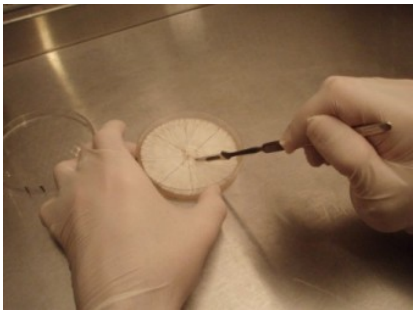
Inoculation of grain spawn with mycelium on agar medium
(Level 3 – mushroom expert)

Recommended tools:

Petri dish (at least 3/4 colonised, not mutated)
Sterilised rye substrate
Scalpel with sterile blade
Bag sealer or strong adhesive tape
Latex gloves
Face mask and hairnet
Disinfectants for workspace and hands
Sterile working area: Glove Bag/Glove Box or
sterile air flow (HEPA-filter, laminar flow hood)

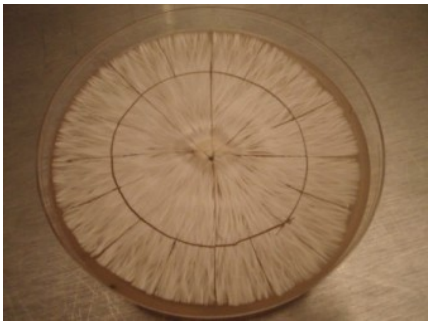



Inoculation

Perform this working step as aseptic as possible to prevent contamination of the grain by bacteria or mould. Clean your worktop, wash hands and forearms, put on face mask, hairnet and gloves and disinfect your worktop well.

Use the mycelium of 1 petri dish for inoculating 1 jar of sterilised grain (about 400 grams of substrate), for grain in a bag take more petri dishes. Avoid inoculation of 1 grain substrate with different mycelium strains; use only copies of the same clone per jar/bag.

Remove the Parafilm from a petri dish only inside the Glove Box or in front of a HEPA-filter. Carve eight lines in star-like array and a circle at half the diameter, centered into the mycelium. Thereby you got 16 small pieces.




Open the jars or the autoclave bags, spear the mycelium slices with the scalpel and transfer them carefully onto the sterilised substrate. Close the jar/bag tight after this is done. Now distribute the mycelium pieces

evenly by shaking the jar or bag to achieve fast and equal colonisation. In case that mycelium pieces stick to the inside walls of the jar or bag, they can be released by knocking gently from the outside, to get them in contact with the substrate again.

Mycelium growth

Store the inoculated substrate in a dark and clean place at convenient temperature for spawn run of this mushroom species. Ensure that the incubator in which the mycelium is placed provides enough ventilation.

After a few days mycelium strands become visible, growing from the small agar pieces and colonising the grain. One or two weeks after inoculation the jars and bags should be given a good shake to redistribute the already colonised matter amongst the unsettled material. This will accelerate the colonisation of the grain. Under convenient conditions rye grain should be fully colonised about 3 to 4 weeks after inoculation.

As soon as all rye grains are overgrown with white mycelium, the grain spawn is ready for inoculation of the fruiting substrate (e. g., compost, straw, wood – depending on the mushroom species). Use 2 to 10 % of grain spawn for inoculation of fruiting substrate, according to mushroom species and type of substrate.

Reproduction of grain spawn

To produce more inoculation material you can use grain spawn to inoculate sterilised rye grain substrate. For optimum results use 10% of ready colonised grain spawn, based on the weight of the fresh rye substrate. Please make sure to perform this step under sterile conditions to prevent contamination.

Unscrew the jars or open the autoclave bags, separate the grain kernels of the colonised spawn by shaking and

plumping them repeatedly. The fresh rye substrate can now be inoculated with grain spawn. Close the jar/bag tightly after this is done. Now distribute the colonised grain evenly into the fresh substrate, by shaking the jar/bag, to achieve fast and equal colonisation.

For mycelium growth stage, store the inoculated substrate standing upright in a dark and clean place at convenient temperature (spawn run) for this mushroom species. Ensure that the incubator in which the mycelium is grown provides enough ventilation.

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;