

Liquid Culture Basics

original source: <https://www.shroomery.org/9145/Liquid-Culture-Basics>

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Notes

Liquid (tissue) cultures are used to expand mycelium into a liquid solution to inoculate your chosen substrate. Like a multi-spore syringe, except the spores have germinated into a network. Since the spores are already germinated; colonization times are substantially faster and inoculated substrates have an edge over contamination with speed.

In addition to the significant speed boost over MS (multi spore) inoculation, mycelium is not harmed by hydrogen peroxide (H_2O_2), so this can be added to your substrate to help reduce contamination. Normally you can not do this until the mycelium has started growing, because H_2O_2 kills the spores.

One spore syringe can be made into gallons of liquid culture. One spore print, agar culture and mushroom tissue can also make gallons.

Liquid cultures are economical, as 1cc from a spore syringe can supply you with a large volume of liquid inoculant which can be used on many jars/bags. Also if the liquid inoculant is a clone (generated from sectorized agar or mushroom tissue), then each jar should show similar growth speeds and maturity.

Liquid cultures are normally at a 4% dilute solution of various sugars and other nutrients in water. This would be 4 grams of sugars per 96 ml/cc water. (Water weighs 1 gram per ml/cc.)

Medium

Some nutrient sources are:

- Karo (You want the clear one that has the red label; "Light with Vanilla". DO NOT get the dark Karo, such as the one containing brown sugar)
- Honey (Non-organic has been known to work, but organic honey is highly recommended.)
- Corn sugar
- Light malt extract (or extra light. The lighter the better, because it will make it easier to observe growth in the jar.)
- Dextrose (glucose)
- While only the above are recommended, because they have been tried and tested, other sources have been successfully used, such as organic maple syrup.

Household sugar (sucrose) shouldn't be used.

Dextrose alone is usually not recommended, but will work, due to the lack of additional nutrients its growth

may be slower but due to its clarity it may be easier to spot contamination. Light malt extract and honey can be used alone. Additional nutrients can be added such as peptone, various flours could be used but it is much harder to determine the stage of mycelium growth due to cloudiness.

1 tablespoon of light malt extract and 1 tablespoon dextrose were weighed out. These are the weights;

1 tablespoon light malt extract = 10.3g

1 tablespoon dextrose = 10.1g

Give or take a point of a gram.

Malt and Dextrose

One member uses -

1 tablespoon dry malt extract or dextrose to 250ml (1 cup) water.

Another -

2% dextrose and 2% light malt extract.

This would equal close to 1/2 tablespoon dextrose and 1/2 tablespoon light malt extract per 250ml (1 cup) water.

Nanook from Nan's Nook uses -

1 level teaspoon dextrose (or light malt extract) to 75ml water.

-OR-

1 1/4 teaspoon dextrose (or light malt extract) to 100ml water.

Honey

4 cc/ml is the exact 4% ratio wanted. A syringe without the needle inserted is good to use as a measuring device. 1 teaspoon organic yellow honey to 100ml water is fairly close.

Note on ratio of solution

If your solution is a little off (3%-5%), don't worry. It'll still put out viable mycelium in most situations. It is better to be too weak a solution than too strong, too strong a sugar solution (around 10%) is toxic for the mycelium, and will not let anything grow in it (why jam is called preserve!)

Mixing

Once you have picked your method (which ever suits you best or is easiest to get) then its time to do some mixing.

Optionally, water can be hot or warm before adding sugars to allow for quicker dissolving.

Wrap top with aluminum foil and place jar in pressure cooker and slowly bring it up to 15 psi. for 15-20 min. Longer with Karo/Honey can cause caramelization.

Allow pressure cooker to cool before removing.

Vacu-tainers

You can bore a small hole big enough for a syringe needle in the top of a jar. (Half pints work best for this) Now put a blob of silicone sealant on it (preferable transparent) on both sides. Swirl it around to make sure it is a centimeter thick around the hole on each side. This is a self healing inoculation point so you can add spores and suck up inoculant without having to open the jar after sterilization. If you band the jar tight before pressure cooking, it will form a vacuum and suck in spores, so you must only prick the injection spot quickly. If

you leave the band loose, you should tighten it right after the pressure cooker has cooled down, as it will not have a vacuum seal. You should always wipe your silicone injection spot and needle (flame sterilize before) with alcohol before inoculation.

Agitation

Some people add a piece of broken glass, a glass marble or a pebble to the jar before sterilization. Agitating allows you to cut up the mycelium which can form into an unsuckable clump in the jar. This is why wide (18 gauge or lower) needles are preferable.

A slightly more advanced method is adding a stir rod (or just a 1" piece of non-insulated wire) to the jar and using a magnetic stirring plate to agitate the mycelium. This is the preferred method of agitation because it doesn't have the potential to get your lid filter (polyfil for example) wet when you shake the jar, which can lead to contamination. Do it yourself (DIY) magnetic stirrers are pretty easy to make and there are a ton of guides available both on the Shroomery.org forums, and the Internet.

(TODO) Add links

Microwave sterilization

It is possible to sterilize honey/Karo in the microwave. Be sure to add more water to the mixture as it will boil off during heating leaving you with a more concentrated sugar solution (this does not occur in a pressure cooker). Do not use metal bands in the microwave. Plastic lids are sold next to the metal ones. Never put thin metal like tinfoil or syringe needles in the microwave. Bring the liquid to a boil, then turn to low/defrost for about 15 minutes. KEEP THE CAP LOOSE! Allow it to cool completely in the microwave for a few hours.

Sediments

Once removed, some sediments may be present.

To fix this, open up the jar and filter liquid through 2 coffee filters, stick liquid back in jar, cap with filter lid, and pressure cook again. If you have a lot of liquid you can decant it carefully into another jar leaving the sediment behind. (The sediments are not harmful but can be mistaken for mycelium growth, it is nicer to see clear growth) This should not be done with Karo/honey. After a few days with honey proteins may sink to the bottom or float around. After shaking these will re-mix.

Aeration

During sterilization/heating most of the oxygen will be driven out of the liquid. Shaking will help the network grow faster, but things like hooking up air flow to jars are not necessary. Be careful not to wet the filter patch (if using one) as this can cause contaminants to grow from the outside inwards through the filter.

Growth

Once inoculated by whatever means (spores, clone, agar), stick in a DARK place with a temp of 82-86F optimally, and room temperature if there is no incubator available. Signs of growth after one week max, and fully done at week 3 max. Some see growth in under a day and fully done in 3. Once growth has slowed down (done), either use immediately or store in a fridge.

Storage

Liquid cultures can be stored in a fridge for 6-8 months (or longer). Some add a little H₂O₂ (approx 1-3cc) at this point since the mycelium is able to handle it, this can help prevent contamination.

Sugar carmelization

With Karo and Honey, if you PC for too long your solution may turn yellowed. This is called caramelization and is over-baking of the sugars which may result in little or no growth at all. If this happens you can still try and grow a culture in the caramelized sugar jar, but if you are pressed for spores it is best to just start over. This is something you want to avoid. Liquids don't take very long to sterilize so you don't get any benefit from PCing for longer than 15-20 minutes max.

Boil sterilization

If you do not own a pressure cooker, boiling can also be used. Bring to a high rolling boil and boil your containers with water at least halfway up the jars for 20 minutes.

Notes

- 1 ml water weighs 1 gram.
- 1 tablespoon dextrose weighs ~10 grams. (may vary slightly)
- 1 tablespoon light malt extract weighs ~10 grams. (may vary slightly)
- 10 ml honey weighs 14 grams.
- 1 tsp (5 ml) honey = 7 g
- 1 tbsp (15 ml) honey = 21 g