

Isolate a new strain from supermarket mushrooms

This protocol explains how to domesticate a supermarket mushroom and turn it into an immortal culture.

Materials: plates with agar medium, supermarket or forest mushroom, hydrogen peroxide (H₂O₂) 3%, tweezers, sharp knife or scalpels, sterile toothpicks (recommended), glass vessel, rubbing alcohol.

Hydrogen peroxide is a highly reactive chemical, which we use to kill microbes. It can be put on wounds to disinfect them (kill the potential pathogens in contact with your wound).



You can get it from the pharmacy. You have to say that the dentist told you to dilute that and wash your mouth, to etch tartar. It's ~5 €.

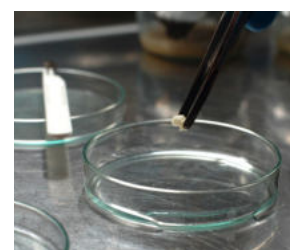
3% means that the solution is composed of 3% of H₂O₂ (hydrogen peroxide) and 97% of H₂O (water). 10% would be better, because it contains more of the active component.

Part 1: Preparation

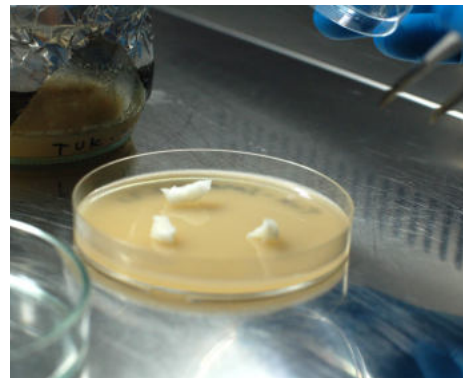
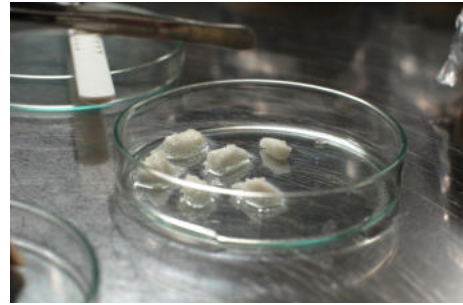
- Buy fresh, edible mushrooms from the supermarket (i.e. Shiitake, Oyster Mushroom, Portobello, button mushroom, whatever you like to hack).
- Prepare 3 agar plates (or more, as needed) and. Also plates-jars from the fridge work well!

Part 2: Cleaning and setting the mushroom in culture

- Prepare your work station; clean your workplace and tools with rubbing alcohol 70%;
Pour some H₂O₂ in a glass vessel (Petri dish here)
- Clean off the outside dirt of the fungus
- Cut away the outer material of the fungus to get access to the material which is inside the mushroom and has not been in contact with any bacteria (yet).
- Lean the scalpel in between and wipe dry on a clean piece of kitchen tissue if you transport a lot of dirt to the cutsite (dirt = material from the outside)

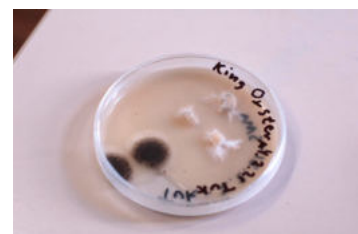


- Once you have access to the inside of the fungus, cut small pieces (like 3x3 mm, the smaller, the better)
- Put the mushroom pieces in the H₂O₂ bath and wait 5 minutes
- Open the lid and with cleaned tweezers or sterilised toothpicks place 3 pieces of mushroom into a PD with fresh medium. Place the pieces at a distance of 3 to 4 cm, making a triangular shape
- Prepare 3 of these plates to maximise your chance to find clean mycelium in the end. Use a new toothpick for each transfer!
(Work in sterile hood, closed box or open flame, to generate upwards air current and avoid microbes to fall on your medium)
- Close the plate with parafilm and incubate it for at least 4 days at room temperature



Part 3: Isolation

- If you spot any other organism growing in the Petri dish, transfer a piece of mycelium to a new Petri dish from the cleanest part of the culture under clean (possibly sterile) conditions
- Observe the growth for a couple of days
- If there are other organisms in the culture, repeat the clean transferring process. If the mycelium is the only organism in the Petri dish, congratulations! The mushroom is ready to be used for further experimentation.



The last petri dish shows an example of contamination (the black colony).