PROTOCOL

HOW TO GROW MYCELIUM BIO-COMPOSITE MATERIALS

Author:

Stefano Roccio – Researcher MNEXT (s.roccio@avans.nl)

Sub-authors:
Fran Ortega Exposito – Project leader MycEoLA & Researcher MNEXT
Ilse Rovers – Senior researcher MNEXT
Shannon Peuling – Researcher MNEXT
Neha John – Researcher MNEXT

STEP 0 – SPAWN PREPARATION

Mother spawn can be used to either replicate the mother spawn, or to inoculate either solid or liquid inoculum media. The inoculum media for the spawn at MNEXT is made from cellulose flocks; very nutritious for fungi. The moisture content of the cellulose is very relevant for proper mycelium growth and should be around 80%, this can be achieved by mixing water to the cellulose. spawn preparation should be performed by a trained laboratory technician, as it is a delicate process that demands strict sterile techniques and skilled laboratory practices. This step should be completed in a single session to prepare all the spawn required for the experiments.

Material & Equipment

- Medicarine
- 70% ethanol (or iso-propanol)
- Malt extract broth powder
- Demineralized water
- Cellulose
- Colonizer agar plate with fungal strain.
- 1L Measuring cylinder
- Erlenmeyer flask
- Cotton-aluminium foil cap, or Erlenmeyer stopper cap
- Scale
- Hot-plate stirrer
- Magnetic stirrer
- Autoclavable bag (SacO2)
- Autoclave tape
- Autoclave
- Monel cup
- Rotator for monel cup
- Inoculation loops
- Laminar airflow hood (LAF)

Procedure

Preparation of MEA Broth

- Dissolve 20g of Malt Extract broth in 1L of demineralized water (or 5g per 100mL broth) using a heated stirring device.
- Prepare a cotton-aluminum cap and autoclave the mixture.
- Allow the broth to cool down to room temperature before use.

Preparation cellulose bag

- Prepare cellulose by adding into an autoclavable bag 1:1.32 per gram of cellulose of demineralized water.
- Massage the bags to ensure even distribution of water within the cellulose
- Label bags (inoculum media – fungal type - date – weight - initials) and add autoclave tape on the bag
- Autoclave the bags containing cellulose mixed with water

- Once the autoclave run is concluded make sure to wait for the bags to cool down to room temperature.

Cellulose inoculation

- Clean the sterile Monel cup on the outside and rotator device with 70% ethanol and place them under the LAF
- Using an inoculation loop, cut a fully colonized petri dish (with the desired fungal strain) into four sections and transfer these sections into the Monel cup.
- Add 50mL of prepared and sterile MEA broth to the Monel cup.
- Place the cup on the rotator and trim at low speed for 30 seconds. Ensure that the entire petri dish has been adequately trimmed by visually inspecting the mixture.
- Pour the inoculated broth into the autoclaved cellulose + water mix and shake well.
- Move the bag to the incubator for 5 days at 30 °C in the incubator.
- Every 2 days shake and massage the bag.
- The spawn should be ready after 7-10 days.

Cellulose inoculum preparation	
Total weight required	**200**
Water saturation	80%
Water content at 100 % WS	71.44%
Water content at RT	0.59%
Water content at desired saturation	57.15%
Cellulose weight (g)	**86.21**
Water weight (g)	**113.79**

STEP 1 - PREPARING SUBSTRATE

To ensure proper sterilization and a safe environment for substrate preparation, it is recommended not to place more than 160g of substrate into each bag. Accurate weighing of substrate and water, according to the specified ratios, is essential for consistent results.

Materials & Equipment

- Medicarine
- 70% ethanol
- Substrate
- Demineralized water
- Scale
- Autoclavable bag (SacO2)
- Autoclave tape
- Autoclave
- Tape or bag sealer.
- Laminar flow (LAF)

Procedure

Substrate preparation

- Weigh the substrate on the scale
- Weigh water to be added accordingly to the ratios in the table below.

Substrate type	Water per 1g substrate
Hay	1.32
Flax	1.38
Cacao	1.32
Wheat straw	1.32
Elephant grass	1.13
Bran	1.32
Hemp fibres	1.02
Hemp shives	1.43
Rapeseed straw	1.65
Wood fibre	1.32
Cellulose	1.31

- Mix thoroughly with your hands (wear gloves) or for large quantities, a cement mixer
- Place mixture in autoclavable bag
- Close bags with bag sealer or tape
- Label the bags (substrate type - date – weight - initials) and place autoclave tape.

Sterilization (autoclave 121 °C, 25 minutes)

- Check water level inside autoclave. Make sure to be able to see the water at the bottom and that the water does not overflow over the metal holes.
- Place bag(s) into autoclave.
- Check water level of exhaust pipe (front side autoclave)
- Keep in mind: the whole autoclave run will take approximately 1,5 hours.

STEP 2 – INOCULATION OF THE SUBSTRATE

This step should be carried out right after step 1, to ensure the substrate bags are still sterile. This step is sensitive and requires high sterility conditions.

Materials & Equipment

- Medicarine
- 70% ethanol
- Sterilized substrate
- Spawn
- Gloves
- LAF
- Bag for hazardous waste
- Sterile spoon
- Weighing boat

- Scale
- Kitchen blender
- Tape or bag sealer.

Procedure

- Remove the bags from the autoclave with oven gloves and wait for the bags to cool down to room temperature
- Clean the laminar flow (LAF) with medicarine first and 70% ethanol.
- Clean beforehand EVERYTHING you put inside the LAF with 70% ethanol
- Clean your gloves before starting and place a bag of hazardous waste inside the LAF
- Blend the inoculated cellulose with a kitchen blender (cleaned with ethanol) for 30 seconds to ensure even homogenous particle dimensions.
- Weigh the required spawn amount with a scale and pour it inside the bag.
- Weigh 10% of spawn of the total weight of the substrate + water (wet-weight) with a scale and pour it inside the bag.
- Seal bag with sealer or tape INSIDE the laminar flow and shake/massage it OUTSIDE the laminar flow to ensure even distribution.
- Label the bags again (substrate type – fungal type - date – weight of substrate – spawn weight - initials) and place autoclave tape.

STEP 3 – MOULDING

The moulding step can happen either right after the inoculation of the substrate, or after 7 days of growth of the inoculated substrate bag inside the incubator.

Materials & Equipment

- Medicarine
- 70% ethanol
- Inoculated substrate bags
- Moulds
- Perforated foil
- LAF
- Bag for hazardous waste
- Scale
- Tape

Procedure

- Clean the mould before putting it under the laminar flow with 70% ethanol.
- Tare the mould on the scale and fill it with the inoculated substrate to cover your mold
- Record the weight to ensure equal density of production for the rest of the moulds.
- Cut the needed amount of perforated foil to cover the top of the molds and wipe it with ethanol.
- Cover the mould with perforated foil and tape it to secure it.
- Label your mould with (substrate type – fungal type - date – weight - initials)

- Place the filled moulds in the incubator at 24 °C and 80% RH for 7-14 days, depending on your fungal type.
- Clean the LAF and tools used with medicarine first and 70% ethanol.
- Flip the samples inside moulds after 4 days of growth. (If applicable).

Cleaning

- When the moulding step is concluded, make sure to properly clean the LAF from substrate particles and disinfect the working table with Medicarine first and Ethanol 70% after.
- Ensure to clean the LAF filters to remove substrate particles.
- Clean the moulds with 70% ethanol.

STEP 4 – DEACTIVATION & DRYING

You can visually evaluate the proper growth of the samples by observing uniform mycelium colonization and the degree of whiteness. As the sample continues to grow, you'll notice an increase in color variations and a thicker mycelium layer. These colors may vary from shades of reds & yellows to greys, dependent on your fungal type. Ideally you want to deactivate when it is completely white and uniformly colonized.

Contaminations are important to detect. The most common contaminations present on mycelium samples come from bacteria or molds. Visually, they can appear: bright green, bright orange, as black patches or dots, pale grey and slimy layer or as uneven colonization of the sample.

- Take the sample out of incubator and out of the mould.
- The **contaminated samples** will be moved and handled **under the fume hood or the LAF.** Procedure: Open carefully the mold and place the contaminated samples and the perforated foil into the red hazardous waste bags. Wipe the molds with medicarine, 70% ethanol, then wash them with soap and water and place them in a UV sterilizer for 20 minutes if applicable.

Procedure

- Place the sample in the oven at 65°C for 24 hours and place baking paper underneath.
- Place your bag label(s) near your samples in the oven, or with tape on the sample.
- If drying a large panel, place a weight on top to prevent warping (with baking paper in between). Take into account that for thick panels, it will require more than 24 hours of drying.
- Weigh the sample at the end of the drying process.

ACKNOWLEDGEMENTS

This procedure was developed and refined over several SIA RAAK MKB projects, reaching its current form in the SIA RAAK MKB MycEoLA project. We extend our gratitude to SIA Regieorgaan for their continued support and to our partners within mycelium-related projects:

To find additional details, refer to the stop-motion video illustrating the production process: https://www.youtube.com/watch?v=BLWsuhXreR8&t=4s