Procedure for Cloning Wild Chicken of the Woods (Laetiporus sulphureus/cincinnatus)

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Prerequisites

The cloning and propagating techniques detailed below require a facility with the following resources:

1. Laminar Flow Hood (99.997% filtration @ 0.03 microns)
2. Antiseptic solution (70% Ethanol/ 30% Distilled Water)
3. Petri Dishes (glass or plastic)
4. Sink with hot water
5. PDA (Potato Dextrose Agar)
6. Erlenmeyer flask (1000ml)
7. Scalpel
8. Inoculation loop
9. Magnetic stir rod
10. Magnetic spinner
11. Aluminum foil
12. Pressure cooker capable of 15 PSI or Autoclave
13. Household dish soap
14. Stretchable petri dish tape
15. Ziplock bags, unused

Procedure

Gathering Samples

Samples were solicited from the general public

Prepare Petri Dishes with PDA Medium

Petri dishes were prepared using the following procedure:

Mixing the PDA Medium

1. Erlenmeyer flask is sanitized using hot water and dish soap
2. Flask is allowed to air dry
3. Magnetic stir rod is added to flask
4. Fask is filled with 10g of dry PDA

5. 250ml of well water is added to the flask
6. Mix flask for 10 mins using magnetic stir rod

Sterilizing the PDA Medium

1. Flask is cover with aluminum foil to protect the solution for excess water during sterilization process
2. Prepare pressure cooker or autoclave to manufactures specifications
3. Process the flask for 15 minutes at 15 PSI

Preparing the Petri Dishes

1. Laminar flow hood work area with an an antiseptic solution
2. When the flask has cooled to 121°F remove from sterilizer and bring to flow hood work area
3. Pour petri dishes using preferred technique in front of the flow hood
4. Allow petri dishes to sit undisturbed until they have cooled to at least 75°F (roughly 3 hours)
5. Use petri dishes immediately, or seal in a sterile bag for later use

Cloning Samples to Petri Dishes

1. Clean work area with an an antiseptic solution
2. Gather the required tools and place them under the laminar flowhood after spraying with antiseptic solution:
    1. Sterilized scalpel
    2. Sterilized (See: "Preparing the Petri Dishes") PDA petri dishes
3. Outside of the lab, gently wash the collected mushroom sample with a drop of dish soap and lukewarm (under 100F) water, ensure the specimen has been thoroughly rinsed off of all visible dirt and soap before proceeding
4. Pat the mushroom sample down with a clean paper towel and place inside a new ziplock bag
5. Transport the mushroom sample in the ziplock bag to the lab area
6. Clean your hands and arms with antiseptic solution, gloves and plastic arm protectors are recommended to ensure no contamination of the sample
7. In front of the laminar flow hood open a sterilized PDA dish under the flow hood, remove the lid and set the lid upside down under the laminar flow
8. Wash the ziplock bag containing the mushroom sample with antiseptic solution
9. Open the bag in front of the flow hood, ensure the flow does NOT blow on top of the petri dish (the mushroom sample must be downwind of the petri dish so contamination from the sample cannot spread
10. Remove the sample from the ziplock bag, spray with antispetic solution and allow to dry in front of the flowhood
11. Using one hand break the mushroom sample in half. Doing this will expose sterile mushroom tissue. Keep the freshly exposed tissue under the laminar flow. The laminar flow should be directly blowing on this newly exposed tissue after the mushroom has been broken in half. The half that is not being used may be discarded or dropped as long as it will not interfere with the laminar flow or the workspace area.
12. With your free hand pick up the sterilized scalpel and scrape a small piece of tissue away from the center of the newly exposed area of the mushroom. If the tissue will no stick to the tip of the scalpel you may need to cut a long 1mm slice in the center of the mushroom at a

45 degree angle roughly 5mm deep. This will allow a 1mm x 1mm section of tissue to be extracted.
13. With the tissue sample on the tip of the scalpel, gently place the sample in the center of the open petri dish. Do not allow the scalpel handle to touch the edges of the petri dish at anytime. Minimize the amount of time the scalpel is above the petri dish.
14. After the tissue sample can be visibly seen on the petri dish, discard the mushroom sample and scalpel
15. Rewash your hands and arms with anti-septic solution
16. Put the lid back on the petri dish, ensure your gloved hands do not contact the inside of the petri dish or its lid at any time.
17. Seal the petri dish with stretchable petri dish tape
18. Incubate between 45F and 85F until mycelium can be visibly seen

Preparing Sterilized Grain Medium

Quart gain jars are prepared with the following procedure:

Preparing Grain (quart) Jars

1. Quart size mason jar is sanitized using hot water and dish soap
2. Jar is allowed to air dry

Preparing Grain

1. Measure out 1/2 of a quart jar of dry rye berries per quart jar to be sterilized
2. Wash grain under cold water until it runs clear
3. Transfer grain into a pot and fill with water until grain is covered by at least 2cm of water
4. Allow grain to soak for 12 hours to germinate heat resistant endospores
5. Transfer the pot to a heating source and bring to a boil for 10mins
6. Ensure the grain is cooked (able to be crushed between two fingers) before removing
7. Drain the grain and spread out on a surface, allow grain to dry until it no longer leaves wet spots when padded with tissue paper
8. When the grain is dry on the outside, but fully hydrated on the inside it is ready to be put into jars, fill each jar to the 2/3 mark

Sterilizing Grain

1. Apply each lid to the mason jar, ensure a snug but not tight fit. Unscrew the lids slightly if they feel any tighter then hand tight. The jars will change under pressure during sterilizing, this step is to prevent the lids from becoming stuck.
2. Cover each lid with a 10cm x 10cm sheet of tin foil. Ensure the sides of the jar lid are fully covered and protected
3. Pre-warm your pressure cooker or autoclave if required
4. Load jars into pressure cooker or autoclave per manufactures instructions
5. Run autoclave/pressure cooker at 15PSI for 1 hour and 30mins

Cloning Petri Dishes to Grain

1. Clean work area with an an antiseptic solution

2. Gather the required tools and place them under the laminar flowhood after spraying with antiseptic solution:
    1. Sterilized scalpel
    2. Sterilized (See: "Preparing Grain Medium") Grain Medium
    3. Colonized petri dish (see: "Cloning Samples to Petri Dishes")
3. Clean your hands and arms with antiseptic solution, gloves and plastic arm protectors are recommended to ensure no contamination of the sample
4. Remove the tinfoil from the quart jar of grain
5. Remove the lid from the quart jar of grain, leave it upside down with the bottom of the lid facing the laminar flow.
6. Remove the petri tape from the colonized petri dish
7. Remove the lid from the colonized petri dish and set it aside, upside down with the bottom of the lid facing the laminar flow.
8. Using the sterilized scalpel cut a 1/8th triangle out of the petri dish. ensure to cut off the edges of the triangle shape. Do not use any agar that has contacted the very edges of the petri dish, as this increases the chances of contaminating the grain.
9. Replace the petri dish lid, do no touch the inside of the lid or dish with your glove hand
10. Close the grain jar and hand tighten the lid
11. Re-seal the petri dish if you intend to use it again later, otherwise it can be discarded

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