Preparation of Grain Spawn

This protocol describes how to prepare grain substrate in a jar for scaling up the biomass production

Materials: pressure cooker, scale, distilled water, rye grains, gypsum (CaSO4 - calcium sulphate), spoon, plate with well growing mycelium, scalpel, gloves, ethanol, jar, wadding

Part 1: Weigh Materials

Component	Quantity	Custom Quantity	
Rye grain	30 g	multiplier	g
Calcium sulphate (gypsum)	(2%) 0,6 g	x	g

Weigh the materials for the substrate and put the weighted gypsum aside

Part 2: Soak Rye Grains and Add Gypsum (1 hour)

- Pour boiling distilled water on rye grains and wait at least 1 hour to let them soak up the water
- Remove the remaining liquid
- Mix the gypsum into the rye grains and stir evenly
- Put the mixed material into the glass jar (jar has to be cooking/pressure resistant)
- Make a hole in the lid, and push wadding cotton through the cooking, and air exchange for the mushroom)
- Close the jars and cover the lid with aluminum foil

Part 3: Sterilize In Pressure Cooker (1 hour)

- Sterilize in the pressure cooker for 1h (No air tight containers, they burst!!!)
- Take off the lid from the cooker, and let the jars cool down (if you add the mycelium now, you will cook it)
- In the meantime while its cooling, sterilize surface, materials, tools and hands with disinfectants / spiritus

  Note: Note on the jar the dry weight of rye used. It will be helpful at the moment of transferring the grain spawn to inoculate new substrate

Part 4: Add Mycelium

- Cut a big piece of agar-mycelium from the PD and put it on the grain surface (take a piece from the outer part of the mycelium, where the young and fast spreading hyphae are growing) (Don't worry about transferring some agar too. Nothing will happen)
- Close the lid of the jar

Part 5: Incubate

- Incubate at room temperature in dark condition
- After 3-6 days, you may mix the grains a bit, to allow a faster growth (with sterilized spoons stirring in the jar, or gently shaking to break the clumps).
- Depending on the size of the jar and speed of growth, it may take 1 to 3 weeks to fully colonize the jar.