

Homebrew Media for the Microbiology Hobbyist

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Introduction

Bacterial growth media are an essential part of isolating and culturing microbes. The correct choice and use of media can select for desired species, control and influence their growth and behaviour, and reveal a great deal about their metabolism. The first decision a microbiologist must make when culturing bacteria is which medium to use, and the results will vary hugely depending.

However, for the amateur biologist, the requirement of sterile and appropriate growth media can be offputting or intimidating. It shouldn't be! Here, I intend to lay out how you can make your own growth media using only off-the-shelf and easily obtained ingredients.

The only part of this procedure that might be any different to what you'd normally do while preparing a meal is the necessity of sterilising by pressure-cooking, which is a skill used in kitchens worldwide already. If you don't know how to use pressure cookers though, do your homework and ask for a demonstration from someone who does. NB: Never seal jars/bottles before autoclaving.

So, why the perceived difficulty? Bacteria are often accused of growing on anything after all, so it stands to reason that creating something to encourage their growth shouldn't be hard. Within reason, this is true. Some kinds of bacteria will grow in almost any “medium” you cook up, which is precisely the reason that things have to be a little more complicated- to prevent contamination, and to select for the type of bacteria you want.

The simplest sort of bacterial medium would likely consist of broth from boiled food and a bit of sugar. In fact, this is described below as “Potato Dextrose Agar/Broth”. This kind of medium provides plenty of nutrients for the bacteria; amino acids, assorted vitamins and minerals etc., while the sugar is the “carbon source”, the bit that gives the bacteria energy and the raw atoms of carbon needed to create the complex molecules needed for growth.

However, the need to sterilise our media before use (so you can grow just the bacteria you wanted in the first place) puts some caveats on our ingredients; sugar, for example, undergoes a hefty few changes as you apply heat, making it unsuitable for prolonged autoclaving (pressure cooking). So, alternatives are used where possible, such as Glycerol. The same may be true of a lot of the components of your broth.

We also need something a little more standardised than “boiled broth and sugar”, because if you

grow something interesting or have a particular culture growing happily in your medium, you may want to share your results with someone else in a way that makes sense. For this reason, purified and partially digested protein (called “Tryptone” or “Peptone”) and Yeast Extract are the standards usually used. An easy way to make homebrew Tryptone will be discussed later.

Let's look at a few media that can be used for some interesting growth experiments..

Lysogeny Broth

The most popular medium used in labs today is called Lysogeny Broth, or LB broth. When it is gelatinised with Agar to make solid plates, it is called LB Agar. It is an ideal growth medium for enterobacteria like E.coli, and simple enough to allow easy customisations. A medically relevant example is adding antibiotics or toxins to see which the bacteria can resist or survive.

LB Broth is composed of the following:

- *1L Deionised/Distilled Water (Can use bottled water)*
- *10g Tryptone** - As a carbon source, and also for energy. Saves us from adding sugars.
- *5g Yeast Extract*** - For vitamins, minerals and nucleic acids.
- *10g Sodium Chloride (Table Salt)*
- *[15g Agar if desired]* – Only needed for making agar plates.
- *Sodium Hydroxide*** (AKA Lye/Caustic Soda) to make pH7.5*

Luminescence Medium

This medium can be used to isolate and grow bioluminescent marine bacteria from fresh fish, shrimp or squid (or just seawater sometimes). It's pretty salty, so most non-marine bacteria won't grow well on it. It requires addition of Glycerol as an energy source: it is more readily digestable by the bacteria to encourage vigorous light production, and some mightn't be able to grow on Tryptone alone. Note that this reduces the amount of Tryptone called for.

- *3ml Glycerol*
- *5g Tryptone**
- *5g Yeast Extract***
- *1g Calcium Carbonate****
- *35g Sodium Chloride*
- *[15g Agar]*

* Tryptone is partially digested protein. We'll look at making this homebrew a little later!

** Yeast extract + Salt = Marmite. Health stores usually stock a no-added-salt Yeast Extract.

*** Both of these are used to make the medium more alkaline, and are probably interchangeable!

Potato Dextrose Broth/Agar (Abb. PDB/PDA)

This simple medium is used to grow bacteria (or molds/fungi/yeasts) that attack plants in the soil, and consists happily of only two key ingredients besides optional Agar. Dextrose, being not unlike normal sugar, will react oddly with other molecules as it caremelises in the autoclave, so sterilisation after pressure is reached shouldn't exceed 15 minutes. You can use PDA to grow common yeasts used in the kitchen, perhaps to breed your own strains or to test for the viability of your yeast before baking an important cake. Or maybe you'd like to grow up broths of Yeast and make your own yeast extract!

- 200g *Potato Infusion* (Boil scrubbed/sliced potatoes for 30mins and filter out solids)
- 20g *agar*
- 20g *Dextrose*

Difficult Ingredients and Unaccounted Additives

With the above recipes, any reasonably equipped lab should be able to make up large broths in short order, but what about the hobbyist or low-budget lab? How do we avoid the added complexity of ingredients like Calcium Carbonate, Sodium Hydroxide and Tryptone?

If in doubt, Google can provide answers. Calcium Carbonate is used in popular antacid tablets like Rennie, forming the most part of a tablet. If you can find one that is solely Calcium Carbonate, much the better! Just avoid sugars or too much of anything but Calcium salts.

So that's one "difficult" ingredient solved! Another one is Magnesium Sulphate, or $MgSO_4$.. also known as Epsom's Salt. You can get this as bath salts, or maybe from a pharmacist. Look for it pure.

Yeast Extract is easy; it's commonly sold as "marmite" in shops, but the salt content might be inconvenient for our uses. Look in health-food shops for extracts without added salt.

Sodium Hydroxide, AKA Lye, AKA Caustic Soda, is available as a kitchen chemical. Try to find one that lists ingredients, so you can get as close to pure Sodium Hydroxide as possible. Ensure the other additives are things you know and understand. Or use Calcium Carbonate instead; both are used as alkaline buffers. If using Sodium Hydroxide, add and dissolve it in water *slowly*. As a strong base, it'll heat up fast. If you can get a working formula using Calcium, it's much safer!

Tryptone is the most awkward one here, because there's probably no off-the-shelf replacement for "digested peptides and amino acids". Fortunately, with some easily available ingredients, you can make your own using soy protein and meat tenderiser or digestive aid tablets!

Making Homebrew “Tryptone”

Tryptone is just milk protein (casein, to be exact), digested by Trypsin, a digestive enzyme found in the stomach. Before you go looking, Trypsin isn't normally available in shops.

However, Trypsin is just another protease, an enzyme that digests any other protein at specific points along the molecule. There are many proteases out there that are routinely used in kitchens. Meat tenderising powders, for example, are usually made with the enzyme Bromelain, which is derived from pineapples. Natural digestive aid tablets often contain plenty of Bromelain or related fruit proteases, which are probably equivalent for this purpose.

Casein should be available as pure protein in some health stores. However, trace lactose may remain in these powders, which could interfere someday if you want to use the LacZ promoter to induce your genetically engineered bacteria to express something. If you know you're not worried about lactose contamination, try making Bromelaine-Tryptone (Bromatone?) and share your results!

However, to establish a standard medium from the get-go that you know won't interfere with your experiments later on, it may be best to use a different protein. Here's a recipe using 100% Soy Protein Isolate to make Soy Phytone, a close analogue of Tryptone. This should be suitable for all applications; BD Biosciences markets a Soy Peptone which supports all manner of microbes.

Phytone – Soy based Peptone

- *Soy Protein Isolate 50g*
- *Deionised or Mineral Water 500ml*
- *Bromelain (or Meat Tenderiser), 1000 GDUs or a generous dose of powder (1+ tsp)*

Dissolve the Soy protein fully in water, then heat the resulting broth until simmering. Lower the heat, but keep very hot for 5 minutes. This may help to degrade any protease-inhibitors that might be lurking in your Soy Protein, which would prevent Bromelain from doing its job. We'll be adding so much Bromelaine that this mightn't matter, but everything helps. Cooking the protein will also help pre-degrade the protein, which may help the Bromelaine digest it.

Allow the mix to cool until it is slightly hot to the touch, and keep it that way. About 45C should be ideal for digestion, but don't worry too much. Bromelain has a wide pH and Temperature range in which it can work. At this point, dissolve in the Bromelain. Keep warm and stir occasionally for about 20 minutes. Then simmer for another 5 minutes to deactivate the Bromelain enzymes.

You can simmer the protein to reduce it down or even dry it out, but you can also use it directly if you calculate the peptone content. Redissolve any residues, measure the remaining fluid in your final mix. Divide starting protein by remaining volume to get Peptone/mL.

Making Media: An Illustrated Account

So, you know how to make peptone and you have recipes for media. You've bought all the ingredients you'll need and you're ready to start. On to the process! Here is a guide to making Luminescent Broth and Agar at home.

<p><u>Step One:</u> Roll call. Assemble your ingredients, check the quantities you'll need against the recipe, and confirm you have enough. Plan and jot down the amounts you'll need for the volume you need/want. Make sure you have the tools you'll need: spoons, measuring jugs (one with as small/accurate a grade as you can get), as accurate a weighing scales as possible, a blender, and a pressure cooker. Alchemist's Skull optional but recommended.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step Two:</u> Measure soy powder desired for final medium blend (5g/L), dissolve in $\frac{1}{4}$ the final volume of bottled/distilled/deionised water. Blending it may help it dissolve. Simmer to neutralise protease inhibitors. Shown in the picture is much more protein than needed; having a very low-resolution scales, I made up a large batch of tryptone.</p> <p>Cool to 40C-45C, and dissolve 1000GDU of Bromelaine. In this picture are shown two digestive aid tablets, each containing around 500 GDU.</p> <p>Maintain at around 45C for 20 mins, stirring occasionally. Then bring to simmer and simmer for 5-10 mins to destroy enzymes.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step 3:</u> Measure and dissolve salts in another $\frac{1}{4}$ volume of water. For 1L of Luminescent broth, we want 35g of Sodium Chloride (sea salt or table salt..possibly not rock salt, as mineral content may be different!) and 1g Calcium Carbonate. Note that “salts” is a general term for dissolved ionic compounds, to biologists; it doesn't necessarily mean table salt at all.</p> <p>Shown is a pair of antacid tablets and the back of the packet; note that each tablet contains about 500mg of Calcium Carbonate, and we want 1g. Hence the pair.</p>	<p>(see this edition instead for pictures)</p>

<p><u>Step 4:</u> Put a little spoon on the scales and set the scales to zero. You can use this spoon to scoop up as much yeast extract as you need without having to mess about too much. Now measure out your Yeast Extract; we want 5g for Luminescent Medium. Dissolve this in a little bit of boiling water (as shown, with the mixing glass also immersed in hot water) to make it dissolvable in the rest of the medium.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step 5:</u> Add 3ml Glycerine/Glycerol and Yeast Extract to another ¼ volume of water. You probably won't have 100% glycerine, as many pharmacy formulations of the stuff are designed to be taken directly for sore throats etc. The one shown here is the closest I could get at short notice; 30% glycerine with only a trace of aniseed. I'm sure the bacteria won't mind the taste of aniseed.</p> <p>However, note that this 30% dilution means that to get 3ml of Glycerol, I'll need 10ml of this stuff. I measured it out with a medicine spoon.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step 6:</u> Mix it all together. Put the three volumes you have prepared together in a blender, and add enough water to make your full volume. In this example, I'm making a litre.</p> <p>Shown are the before and after. I had to wait a while for the froth to recede that much. The ingredients really lend themselves to a fine froth, but it's not that important.</p> <p>More important is the detail perhaps visible on the before picture; a slight scum on the surface of Calcium Carbonate and a murky appearance indicates that it's not properly homogenised. If I divide this up before blending it, I may end up with different formulations in different containers; bad news for repeatability and standardisation.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step Seven:</u> Optionally divide and add agar. I wanted Agar plates to streak out bacteria, so I could try to isolate single colonies to culture in the broths.</p> <p>So I separated out about 200ml from the main stock, and added a proportional amount of agar according to the recipe, plus a little (it's better to be a little too hard-set than a little too soft).</p> <p>Repeated microwaving didn't dissolve all the agar, but as long as it's moderately homogenous it doesn't matter.</p>	<p>(see this edition instead for pictures)</p>

<p><u>Step Eight:</u> Pour Plates, before or after. If you have a sterile workspace (such as near a bunsen burner or under a flow hood) you can pour your plates after autoclaving before the gel cools fully and sets. Having no such clean area at home just yet, I instead poured my plates in advance of autoclaving.</p> <p>Each “plate” (jam jar lid or creme-brulee cooking dish) was seperately wrapped in tin foil to preserve sterility, then stacked carefully. I used lengths of tinfoil card to help them stack neatly.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step Nine:</u> Aliquot remaining broth, seal and prepare to autoclave.</p> <p>Aliquoting, as the practise of separating into smaller fractions is known in the lab, is a great way to prevent a whole batch being exposed to potential contamination when you only need to use a little. It's better to divide your broth into your working fractions (like separate culture jars/tubes/flasks) before sterilising to minimise chances of contamination.</p> <p>If you don't have suitable lids that'll survive autoclaving, you can use tinfoil.</p> <p>If you use lids, be sure to leave them one turn loose when you put your stuff in the Autoclave; due to the pressures involved in autoclaving/pressure cooking, they will shatter if pressure cannot equalise. Leave the lids on but loose.</p> <p>Use something to keep your vessels off the floor of the cooker so they don't break: a fold-out colander with metal feet would be excellent for this task. For want of an appropriate colander, I raised a stack of foil-card discs off the floor of the cooker using metal napkin rings.</p>	<p>(see this edition instead for pictures)</p>
<p><u>Step 10:</u> Autoclave!</p> <p>If you've never used a pressure cooker, now's a good time to ask for a lesson from someone who has, for safety. In my case, I looked up some youtube videos and read the manual a few times. What I describe is what I did, but don't take it as canon. Play it safe and get a second opinion.</p> <p>Use some water at the bottom of the cooker to provide steam and prevent drying out and overheating of the cooker. I used two large mugs for this 11L cooker; it's better to have more than you need than less than you need, so don't be afraid to put in extra.</p> <p>Make sure your rubber seal is in the lid before affixing it. Seal the lid securely by rotating into</p>	<p>(see this edition instead for pictures)</p>

place. If you have a weight that sits on the main valve to set the pressure, leave it off for now.

Apply heat slowly, especially if you're not using proper borosilicate glass. Listen for the sound of simmering and look out for steam to come from the valves to indicate boiling has started. The pressure interlock on your cooker should kick in. When there's a good amount of steam emerging, pop on the weight to apply pressure.

Step 11: Cook and Cool

Leave the cooker for 20 minutes once it has reached full pressure (the weight rises and starts sputtering lightly at full pressure. If it gets too loud and agitated, you're too high; reduce the heat!). Stick around and ensure you can always hear boiling water in there and see plenty of steam; if it dries out, take it off the heat immediately and force cool it with wet cloths to reduce pressure. Discard the lot if this happens.

If all goes well after 20 mins you can take the heat away. Don't force cool the vessel, as this will create a sudden reduction in pressure and cause the containers within to boil over (especially messy when agar is involved). A good rule is, wait for the pressure interlock on the handle to drop, and wait another 15 minutes before opening.

If you plan to add antibiotic to your broths or agar, you should do it after autoclaving once it becomes cool enough to hold without pain, but before the agar gels. This is because most antibiotics are heat-sensitive, and are easily destroyed at temperatures above 45C-50C or so. Normally one would filter-sterillise antibiotics to ensure they do not contaminate the medium. If you have pre-made sterile antibiotics, all the better.

If you're not going to use your media or plates right away, seal the lids that you cleverly left loose earlier, and put them in the fridge. Properly autoclaved, a sealed container of medium should remain sterile forever. Petri dishes allow airflow, so they are less long-lived, but they should still be sterile for a long time if undisturbed.