TYROLER glückspilze

Mushroom Cloning
(Level 3 – mushroom expert)

Gaining new cultures out of a fresh mushroom involves asexual reproduction. A mycelium achieved through cloning is called „pure culture“, it is genetically identical to the original mushroom. The successful breeding of a mycelium out of spores on the other hand is the way of sexual reproduction- the creation of a new organism with a new genetic code. When working with pure cultures, only one individual develops on the substrate, so there will be no competition for nutrients, leading to a higher and well predictable yield with stable quality.

Recommended Tools:

Hairnet and face mask
Latex gloves
Alcohol lamp
Scalpel with sterile blades
Workspace disinfect
Hand disinfect
Petridishes with antibiotic agar medium
Parafilm to seal the petri dishes
Glove Bag (sterile workspace)

Inoculating the nutrient medium with a mushroom clone:

Only select the most vital and gratifying mushroom fruit bodies. If you are not able to make a clone immediately after picking the mushroom, keep it neatly stored in the refrigerator for max. 48 hours. Do not store mushrooms longer than absolutely necessary because it will be harder to gain germination of mycelium out of older fruit bodies and the risk of bacterial contamination increases.

As in every laboratory working step during the life cycle of mushrooms, clean and sterile working is essential for success! Put on face mask, hairnet and gloves; clean the working area in front of the HEPA-filter or the Glove Box in-depth and disinfect working area and gloves. If you intend to clone more than one mushroom, disinfect the working space, change gloves and put on a fresh scalpel blade (or flame it until glowing red and let cool down) before carrying on with a new fruiting body.

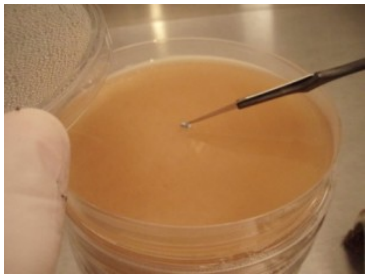
Fruiting bodies are basically composed of compacted mycelium, thus flesh intended to clone can be taken from any part of the mushroom, but most suitable are interior parts of the upper stem and cap. Split the near-ground part of the stem half a centimetre deep and tear apart the mushroom without touching the inner flesh.

Flame-sterilise the scalpel until glowing red over the alcohol lamp, let cool off and put on a sterile blade. For tissue harvesting we recommend to graft tissue from the most internal parts of a mushroom which provides greatest cleanliness and the lowest contamination risk. A piece sized 3x3 mm will suffice. Impale the small piece of flesh on the scalpel and transfer it onto the antibiotic agar medium. Seal the inoculated petri dishes with Parafilm and shelve them in a dark place to stimulate the mycelium growth. The adequate temperature depends on the mushroom to cultivate. A few days up to one week later the mycelium starts germination.

It has proven to be useful to inoculate several petri dishes with internal mushroom grafts of the individual you wish to clone since the results sometimes strongly vary. Even under best laboratory conditions you will have to face failure rates up to 10%. Amateurs and newbies should expect failure rates around 25% or even more, but should not get discouraged, with a little practice in cloning you will improve your skills quickly.

Label the petri dishes giving information about date, genus name, strain (strain means different species within one genus – comparable with different kinds of apples) and consecutive number. A CD-marker or permanent marker will do.





Mycelium growth

During the spawn run (development and growing of the mycelium) the sealed petri dishes are stored in a dark and neat place. The appropriate temperature depends on the genus being cultivated. Ensure that the incubator in which the mycelium is grown provides enough ventilation. After three to five days (depending on species) new mycelium filaments will start to grow out of the piece of flesh. As soon as rhizomorph mycelium strands become visible, they are ready for selection (look up the instructions for „Selection of mycelium strands“).

References:

„Mycelium running/ How mushrooms can help save the world“, Paul Stamets; Ten Speed Press, Berkeley/Toronto;
„The Mushroom Cultivator: A Practical Guide to Growing Mushrooms at Home", Paul Stamets, Agarikon Press; First Edition (December 1983);
„Growing Gourmet and Medicinal Mushrooms”, Paul Stamets, Ten Speed Press, Berkeley/Toronto;