

Yeast Culturing

By Stephen Elgey. MBBS(Hons), B.App Sc – Medical Laboratory Science.

At some point, many brewers discover that dried yeasts do not allow them to produce the range of beers required to satisfy their quest for their perfect beer. This is not to say that dried yeasts are inferior (Safale produces an excellent range), but most strains of yeast are not able to be dried and packaged as they don't readily tolerate the drying process. Many of these strains are available as Liquid yeasts (eg Wyeast, Whitelabs), but cost upwards of \$14 per brew. Additionally, some of your favourite brews may be live conditioned and you can retrieve the yeast for use.

Yeast banking can be handy as you have the yeast that you need for the right beer, but it can be a lot of work and you need to begin preparing your yeast for up to 1 week before brewday. You can't decide to brew on the spur of the moment.

Here are some techniques and methods that I have used successfully for several years.

Culture Media (Liquid or Solid Media)

This media, although not sterile by microbiological standards, is pasteurised. We are only trying to inhibit the growth of fungi and common beer spoilage organisms. Spore forming bacteria such as Clostridia spp (eg *C.botulinum* - Botulism) will not be destroyed. However, wort is not a good growth media for these organisms so they will not multiply to any significant extent if stored correctly.

If you really want to sterilise, then I suggest that all media is autoclaved, or failing that cooked in a pressure cooker for 15 minutes at pressure.

Equipment

500+ ml Glass jar with heat resistant lid.
Measuring cup and spoons
Microwave oven
Pressure cooker (Optional)

Materials

1/2 cup light dried malt extract
3 Tsp Agar (for solid media – from Chinese grocery))
Clean Dechlorinated water to make up to 500ml

Technique

Add dried malt to clean jar or glass measuring jug. Top up with water to 500ml, place on cap and shake thoroughly to break up balls. This should give a wort of approximately 1.040 SG. Loosen lid and microwave on high to bring to the boil. Remove and tightly cap.

NOTE : You must watch carefully. As soon as the solution begins to boil it will form a large amount of foam that WILL overflow. Turn off as soon as boil is reached.

This is the base media that can be used for liquid cultures. To make solid cultures, then add the agar, shake vigorously and again bring to the boil carefully. This solution really will foam vigorously. Tightly cap after boil.

At this stage you can pressure cook to sterilise, pour into petri dishes for immediate use or cool and place in refrigerator for later use.

Pouring Solid Media

Agar has a unique property of having different melting and setting temperatures. It will melt at approximately 90C, but will not set until it falls to around 40C. What this means is that once set, it will not melt at temperatures used for culturing (unlike gelatine).

Solid media once set should be stored and used UPSIDE DOWN to prevent condensation from dripping onto your media. I do not recommend making more than is needed unless your media is sterile and you have a perfect technique.

Equipment

Flame source, eg gas cooktop
Petri Dishes, or small containers with lids that can tolerate boiling water. (If containers are not sterile, clean thoroughly, add some water and microwave for several minutes to pasteurise)
Microwave oven

Materials

Solid Culture Media (see previous)

Technique

Light flame source and place dishes bottom down with lids on near to this. This provides an updraft that keeps wild yeast etc away.

Melt solid media in microwave with lid loosened. You only need to melt enough that you wish to use.

Near your flame, remove the lid and run the lip of the media jar through the flame, then lift off the lid off your dish and pour a layer of media about 3-5mm in depth. Run lip of jar back through flame and cap.

When set, turn dishes upside down and allow to cool.

Using Solid Media

To use solid media, you need to be quick and organised. The idea is to get growth of even single colonies of yeast that can be picked off and used to produce a starter.

To achieve this, you need to 'streak' a plate. I recommend you use the '12 Streak' technique.

Producing a 12 Streak Plate

Equipment

Flame source. Eg gas stovetop
Microbiological Loop
Culture chamber with water reservoir

A Microbiological loop is made from a thin wire twisted together with a loop of approx 2-3mm at one end, and a handle on the other. The essential quality is that it heats to red hot quickly, and cools quickly. Thin stainless is ideal. Commercial varieties are made of platinum.

A culture chamber can be as simple as a lunchbox with a film canister of water in it. As long as it holds your dishes and has a lid, it is perfect.

Materials

Petri Dish or Culture Dish. Store lid down
Yeast source (liquid)

Technique

To produce single colonies, a 'lawn' is created, from which you streak the loop through 4 times from edge to edge. The loop is flamed after every set of streaks. You then streak the loop through the first 4 streaks four times, flame and repeat for the next four streaks. A single final streak follows. You hope to produce single colonies by the final 4 streaks.

The 'lawn' is made by flaming your loop until red hot, opening your yeast source and dipping the loop in. A thin film of liquid will adhere to the loop. You seal your yeast container then lift the dish off the lid and run the loop all over **1/3rd of the dish area**. You may need to repeat the dipping to cover the area. The colonies that grow here will be confluent.

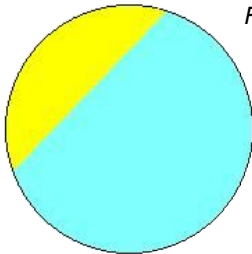


Fig 1. Lawn spread over 1/3rd of plate area. This lawn is not used to collect yeast for starters.

Flame the loop and dip near the lower corner of agar to cool. Streak from an outer edge in the lawn to an opposite edge of the clean agar. Repeat 3 more times and then flame loop

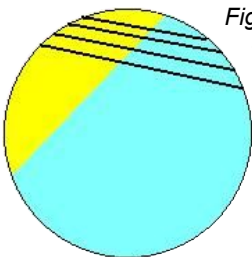


Fig 2. 4 streaks from lawn across to opposite edge. Observe how the streaks are close, but do not overlap.

Cool the loop in a clean part of the dish, and streak 4 times through the first 4 streaks. Flame loop.

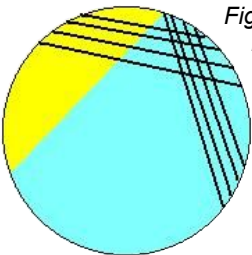


Fig 3. 8 streaks completed. Note how these streaks **DO NOT** pass into the lawn.

Cool the loop and run 4 streaks through the prior 4 streaks.

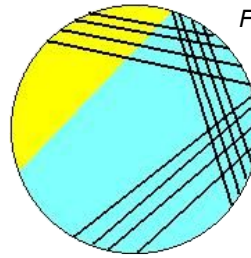


Fig 4. 12 streaks. Again observe how the streaks do not pass through the original 4 streaks. You only cross over the preceding streaks

Finally, flame and cool the loop, then run a single squiggly line through the last 4 streaks. This completes the plate. Relace dish upside down onto lid and flame loop.

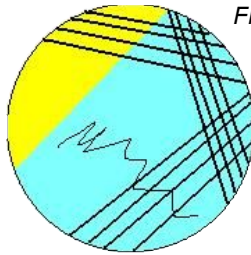


Fig 5. Completed Plate. This method ensures that you will get single colonies.

Put plate in culture chamber upside down. Place small amount of water in chamber, seal, and leave in a warm place to grow. Do not expect to see colonies or growth for at least 24-48 hours.

The area of the lawn will be a mess of white yeast. You will see lines of yeast along the first 4 streaks, and then individual colonies will begin to appear. Yeast colonies have a white creamy appearance. If it is slimy or opaque, then don't use it. It may be contaminant yeast or respiratory deficient mutants.

Harvesting Yeast from a Dish

You need to pick off individual colonies with your loop and transfer these to whatever you want to culture them in. The loop will only hold about 1/2 to 1 colony depending upon the size. I find it easiest to flame the loop, cool in a clean part of the agar, and then scoop the colony off. Cleanliness is paramount here, so work near your flame source.

Equipment

Flame source, eg gas cooktop
Microbiological Loop

Materials

Petri Dish with Active yeast
Liquid Culture/Storage Media in small bottles

Working over your flame, open your Culture bottle and flame the lip. Flame the loop. Lift the petri dish off the bench and dip loop in agar to cool. Select a nice looking colony and lift off, put dish down and transfer to bottle. Flame loop and repeat collection process until sufficient colonies obtained. I tend to collect yeast from around 5-10 colonies for starters.

If a starter is being made, incubate at desired temperature until activity is seen. From here, step up gradually to a full size starter

If storing yeast, then transfer bottle to appropriate storage compartment.

Storing Yeast for Future Use (Yeast Banking)

Yeast can be stored unused for several years under the right conditions. Live conditioned beer is a case in point. It is not always practical to store like this. There are 2 methods that I use – storing frozen, or in sterile water at fridge temperatures. Either method is acceptable, but frozen yeast if properly prepared and stored should remain viable for 5-10 years at least. I have cultured yeast from water storage at up to 2 and half years.

The disadvantage of frozen yeast is that you are not able to continually freeze, thaw, then refreeze etc. the yeast will die after 2-3 freeze/thaw cycles. What you can do is freeze some of your yeast to act as a disaster recovery in case your fridge cultures get contaminated. The yeast stored in the fridge I use to make the 12 streak plates for starters.

It is recommended that if you want to store yeast, you must have access to a pressure cooker or autoclave, as boiling media and pasteurising is not good enough.

Freezing Yeast

If you freeze yeast in water or beer it will die. The ice crystals formed will rupture the cells and they will leak their contents. This can be prevented by several methods, but all use the same principle.

If you add an agent that allows freezing, but prevents crystal formation, then the yeast will not be excessively damaged. About 10% of the yeast will still die, but there will be more than enough left to compensate for this. Agents that can be used are Glycerol, Skim Milk, or DMSO(DiMethyl Sulfoxide). DMSO is not readily available to the homebrewer, and skim milk does not sterilise well. I prefer to use glycerol as it is cheap and readily available. The only disadvantage is that what you buy from the shops is not sterile, and everything MUST be sterile. Sterilise in your pressure cooker, or through 0.2 micron filters.

Cryopreservative Solutions for Freezing and Refrigeration

Use water as clean and pure as possible. Tap water is no good as it contains chlorine and chloramines that may kill the yeast. Try water used for ironing as it is generally mineral free and produced by distillation or reverse osmosis.

Equipment

Heat resistant Container with lid
Small Sterile containers. Ideal size 1-5ml
Measuring cups.
Pressure Cooker

Materials

Glycerol(for freezing)
Clean Water

Technique

Make a solution of 20% Glycerol by volume in heat resistant container. If refrigerating, make a 100% solution of water. Place in pressure cooker. Allow to cook for 15 minutes at pressure.

If canister is sealed, do not vent pressure at end of cycle, but allow cooker to cool and lid to fall in. If Canister lid is loose, then vent pressure and tightly seal. Cool to room temperature.

This solution can be dispensed immediately into small containers. I find 1-2 ml is sufficient.

Storing the Yeast

The yeast for storage should be collected from clean colonies, the same as for culturing. You should only produce quite thin suspensions, as large amounts of yeast will not freeze or store well.

Yeast for refrigeration can be placed directly in the refrigerator in a dark corner to avoid light. Retrieve when you want to produce yeast for a starter and streak the yeast onto a plate.

If freezing, the ideal rate of cooling is $-1\text{C}/\text{Min}$. We cannot possibly hope to achieve this, so forget about it. Place yeast in freezer in some sort of storage canister and retrieve when needed. The colder your freezer, the longer the yeast will remain viable for. As a rule, I tend to reculture every 2-3 years, but you may be able to get longer.