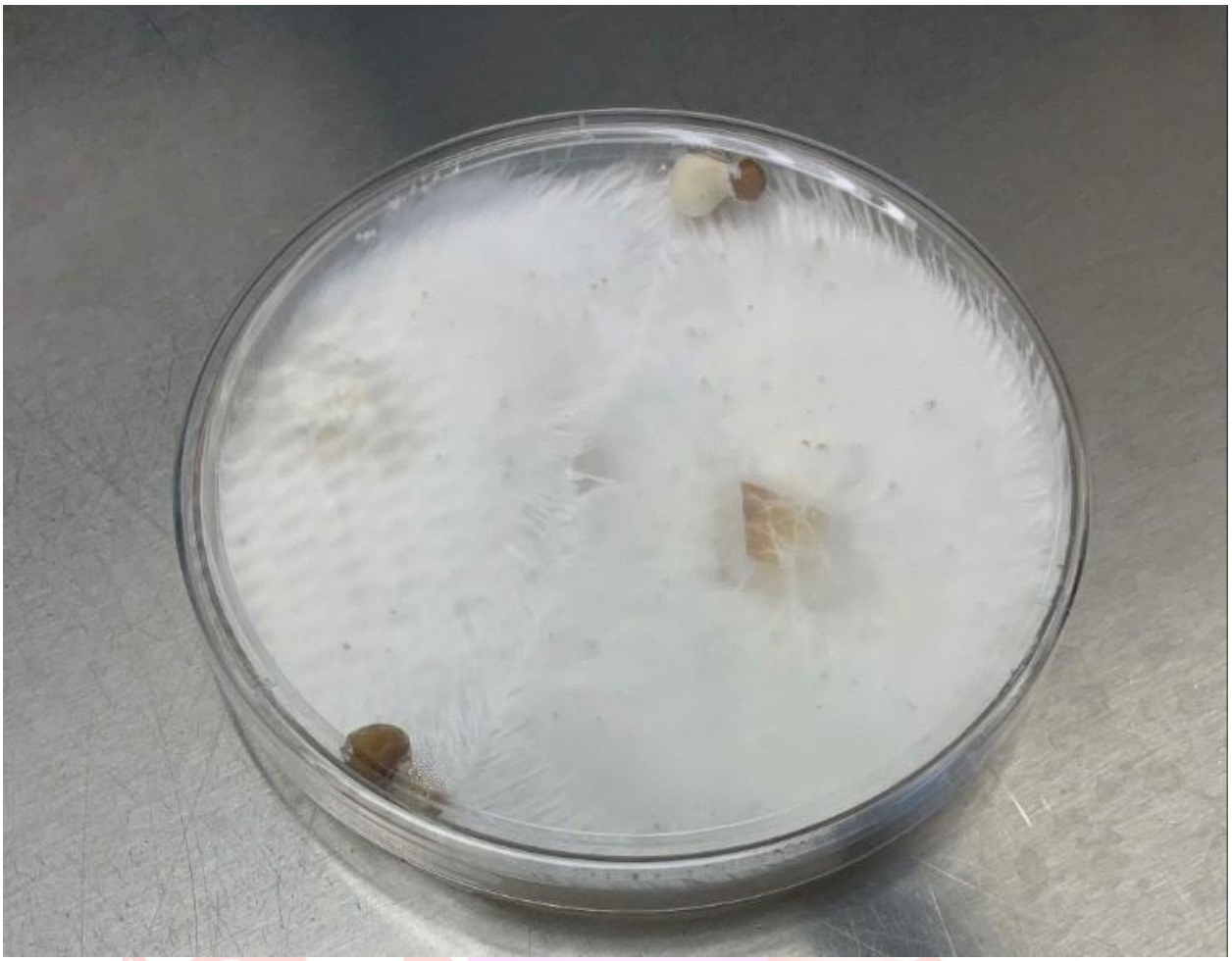
Pictured above: Two different agar plates, Rusty White transfer two from spores showing a small yellow/brown bacterial growth forming near the edge of the agar plate. Bull Run baseball gill agar plate with another trichoderma spot growing near the edge of the plate.

Pictured above: A failed Hanoi liquid culture test. Mycelium being surrounded by trichoderma after 72 hours of incubation.

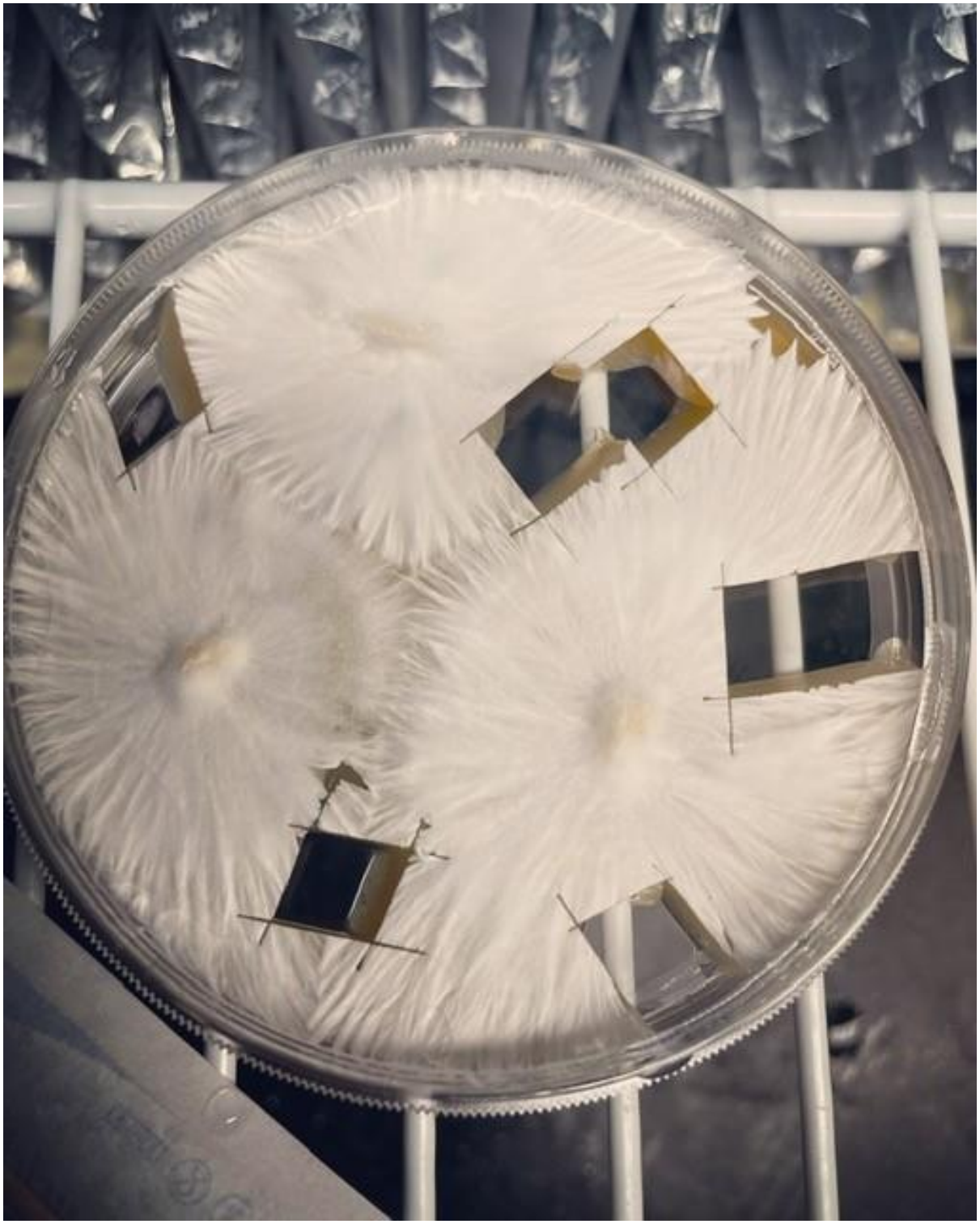


Pictured left: Other examples of contaminated plates. Bacteria, yeast and more trichoderma.

Disclosure: I am not formally microbiology educated. My experience solely comes from working with P. Cubensis genetics. I come across a few contaminants that I am unfamiliar with. My rule of thumb is hazard disposal of anything that looks suspicious and lastly do not touch.



Picture above: Fully colonized agar plate with two baby mushrooms growing at the both top and bottom. This plate was left alone and after sometime healthy plates will fruit right on the agar itself. The plate has two transfer cuts on each hemsphire that have grown together and shown compatiblity.



Pictured above: Fully colonized plate that has three different sections of tissue pieces. Grown together after days on a plate. The missing pieces are the next transfer or transfer 2.0. I like to take transfers from overlapped mycelium.

THANK YOU AND IF THIS HELPS IN ANYWAY, PLEASE LET ME KNOW!

References:

Nicholas, L., and Kerry Ogamé. *Psilocybin Mushroom Handbook: Easy Indoor and Outdoor Cultivation*. Illustrated, Quick American Archives, 2006.

Cappuccino, James, and Chad Welsh. *Microbiology: A Laboratory Manual (10th Edition)*. 10th ed., Pearson, 2016.

https://www.horticulturesource.com/fresh/product/1000ml-glass-reagent-media-storage-bottle-gl45-screw-cap/?gclid=CjwKCAiAk--dBhABEiwAchIwkZJKT3f3ljNCSrsYvbFov7rAFyvzt0NC9Ma0uhZE4lwkphnhrdALMRoCvj4QAvD_BwE