

Preparation of grain spawn

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

Materials: stove, pressure cooker, scale, distilled water (more clean than tap water), 100 g rye grains, gypsum (CaSO₄), petri dish (PD) with growing mycelium, toothpicks, tweezers, forceps, gloves, ethanol

Part 1: Weigh Materials

Component	Quantity	Custom Quantity	
Rye grain	100 g	multiplier	g
Calcium sulphate (gypsum)	2 g	x	g

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

Why add gypsum? It acts as a buffering agent to prevent acidification of the medium

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

- Pour boiling distilled water on rye grains for 1 hour and let them soak up the water
- Remove 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)



soaking rye grains

Part 3: Prepare Jar Lids

- make one hole in the metal lid and pull some wadding through it (the type of cosmetic cotton wadding used to remove makeup), to let oxygen in, but other organisms out
- Screw the lid on the jar, then cover with aluminum foil

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lid with wadding seen from top and bottom



jar covered with lid and foil



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Part 4: Sterilize In Pressure Cooker (1 hour)

- Sterilize in the pressure cooker for 1h (No air tight containers, they burst!!!)
- Take off the lid, and let the container 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

Part 5: Add Mycelium

- With a quick and determined movement, cut a piece of mycelium from the PD and put it on the grain surface
- close the lid of the jar

Part 6: Incubate

- Incubate at room temperature (25 – 30 °C) 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, when no tough clumps are inside).
- Depending on the size of the jar and speed of growth, it may take 1-3 weeks to fully colonize the jar.



Grain spawn from jar



Well grown mycelium in jars