

Yeast Propagation and Maintenance: Principles and Practices

by

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Yeast is that wonderful microbe which converts sweet wort into an enjoyable alcoholic beverage. In addition to converting sugar to alcohol, yeast can also influence the taste, flavor, bouquet, and even the color of beer. They do this by secreting a variety of compounds at very low levels. Different yeast strains produce different levels of these compounds and therefore impart their own subtle characteristics to the wort in which they are pitched. Although yeasts may all be the same species, *Saccharomyces cerevisiae*, they are not created equal. Indeed a bread yeast does not make good beer and the same may be said for some wine yeasts. Beer yeasts also differ and anyone who has split a batch of beer and pitched different yeasts will attest to the difference they can have in brewing. So just as hops, malts, and water must be chosen for a specific beer, so must the yeast. The propagation and maintenance of yeast at home adds yet another level of control to the brewing process, allows for experimentation, and aids in the consistent production of unique high quality beers.

The development of pure yeast strains and their importance in the brewing process has been going on for over a century and is still an active area of research. In 1883, Emil Christian Hansen described the first techniques for successfully isolating single yeast cells and propagating them to a larger scale. This was a landmark finding since up until then all yeasts were a mixture containing various forms of brewing yeast, wild yeast, bacteria, and molds. Brewing with these mixtures of micro-organisms was difficult. Beer spoiling was common and there was wide variability in beer quality. Hansen's techniques changed all that and were quickly applied to improving large scale beer production; first in the Carlsberg brewery and a few years later in American breweries. Current propagation techniques remain similar to those first described by Hansen. Further characterization of yeast physiology and fermentation technology, however, have also influenced the current methods used to propagate and maintain yeast. The following is a discussion of some of these aspects of yeast physiology and fermentation and how it applies to homebrewing practices. Methods for maintaining yeast and their effectiveness with regards to yeast viability and stability will also be discussed.

Principles of Yeast Growth and Fermentation

Yeast is a facultative anaerobe which is just a fancy way of saying that it can survive and grow in the presence (aerobic) or absence (anaerobic) of oxygen. The presence of oxygen determines the metabolic fate of the cell. In terms of the yeast cell, its survival, growth and metabolism is optimal in the presence of oxygen. In this case, yeast will rapidly grow to high densities and will convert sugar (glucose) to carbon dioxide and water. Under anaerobic conditions, yeast grows much more slowly and to lower densities and glucose is incompletely metabolized to ethanol and carbon dioxide. It is important to realize that optimal yeast growth is distinct from fermentation. Therefore, the conditions and methodologies used for propagating and maintaining yeast need not be identical to those used for fermenting wort. The purpose of a yeast starter is not to produce an enjoyable fermented beverage but rather to produce a sufficient quantity of yeast for subsequent fermentation. Propagation conditions should be such that a maximal amount of yeast is produced which provides optimal fermentation performance once pitched. What do we mean by fermentation performance? The main criteria for fermentation performance is based on the rate and extent of fermentation as well as the production of a beer with a balanced sensory profile with no off-flavors/aromas or inappropriate esters. The former refers primarily to attenuation (technically referred to as the apparent attenuation) and is usually indicated by the percent reduction in gravity or:

$$\text{Apparent Attenuation} = \frac{(\text{O.G.} - \text{F.G.})}{(\text{O.G.})}$$

For a normal 1.050 original gravity wort, the terminal gravities should be near 1.012 or 76% attenuation. Apparent attenuation between 70-85% are normal for most yeasts. Also fermentation should occur rapidly and be completed within 3-5 days.

Factors influencing yeast growth

Several factors influence both yeast growth (and fermentation) and therefore should be considered when propagating and maintaining, and yeast. The most important are oxygen, pH, temperature, and wort composition.

Oxygen. As mentioned above oxygen or aeration is essential for good yeast growth and is the driving force behind many aspects of yeast metabolism including fermentation. Oxygen is quickly absorbed by yeast and is used to synthesize unsaturated fatty acids and sterols which form the cell membrane. These molecules are important for both growth and fermentation and serve as a means of storing oxygen within the cell. They are also necessary for increasing cell mass (growth), improving the overall uptake of nutrients, and determining alcohol tolerance. Oxygen also stimulates synthesis of molecules necessary for yeast to metabolize and take up maltose, the primary sugar in wort.

What does this mean in terms of brewing. Well since oxygen directly correlates with rapid growth and increase in yeast mass (cell number), aeration during yeast propagation should increase the overall number of yeast cells. In other words, **your starters need to be well-aerated.** I have been a long-time advocate of not using airlocks on my starters, slants, etc. In fact, I like to shake my starters as much as possible. This will not only help introduce air into the wort, but will also keep the yeast in suspension and exposed to all the nutrients. Alternatively you can intermittently inject air or oxygen into a starter using a sterile filter and aeration stone. Foaming is usually a problem and therefore aeration is usually intermittent. Another alternative which I use quite often is to continuously stir starter cultures. A magnetic stir bar is placed in the starter vessel and the vessel is put on a magnetic stir plate. The stir plate causes the bar to rotate thereby mixing the contents of the starter vessel. Most stir plates have a dial so that you can adjust the speed at which the bar spins. In each case the tube or vessel is loosely capped so that gas can be exchanged. Comparison of the number of yeast cells in starters which were mildly aerated (shaken intermittently), moderately aerated (injected with air intermittently) or highly aerated (continuously stirred) suggest that an increase in aeration/agitation does correlate with an increase in yeast cell number (Figure 1). Continuous agitation/aeration can yield as high as a 10- to 15-fold increase in yeast cell number. This translates into a 10- to 15-fold higher pitching rate than what is observed with a traditional airlocked starter. This means you can generate a much larger amount of yeast using less wort!

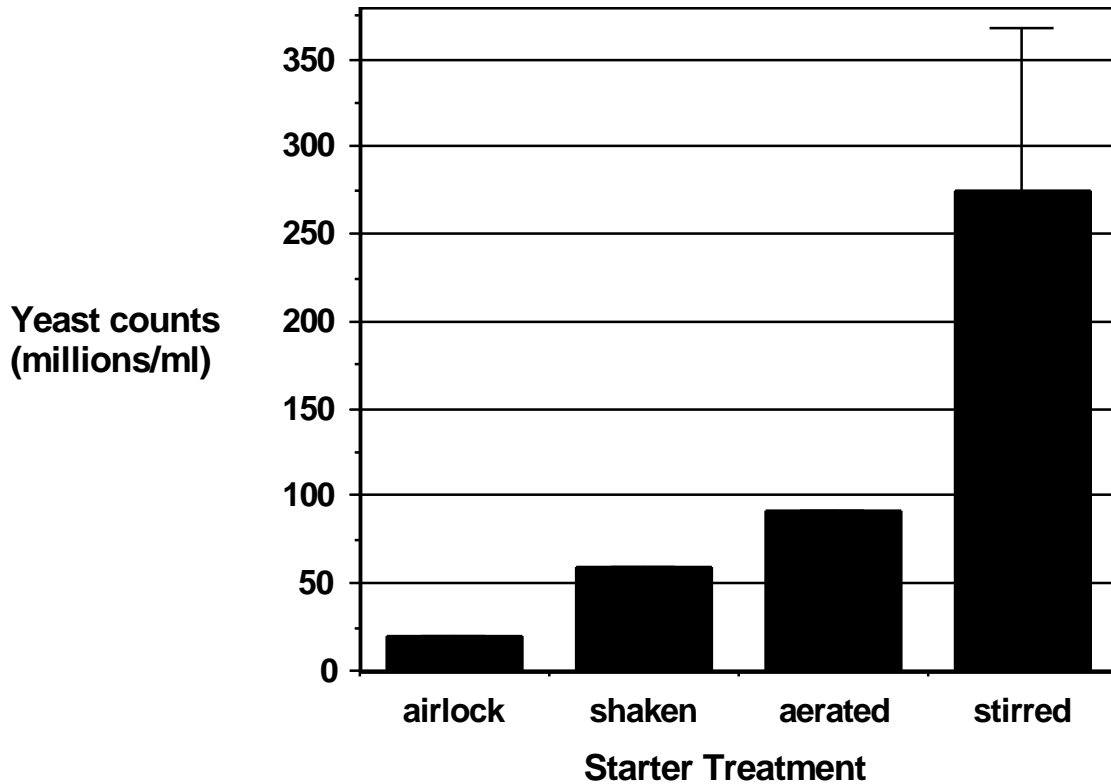


Figure 1. Effect of aeration on yeast cell number. 500 ml of BrewTek Superwort was pitched with a saturated 10 ml superstarter culture of BrewTek yeast and incubated at room temperature (75 °F) for two days. Cultures were either shaken 3-6 times a day, aerated with BrewTek aeration system for several minutes (foam permitting) 3-6 times a day, or continuously stirred on a magnetic stir plate. Yeast cell concentration was determined on the BrewTek hemacytometer. Traditional starter (with airlock) were taken from numbers published by Ray Daniels in HBD #1746 using Wyeast packet as inoculum.

In terms of fermentation, aeration is also important but only in the early stages (first 6-24 hours). Aeration in later stages can oxidize beer constituents and lead to the development of off-flavors. Since aeration sets the stage for maltose fermentation and alcohol tolerance, it is easy to envision why insufficient aeration could lead to stuck fermentations or incomplete fermentations. Incomplete fermentations can be manifested as either high finishing gravities or the production of off-flavors especially diacetyl, acetaldehyde, and hydrogen sulfide. Insufficient aeration is also associated with excessive ester formation. The profound effect of aeration on yeast is further illustrated in studies where yeast from a poorly aerated beer was repitched into aerated wort and still did not perform well. Thus insufficient aeration can have a long-lasting effect on yeast.

In general, it is difficult for homebrewers to achieve sufficient oxygen levels. The levels of oxygen necessary for optimal fermentation vary depending on the yeast strain. **Ale strains usually need between 8-12 part per million (ppm) while**

lager strains require slightly higher amounts (10-15 ppm). At atmospheric pressure the maximum level of dissolved oxygen in wort is approximately 8 ppm and the saturation level decreases further as the gravity of the wort increases. Thus unless special steps are taken to introduce air or oxygen into the wort, it is difficult for homebrewers to achieve adequate aeration. Recent studies have shown that oxygenation is by far more efficient than aeration. Injection of oxygen through a 2 micron diffusing stone can actually supersaturate the wort with 10-12 ppm of dissolved oxygen being reached in 5 gallons of wort by a single 60 second blast of oxygen!

Temperature. Another important factor which influences yeast growth and metabolism is temperature. Temperature is somewhat neglected in terms of its role in influencing growth rate and fermentation performance. Most brewing yeasts will actually grow and ferment at temperatures up to 98 °F (37 °C). These high temperatures are not optimal for yeast propagation or fermentation, since they produce numerous esters and affect the overall viability and stability of the yeast. 86 °F (30 °C) is the usual temperature for the growth and propagation of laboratory yeast but this is still too high for brewing yeast. **Room temperature or 77°F (25 °F) is the recommended temperature for propagating brewing yeasts.** At this temperature rapid growth and fermentation occurs without any adverse affects on subsequent fermentation performance. Although ale yeast will grow just fine up to near 90 °F, lager yeasts start to lose viability at high temperatures. The mid 70s are optimal for growing lager yeasts and higher temperatures should be avoided.

In terms of fermentation, **lager yeasts are routinely fermented between 40-54 °F while ale yeast are used between 55-70 °F.** The optimal fermenting temperature of a yeast varies considerably. **Some ale yeasts for example, do not perform well below 65 °F.** The Sierra Nevada strain is notorious for this as well as some Belgian and wheat beer strains. Again fermenting too cold can lead to incomplete or extended fermentations. Common symptoms of fermenting too cold are stuck fermentations, poor attenuation (high finishing gravities) and off-flavors especially diacetyl. One possible remedy for a stuck fermentation is to warm it up! Be forewarned that fermenting at temperatures above 70 °F can lead to excessive ester formation and may lead to undesirable flavor profiles. In fact, most homebrewers pitch their yeast in worts about this temperature. Lagers, however, should be pitched at lower temperatures (60 °F or lower). In this case it may be necessary to acclimate the starters to a lower temperature to prevent cold shocking them. This can be done by slowly lowering the temperature of the starter the day before. Yeast growth and fermentations are energy generating processes and therefore generate heat. The temperature within the fermenter can be as much as 8 °F higher than outside of the fermenter during the first few days

of fermentation. So beers that are fermenting in refrigerators set at 65 °F are most likely fermenting at about 72 °F.

Wort or media composition. Wort (or media) composition also determines yeast growth and fermentation performance and is important in maintaining and storing viable, stable yeast. In terms of fermentation, standard brewing wort contains most of the ingredients necessary for fermentation. Problems arise only if the nitrogen composition is low. This occurs only if a cheap or poor quality malt extract is used or if there are a large amount of adjuncts added. In terms of propagation, the closer the starter media is to the fermentation wort the better. **A wort with an original gravity of 1.040 works well for most fermentations and is recommended for use in most brewing situations.** If pitching into a high gravity wort, a standard starter may get shocked from the change in osmotic pressure. In this case a higher gravity starter (O.G. =1.065) may be necessary. Lower gravity starters (O.G. = 1.020) are commonly used by homebrewers and routinely produce higher concentrations of yeast but do not perform well when pitched into normal brewing worts. Presumably this is due to osmotic shock.

The addition of yeast nutrients and certain salts can also improve yeast growth and are a worthwhile addition to starters. Yeast nutrients usually are of two types, one which is ammonium phosphate-based, and the other which is amino acid/peptide and vitamin-based (similar to the peptone and yeast extract in the laboratory media described below). Both serve the same basic function which is to increase the nitrogen content of the wort and yeast. A mixture of different nitrogen sources have been shown to enhance both growth and fermentation and suggest that the amino acid/peptide-based nutrients may be more appropriate than diammonium phosphate. **Also rapidly growing yeast such as those in starters have a higher than normal nitrogen requirement. Thus starter worts should be supplemented with yeast nutrients so that nitrogen is not limiting.**

Ammonium phosphate-based nutrients impart very little to the flavor profile. The same is not true of the amino acid based extracts which tend to impart an autolyzed flavor (bloody, bouillon-like, and metallic) if used in excess (greater than a Tablespoon per 5 gallons). Thus amino-acid based nutrients should be used sparingly (if at all) in the fermenter. This is an important consideration when making meads since honey is very low in nitrogen and delicate in flavor. Therefore diammonium phosphate is the preferred nutrient in meads although a small amount of the vitamin/amino acid-based extract should be added (1 tsp) to provide additional vitamins and minerals.

We have recently tested the effects of a variety of food-grade amino acid/peptide-based nutrients on yeast growth (Figure 2). These experiments indicate that the addition of certain yeast nutrients (especially nutrients #3 and 4)

can increase the rate of yeast growth but not the overall concentration or yield of yeast. Thus the addition of yeast nutrient to starters can help accelerate their growth. This is important for homebrewers since we rarely sterilize our worts and rapid growth is necessary for the yeast to take it over the starter before any potential bacteria can grow. Unlike the fermenting beer, yeast nutrients can be added at relatively high concentrations (1% or about 1/4 tsp per quart) to starters since flavor is not an issue. If you are worried about the flavor of the starter contributing to your beer flavor, you can always let the yeast settle and decant off the majority of the liquid.

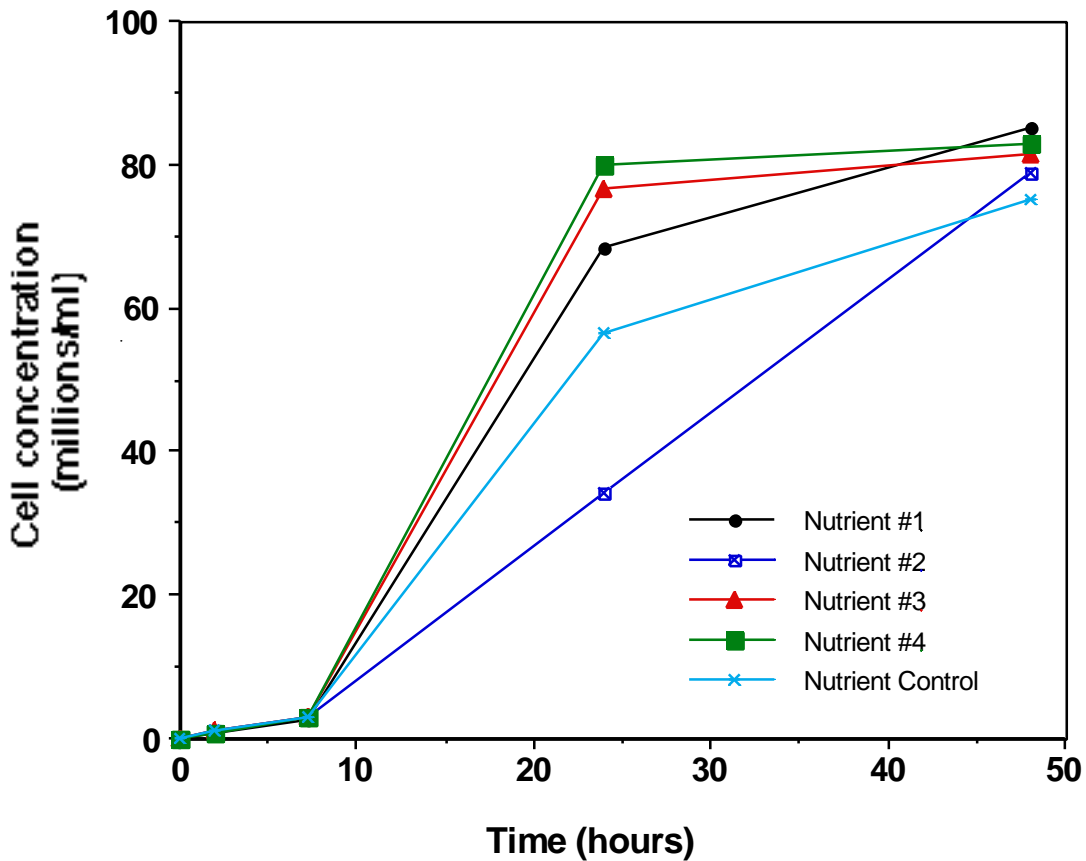


Figure 2. Effect of various yeast nutrients on yeast growth. 0.1 ml of actively growing yeast are pitched into 10 ml laboratory wort containing different nutrient. All nutrients tested were amino acid/peptide/vitamin-based and were added to a final concentration of 1%. Yeast cell counts are determined at various time points using a spectrophotometer where 1 OD₅₉₅ = 30 million cells/ml. Note that the addition of certain nutrients especially samples #3 and #4 lead to maximum yeast growth in a shorter period of time (24 versus 48 hours).

Zinc also supposedly improves yeast growth and fermentation and is added to the propagation tanks in some British breweries. 0.5 ppm zinc is optimal. Interestingly some yeast nutrients may also contain zinc. For example, the BrewTek Yeast Nutrient if used in starters at 1/4 tsp per quart should yield

about 2-5 ppm. This is another reason for supplementing starters with yeast nutrient. Information regarding the importance of zinc (as well as other minerals) on yeast growth and fermentation is limited and has not been tested to the same extent as some of the nitrogen sources.

Yeast grows perfectly fine in simple sugar solutions supplemented with a rich nitrogen source. The standard laboratory media for growing and maintaining yeast include YPD, potato dextrose, and sabouraud dextrose. The composition of these media are summarized in Table 1. These media are designed for laboratory yeast and do not contain a complex source of fermentable sugars. **Laboratory media is not recommended for extensive propagation of brewing yeast since this may ultimately affect fermentation performance.** More suitable media appropriate for brewing yeast are listed in Table 2. These media can be used for slants, plates and limited liquid propagation but should not be used as starters because of the lower gravity.

Table 1. Laboratory media

YPD	Potato Dextrose	Sabouraud Dextrose
1% Yeast Extract	20% Potato infusion	1% Neopeptone
2% Peptone	2% Dextrose	4% Dextrose
2% Dextrose		

Table 2. Recommended Media for Maintaining Brewing Yeast

Wort	YM (or MYGP)
1.500% Malt extract	0.3% Malt extract
0.078% Peptone	0.5% Peptone
1.275% Maltose	0.3% Yeast Extract
0.275% Dextrin	1.0% Dextrose
0.235% Glycerol	
0.100% Dipotassiumphosphate	
0.100% ammonium chloride	

Table 3. Recommended Media for Starter

Starter Wort	Amount for 1 Liter (qt)
Dry Malt Extract or ~1.040 O.G. wort	1 cup or 0.25 lbs DME
1% vitamin based yeast nutrient	1/2 tsp or 10 g
Hops (optional)	1 pellet or a few drops of iso-alpha extract.

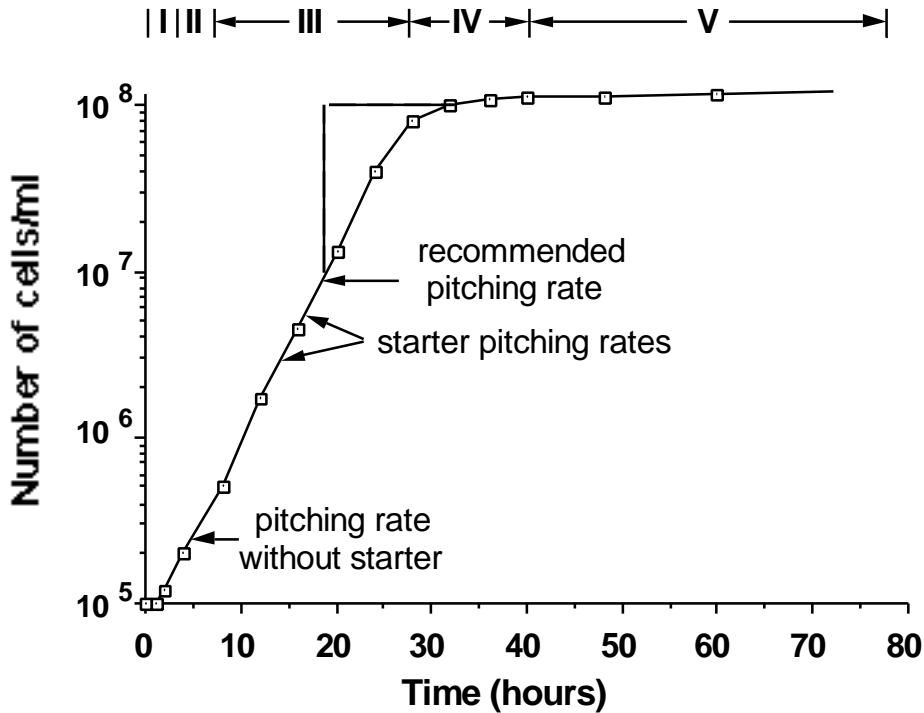
pH. The last factor to affect yeast growth is pH (a measure of acidity). Yeast grow well at acidic pHs. They grow best between pH 4 to pH 6. Normal wort is acidic with a pH near 5.2. During growth and fermentation the pH drops to about 4.1-4.2 and in some cases even lower. The further acidification of the wort helps to prevent bacterial infection. **(Most bacteria cannot tolerate acid pH).** Yeast can survive at very low pH, as low as 2.0. This is the basis of acid washing where the bacterial load of a yeast slurry is reduced prior to repitching by lowering the pH to 2.2. Most bacteria will be destroyed at this pH while a good percentage of the yeast will survive. Interestingly, diluted unfermented honey is more acidic than wort and the production of more acid during fermentation actually slows down its fermentation. To make matters even worse most meadmakers add acid blend to the honey. Although the acid does help to balance out the flavor, it will inhibit fermentation and therefore should only be added after fermentation is complete. In fact some meadmakers will add a small amount of calcium carbonate to buffer the acidity and raise the pH. This can significantly accelerate fermentation. I have successfully fermented a mead out in a week using this method (and a good healthy supercharged starter).

Artificial media such as those described in Tables 1 and 2 are neutral (pH \approx 7) when prepared, therefore, it is a good idea to adjust the pH to below 6.0. This will help minimize bacterial contamination during propagation procedures. Hops also supposedly have anti-bacterial effects and can also be added to these media and starters.

Summary. Based on the above information, a yeast starter should be composed of a 1.040 gravity wort that is supplemented with amino-acid based nutrients (BrewTek nutrient or Superfood). It should be aerated well before adding yeast. Once the yeast is added it should be kept at room temperature (\sim 75 °F) and shaken as often as possible (or better yet, constantly stirred). All media used to store brewing yeast (slants, plates, etc.) should contain yeast nutrients, some malt, and should have an acidic pH.

Stages of Yeast Growth

When yeast is added to wort or media, it goes through 5 basic stages of growth. (Figure 3).



- I. Lag phase - period of adaptation
- II. Accelerating growth phase- initiation of yeast growth
- III. Exponential growth phase - rapid yeast growth, doubling every 2-3 hours
- IV. Decelerating growth phase - nutrient supply depleted
- V. Stationary phase - no yeast growth, cell viability begins to decline

Figure 3. Growth curve for brewers yeast. Diagram showing typical changes in yeast cell number when introduced into liquid media at very low concentration. The various phases of growth are shown. Note that the actual time it takes to reach stationary phase varies depending on growth conditions, yeast strain, and amount of yeast inoculated.

I) Initially there is a **lag phase**. This occurs during the first few hours after addition of the yeast. During this time there are no apparent signs of fermentation or growth. The yeast are becoming acclimated to their new environment. If the previous media (or starter) is similar to this new one, acclimation will occur rapidly and the lag phase will be short. If there are major differences in the gravity, temperature, or wort composition, the yeast may be surprised or shocked and it may take some time to adjust to this new environment. Major changes occur within the yeast at this time, they are

absorbing all of the oxygen in the wort, using it to synthesize all the enzymes and other metabolic machinery necessary for growth and fermentation, and storing oxygen up in the form of sterols for later use. This stage is critical to fermentation and should occur as rapidly as possible, preferably within a few hours.

II) The second phase is the **accelerating growth phase** during which yeast cells start to grow and divide. Signs of fermentation will also become apparent. The yeast begin storing sugar in the form of glycogen for later use.

III) The third phase is the **exponential phase** where yeast reproduction and metabolism is in high gear. Cells are dividing every 90 - 180 minutes and fermentation begins. During this time the number of yeast cells may increase as much as 1000-fold (or 3.0 logs) within 24 hours. The extent to which the cells divide is dictated primarily by the pitching rate. **If appropriate pitching rates are used, the yeast are pitched at high concentrations (5-15 million yeast cells per ml) and undergo approximately 3 generations (2^3 - or an 8-fold increase in cell number) to yield 80-100 million cells per ml.** 100 million cells per ml is about the maximal concentration of yeast attainable in fermenting wort (Figure 2 & 3). Fermentation is also very active and a krausen may be beginning to form.

IV) The fourth phase is the **decelerating growth** which should occur 12-24 hours after pitching. At this time the oxygen is fully depleted and fermentation and CO₂ production is taking over. Fermenting wort should be in high krausen. **Maximal fermentation occurs during 12-48 hours;** heat is being generated and there should be rapid CO₂ evolution (bubbling).

V) Finally several days later, the yeast enter a **stationary phase**. During this time the fermentables and nutrients are completely consumed. All yeast growth has stopped and they are beginning to fall out of suspension or flocculate. The sterol and glycogen stored up during early growth are beginning to be broken down and used to continue growth. Prolonged exposure in this phase (weeks) can lead to autolysis or total breakdown of the cell.

Propagating procedures for the preparation of pitching yeast

Why are these growth curves important with regard to brewing. Basically this is what is happening in your starter. **When a small amount of yeast from a slant is inoculated to a tube containing 10 ml of wort or media, it will undergo the growth curve shown in Figure 3 over a 1-3 day period.** The precise length of time will vary depending on the yeast strain, how old the slant is, the media used, the level of aeration, etc. Note that use of a larger starting volume than 10

ml will increase the amount of time it takes to reach the stationary phase. 10 ml is a good volume since it is small and will reach saturation in a short amount of time and therefore should minimize potential contamination.

Now that we have an idea of how much yeast is in a 10 ml culture and how fast it will grow. We can start estimating how large a volume of yeast we need to pitch into a fermenter and how long it will take to reach that volume. This brings us to pitching rates. Pitching rates have been shown to have a profound effect on fermentation performance and are one of the main factors which contribute to consistency between batches. **The pitching rate is just the amount of yeast added to the fermenter. It is usually expressed as the amount of yeast found in 1 ml of wort after pitching.** The recommended optimal pitching rates are 6-10 million cells/ml for ales and 10-15 million cells/ml for lagers. (George Fix recommends 10 million cells/ml for ales, 15 million cells/ml for lagers.) Higher gravity worts require even higher pitching rates.

$$\text{Ale pitching rate} = 6 - 10 \text{ million cells/ml} \times \frac{(1 - \text{O.G.})}{48}$$

$$\text{Lager Pitching rate} = 10 - 15 \text{ million cells/ml} \times \frac{(1 - \text{O.G.})}{48}$$

For example a 1.096 gravity ale wort should be pitched with 14-20 million cells/ml.

What does this mean in plain English. A 5 gallon fermentation contains approximately 20,000 ml. This means that for 1.050 gravity ale you would need to add between 120 billion (20,000 ml x 6 million cells/ml) and 200 billion cells (20,000 ml x 10 million cells/ml).

Table 4. Pitching Yeast Characteristics

Yeast Type	Conc. (millions/ml)	Volume (ml)	Total Yeast (billions)	Pitching rate for 5 gal‡ (millions/ml)	Viability¶ (buds)
Dry (5 g)	620	200	124 (1011)*	6.2 (4.96)	80% (0%)†
Liquid #1	71	50	3.6 (1-5)*	0.18 (0.045)	25% (0%)
Liquid #2	58	50	2.9	0.145 (0.029)	20% (5%)
Starter (shaken)	60 (20)*	500	30 (10)*	1.5 (1.43) (0.5)*	95% (100%)
Starter (aerated)	92	500	46	2.3 (2.25)	98% (100%)

Starter (stirred)	180-360	500	90-180	4.5-9 (4.5)	100% (100%)
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‡ Based on exclusion of methylene blue.

† Rehydrated yeast may exhibit altered methylene blue uptake.

* Data adapted from those published by Ray Daniels in HBD #1746. This difference may be due to intermittent shaken as well as media, etc.

‡ Pitching rate based on total yeast; numbers in parenthesis are corrected for viability.

This is a lot of yeast!! So how do we go about propagating 200 billion yeast cells so that we can hit this pitching rate. Above is a table which shows the yeast cell counts for various yeast sources as well as for 2 cup (500 ml) starters propagated in nutrient fortified wort (see Table 3 for recipe). Aside from dry yeast, the only method which can yield a suitable pitching rate with two cups is the stir plate method. This is my method of choice since it is efficient and non-invasive which means that I have minimal chances of infection during propagation. The main disadvantage is that stir plates are expensive (\$75-\$150 new) although you may be able to find some relatively inexpensive ones at a scientific surplus or electronic stores.

Most homebrewers start out pitching a Wyeast packet. How much are you actually underpitching with one of these 50 ml pouches? Assuming all the yeast in a Wyeast packet are viable (only about 25% truly are!), we are adding only 50 ml of about 60 million/cells per ml. This translates into a pitching rate of 150,000 cells per ml (Table 4). **Thus with a single Wyeast packet you are underpitching by a factor of at least 35 for ales and almost 100-fold for lagers.** What is the big deal about underpitching. Well remember that very little yeast growth should go on in the fermenter. There should be no more than 3 or 4 cell division which should take place during the first few hours of fermentation. If underpitched the yeast will spend much more time trying to grow to adequate quantities. During this extended growth period the yeast tend to secrete more esters and fusel alcohols. Moreover they may not have a sufficient number to adequately metabolize (digest) all of the fermentable sugars. So what you end up with is a beer with off-flavors (such as esters, fusel alcohols, diacetyl, acetaldehyde) and a high finishing gravity. Thus it is important to always make a starter and make it a relatively big one. Remember that **you want the yeast to spend most of their energy making alcohol not babies in a fermenter!!**

So how big a starter do you need to make to hit the pitching rate. Table 5 estimates the approximate volumes of starters necessary to pitch 5 gallons of wort at 10 million cells per ml. **The traditional airlock starter is very inefficient at generating yeast** and it would take almost 2.5 gallons (5-10 liters) of starter to generate enough yeast for a 5 gallon batch! Mechanically shaking your starter

intermittently so as to resuspend the yeast is moderately effective and is the easiest and most cost-effective thing you can do to improve the efficiency of your starter. In this case you would need about a 0.75 gallon (3 Liter) starter to generate enough yeast. As mentioned above the stir plate is by far the most efficient.

Table 5. Approximate Starter volumes to achieve 10 million cells/ml.

Starter treatment	Conc. (millions/ml)	Starter Volume for 5 gallons
traditional	20	10 quarts
shaken	60	3.3 quarts
intermittent aeration	92	2.2 quarts
continuous stirring	180-360	0.75 quarts

The next issue is how long will it take to generate that big starter and how do I go about doing it. Once we have a 10 ml saturated culture going it should take only a few days to reach these volumes. Close examination of Figure 3 indicates that yeast should not be diluted more than 200 times the previous volume. In otherwords 10 ml should not be stepped up to more than 2 liters. The rationale behind this is simple. During yeast propagation it is important to keep the yeast growing exponentially (Figure 3, phase III). Diluting the yeast out too far will slow down their growth and give bacteria a chance to overtake the culture. Since **bacteria can grow as much as 6 times faster than yeast** (the average doubling time for a bacterium is 20 minutes!), it is important to keep yeast growing rapidly. If more than 1 or 2 liters of starter are required, it is best to do more than one step-up. In most cases it is best to do a 500 ml intermediate starter and after 1-2 days step this up to as much as 1-2 gallons. In each case the yeast should reach saturation in 24-48 hours. Figure 4 outlines the two different propagation strategies.

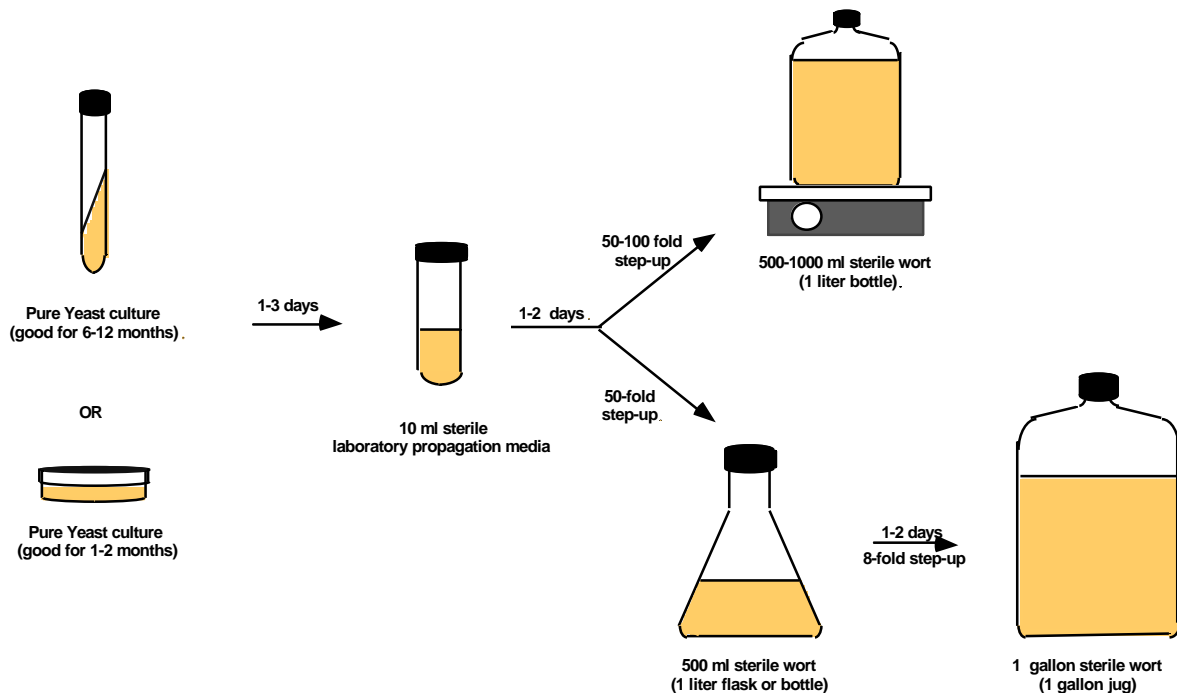


Figure 4. Propagation of yeast for pitching. The various steps involved in building up enough yeast to pitch is depicted. The stir plate method (top) is shown with the intermittent shaking method. Liquid yeast packet (~50 ml) could be substituted for 10 ml tube. Each step-up should be incubated for at least one day and as long as 3 days at room temperature (75 °F).

Although a 100-fold dilution or step-up seems reasonable for yeast propagation, most breweries (or at least what is taught by brewing schools) advocate even smaller dilutions. The Siebel Institute recommends that yeast be stepped up in 8-fold increments, the British Brewing schools recommend 10-fold increments, and the German Brewing schools recommend 4-fold increments. Why such small increments? Well the answer lies in fermentation performance. Larger step-ups supposedly alter the efficiency of metabolism of the fermentable sugars in wort. Those affected are ones which are fermented last, primarily maltose and maltotriose. Thus yeasts which are propagated by large step-ups will be susceptible to lower attenuation and sweeter finishes. I should also point out, however that most breweries start with a 10 ml culture and step-up to 500 ml or 1 liter initially and that it is only in the later stages that the step-up size is reduced. Figure 5 outlines a typical brewery propagation. The disadvantage of this additional step is that it requires further manipulation of the yeast and therefore increases the chances of bacterial contamination. Having brewed beers preparing starters each way, it is not clear to me whether this actually makes discernible differences in the end-product. Although I have not done a rigorous analysis. My overall impression is that it may not be relevant to homebrewers, but then again I am not trying to make the same beer consistently.

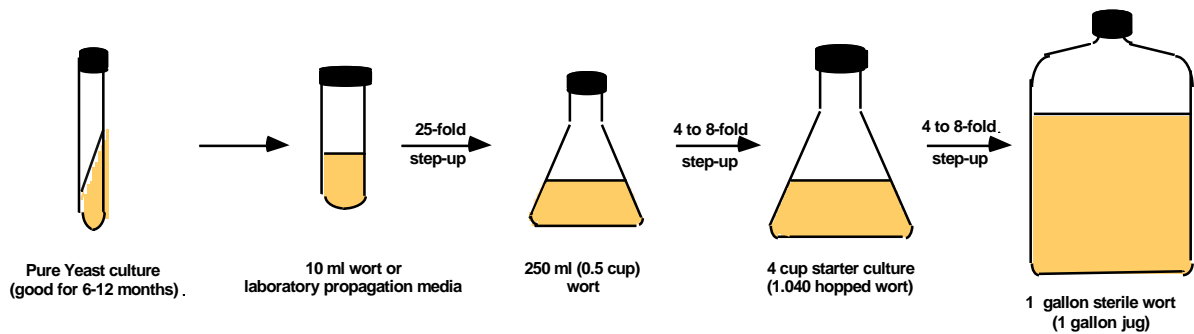


Figure 5. Brewery Propagation of yeast for pitching. The various steps involved in building up enough yeast to pitch is depicted. Typical brewery propagation scheme is shown where later step-ups are limited to 8-fold increases. Each step-up should be incubated for 1-3 days at room temperature (75 °F) with intermittent shaking or aeration.

In general using a clean, healthy 2 liter yeast starter, aerating well, and fermenting at an appropriate temperature will produce outstanding beers. Underpitching by large amounts should be avoided since they will increase yeast growth and may lead to excess ester formation, unreduced diacetyl, hydrogen sulfide, or stuck fermentations. Another alternative is to save the yeast from the primary fermenter in a sanitized jar (store it as cold as possible without freezing it) and repitch all or part of it into the next batch. It will keep fine in the residual beer its suspended in. Prior to pitching, the trub (cold break) can be removed by washing the yeast with sterile water or acid. I usually don't bother; instead I only resuspend the top two-thirds of the yeast for pitching and discard the remainder. If you plan on storing the yeast for more than a couple weeks, it is best to wash the yeast with some yeast after a day or two in the refrigerator. Let the yeast settle out, then pour off the residual beer and resuspend the top two-thirds of yeast in sterile water and transfer to a new sanitized jar. Storing yeast on the trub supposedly reduces its viability and therefore reduces how long you can store it. The amount of yeast slurry you should pitch depends on the yeast viability. Yeast viability will vary depending on the yeast strain, how long it's stored, how cold it's been stored, and whether it's been acid washed. The only good way to quantify this is by staining some of the yeast with methylene blue and examining it under a microscope. If it's been several weeks, it is usually a good idea to reactivate the yeast by pitching some (about half) into a quart of wort the day before brewing. Breweries tend to repitch one-third to one-fifth of the last batch, but this is straight out of the primary fermenter into a new batch. Note that breweries usually collect only the middle third out of the conical fermenters. Repitching yeast is really the cheapest, easiest way to achieve appropriate pitching rates for lager and/or high gravity beers or if brewing large batches. In general, I do not recommend reusing the yeast more than 2 or three times because of the possible contamination. Some brewers like to acid wash their yeast at pH 2.2. This reduces the amount of bacteria picked up during

fermentation, but will also reduce yeast viability. It also does not get rid of wild yeast which is a common problem in home breweries.

Tips for handling yeast cultures and starters at home

When I first started brewing I was amazed that very few people were culturing their own yeast. The main reason seemed to be that most homebrewers thought that it was necessary to have a glove box to successfully propagate yeast at home. I knew full well that this was a misconception since I had been culturing micro-organisms on the benchtop of research laboratories for years. This also seems to be common practice in brewery laboratories. One of the biggest misconceptions regarding microbiology is that bacteria can "crawl" into containers and infect it. Airborne bacteria do not have wings or legs. They get transported about by dust particles. The classic experiment illustrating this point was done by Louis Pasteur. He designed a gooseneck flask to be open at one end and contain sterile broth at the other. Although air could pass freely into the tube, dust particles became trapped in the curved portion. At no time have micro-organisms grown in this sterile broth. I've heard that this flask is still on display in the Pasteur Institute in France! This is also

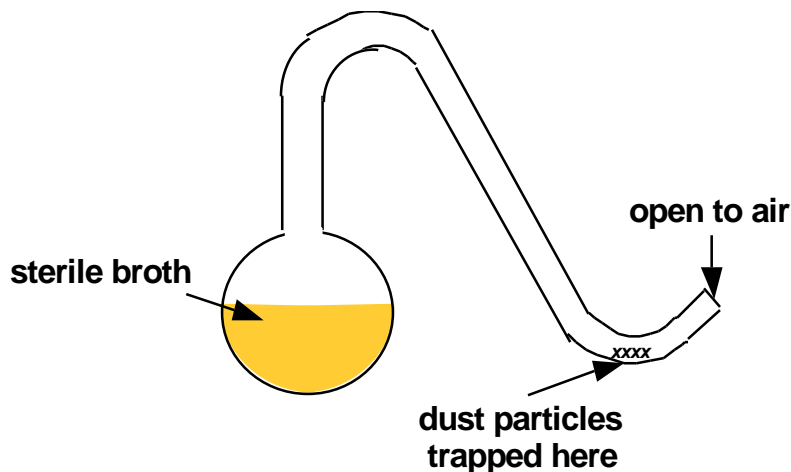


Figure 6. Pasteur's gooseneck flask. Only air and not dust particles can reach the broth. Therefore it remains sterile.

why plates can be left unsealed and stay sterile or why it is sufficient to cover sterile glassware with a sheet of heavy duty aluminum foil. In all of these cases it is nearly impossible for dust to get into the sample. Below are a few tips which can help create a bacteria-poor environment at home.

1. Work in a draft-free clean area. All manipulations should be performed within a 2-4 square foot area. I've used a desk, coffee table, or dining table. The

kitchen is not recommended since there tends to be a higher concentration of bacteria (especially lactobacillus) in that area. Clean the working area and your hands. The new antibacterial soaps on the market would be good for this. Sanitize the area and your hands by spraying them with 70% rubbing alcohol or ethanol before starting.

2. Perform all manipulations near a flame source. Good flame sources include propane torches or alcohol lamps (we don't recommend using your stove since the flame source is too low to effectively perform your manipulations). The flame and heat from the torch keeps all the bacteria-containing dust particles well above your working space and out of your yeast and wort. Flaming the mouth of all jars, bottles, and fermenters will also help get rid of air-borne or surface contaminants. Flaming is a standard microbiological practice, and although it may not be necessary, many consider it as a prayer to the sterility gods.

3. Keep containers open for as little time as possible. It is best to loosen all caps and rehearse exactly what is going to transpire before actually doing it. For example if you are going to transfer some yeast from one tube into another. First loosen the cap on the tube of yeast, then the cap on the broth. Both should be situated in a rack or area for easy access. Then figure out exactly what hand you are going to hold each tube in and how exactly you are going to make the transfer. Are you going to put the cap down or hold it with your little finger of the piping hand? Are both tubes going to be held in the same hand or is one going to be left in the rack? If you cannot handle, (or are not coordinated enough to hold) the caps while you are opening and closing the various tubes and vessels, it is okay to put the caps down on the table. Be sure to place them with the sterile side facing down and if possible, place them on a paper towel wetted with alcohol. (Special precautions however, should be taken to keep the flame source away from any alcohol since it could ignite). Know exactly what you want to do before starting. This will lead to smooth easy transfers. The more you do this the more adept you will get at it. In all cases it is necessary to avoid touching any part of the pipet to the outside of the tubes or the surface of the bench. Transfers should be done quickly but not rushed so as to drop things or make mistakes.

Methods of Yeast Maintenance

Maintaining and storing your own yeast stocks is both convenient and cost-effective. Three major things must be considered when choosing a method of yeast storage. These are yeast strain purity, viability and genetic stability. Each of these differ depending on the method of preservation. The one most suitable for homebrewers is somewhat controversial. Each method has its own

advantages and disadvantages and depends on personal preference as well as access to specialized equipment.

Media preparation. It is important to point out that the media used for long-term storage should be sterile. That means all micro-organisms including spores are destroyed. This can be done by heating in an autoclave or pressure cooker for 15-30 minutes at 15 psi. If this equipment is not available the media can be sterilized by tyndallization. This is done by boiling the media for 15 minutes every other day for a week. Note that this is similar to canning where the media is immersed in a pot of boiling water and boiled. At least two to three successive boilings are necessary for complete sterilization. Propagation media such as that used for starters need not be sterilized but has to be sanitized. In this case it should be boiled for at least 15 minutes and used within two or three days of preparation. Propagation media which is stored for any extensive length of time should be sterilized by one of the methods described above.

Master stocks. In general, it is a good idea to keep two stock preparations of yeast; one which is referred to as a working stock and the other, a master stock. The working stock is for routine use such as initiation of yeast propagation. The master stock is used to preserve the integrity of the original yeast strain. It is only used to replace the working stock or to propagate new master stocks. New master stocks are prepared when viability of the current master stock may be diminished. When this needs to be done depends on the yeast strain and the method of storage.

Liquid Media. This is a common method of storage for homebrewers and has also been referred to as yeast ranching or parallel yeast culturing. The best media for this method is wort or wort-containing media. Yeast is inoculated into 10 - 20 ml of media and grown until it reaches the stationary phase of growth (approximately 3 days) then stored in the refrigerator as cold as possible (40 °F). That means don't keep it on the door. Stocks should be made in duplicate; one to use for brewing, the other as a stock. Some homebrewers prefer to build the 10 ml culture up to a larger volume and then dispense it into 12 oz. bottles. Storage in culture tubes or small jars also works fine. If stored properly, these cultures are stable for up to 6 months and then must be recultured (preferably from the untouched master stock). There are reports that storage in 10% sucrose after growth in wort can increase the shelf-life of yeast to as long as 2 years. In this case, it seems to be necessary to remove all residual nutrients or wort since direct addition of sucrose to the stationary yeast leads to continued fermentation even at 40 °F. Other bona-fide non-fermentable sugars such as lactose or glycerol may be more suitable but have yet to be tested for improving yeast's shelf-life. Yeast strains vary in their sensitivity to storage in liquid wort. In general, only a small percentage of the cells survive storage. Therefore, it may be necessary to store in

volumes larger than 10 ml especially if longer storage periods are used. Culturing in wort has been extensively characterized by the National Collection of Yeast Cultures (NCYC). They have cultured yeast for periods of up to 60 years and find that the mutation rate can be high. Of 600 strains studied as many as 50% with specific nutritional markers had lost at least some of their specific markers after culturing for 10-25 years (that's after 20-50 passages). This was for all types of yeast strains including brewing yeasts. 10% of the 300 brewing yeast strains tested showed changes in flocculation behavior after 10 years or 20 passages. Thus storage in liquid media is feasible, but it is not the method of choice for long-term storage since it can undergo considerable genetic drift from the original stock. It is not clear whether minimizing the number of passages will also reduce the overall mutation rate.

Solid media (agar). The standard method for maintaining yeast and bacteria is on some type of solid media either in the form of plates or slants. Agar is typically used as a solidifying agent and is added at a concentration of 1.5-2% (1.5-2.0 grams per 100 ml liquid). The base media can be wort or one of the laboratory media described above. Agar is insoluble in wort or media and needs to be boiled for a few minutes to dissolve. After pouring plates or slants it is important that they be sufficiently dried at room temperature (2-5 days) before using them. Otherwise condensation may form on the sides of the tube or petri plate during storage which can lead to contamination especially by mold and fungus.

Agar Plates- Agar plates are made by pouring a sterile agar solution into pre-sterilized glass petri dishes or disposable petri dishes. Once solidified and air dried, yeast can be applied to the plate. This is done with a sterile inoculating loop. A small amount of yeast is added to the plate at one end and then spread across the plate. If performed properly, the yeast will be diluted to a point where a single yeast cell will be deposited. After growing for 3 - 5 days that single yeast cell will develop into a small round white mound of cells on the plate. This growth is referred to as a colony or clone. Colonies originating from single cells are round since they grow and expand outwards from the center. They do not have arms and legs and cannot move around on the plate. So nice distinct round colonies on a plate represent one yeast cell from the original yeast and should be free of bacteria and other contaminants. Bacteria and other contaminants may exist in other areas of the plate depending on the quality of the yeast used. Streaking on plates is classically used to purify yeast away from contaminants. In this case, a single colony is removed with an sterile inoculating loop and transferred to a fresh plate, slant, or tube of liquid media.

There are a number of procedures used to for spreading or streaking yeast. The so-called quadrant technique consistently produces single colonies and is

depicted in Figure 4. This method involves dragging lightly (like writing with a pencil) an inoculating loop containing yeast over one quarter of the plate (quadrant) using a back and forth motion. The inoculating loop is then resterilized, cooled and used to transfer a small amount of the spread sample into the next quadrant. Again this diluted sample is then spread back and forth in the upper area of the quadrant. The inoculating loop is resterilized, cooled and used to transfer some diluted sample from the second quadrant into the third quadrant. After spreading over the third quadrant, the inoculating loop is resterilized and cooled, then used to transfer diluted sample from the third quadrant into the fourth and last quadrant. This sample is spread back and forth and should fill in any remaining unstreaked area on the plate. By sterilizing the loop between each transfer to a new quadrant, a dilution is made such that finally a single yeast will be deposited on the plate. After incubation at room temperature for 3-5 days, these single cells will divide many times and finally form a dull white mass of growth on the agar surface. Streaked plates are incubated inverted (agar side up) since condensation can form and drip onto plate and disperse the colonies. Plates are sealed with electrical tape, saran wrap, or parafilm and store inverted in the refrigerator.

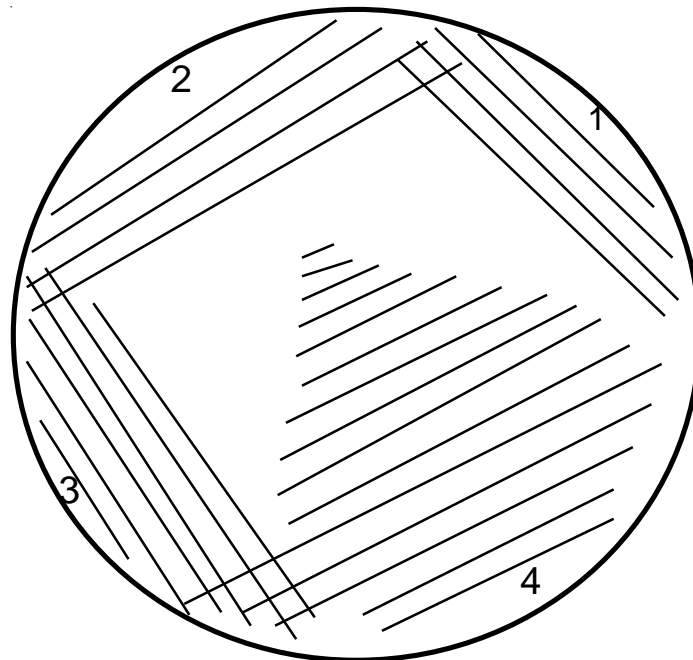


Figure 7. Quadrant streak method for applying yeast to an agar plate. Schematic diagram showing the 4 areas or quadrant over which yeast is applied and the approximate number of back and forth streaks to be used in each quadrant. Note that the inoculating loop is sterilized between streaking of each quadrant and therefore should dilute the yeast such that a single yeast is applied.

The main advantage of agar plates allow separation of yeast from possible contaminants. Also the large working area is readily visible and easily accessed.

The disadvantage is that its accessibility makes it less reliable. Not only is it much more susceptible to mold contamination, condensation can also be a problem and if it isn't, then the plates may dry out. The larger surface for air (oxygen) exposure appears to diminish the shelf life of the yeast as well. There have been claims of yeast being stored on plates for a year or longer, but usually they are only stable for a few months. The shelf-life may actually vary depending on whether glass or plastic plates are used, how dry they are, and how well they are sealed. Many homebrewers prefer agar plates over slants, yet plates are not even considered as a method of storage by professionals. Because of the reliability factor, I prefer to use plates primarily for purification of single yeast colonies and not for yeast maintenance.

Agar Slants- Agar slants or slopes are made usually by adding a molten agar solution to glass culture tubes, sterilizing them, then allowing them to solidify at a 30°-45° angle. Yeast is applied with an inoculation loop and is streaked back and forth from the bottom up. The culture tube is incubated upright at room temperature (3-5 days) with the caps turned back one-half turn. Once a nice lawn of yeast is present the tubes are tightly capped and stored in the refrigerator.

Unlike plates, slants tend to be less susceptible to mold contamination and to drying out, and therefore are more reliable. They are slightly more difficult to work with since you have to deal with caps and you can't see the yeast as well. The major advantage is that yeast can readily be maintained on slants for 1-2 years. However, reculturing every year is recommended. The quality of the tube and the how well a seal the cap forms may be a determining factor in the shelf-life of yeast on agar slant. A sterile overlay of mineral oil has been reported to extend the shelf-life of a slant by up to a year but this is can be messy. Presumably the oil overlay helps keep the air out and prevents the yeast from oxidizing.

Stabs- Stabs are upright tubes or bottles of semi-solid agar media. They contain only 0.7-1.0% agar. These are best prepared in screw-cap container with rubber inserts in the caps such as bijou, McCartney or Universal bottles but standard culture tubes may also be used. Yeast is applied with an inoculation loop and is inserted or stabbed into the agar all the way to the bottom of the tube or bottle. The stab is incubated and stored similar to a slant. Stabs are a common method used for long term storage of bacteria. Like plates, I have not seen any reference to this technique for yeast. The main advantage of this procedure is that it minimizes exposure to air which appears to be the primary limiting factor for shelf-life and stability. The disadvantage is that they are difficult to work with since the yeast is embedded in the agar and difficult to see. Also a fair amount of agar is usually picked up during transfer. It is unclear what the shelf-life is for stabs but my guess is that it would be at least 2-4 years (using tight-sealing

bottles). Stabs therefore are a good for long-term storage, but not appropriate for routine propagation.

Dried yeast- Yeast is spotted onto a 1 inch square of sterile filter paper (Whatman 3MM) or a thick paper towel; wrapped in foil and dried (by desiccation) in the refrigerator for 2-3 weeks. Yeast can be spotted in growth media but it is better to add yeast that is suspended in condensed skim milk! Just let the yeast settle out of suspension, decant off the majority of liquid and resuspend in a small amount of evaporated skim milk from the grocery store. Store the dried yeast in an envelope in the refrigerator. Although yeast maintained by this method is stable for 3-6 years, their manipulation during drying and resuscitation makes them more susceptible to contamination. Dried yeast can be resuscitated by placing in liquid media or on a plate. In general it should be streaked out on plates prior to use. This method is commonly used for genetic strains of yeast. I have successfully used this technique on brewing yeast, although the shelf-life and stability has yet to be determined. Supposedly the Siebel Institute is exploring this issue. In any case it is a great way to send yeast around world.

With regard to the dry yeast packets available at most homebrew shops, not all brewing yeast are amenable to the propagation and drying procedures used in the dry yeast industry. These yeast are typically grown to mass quantities in a dextrose/molasses mixture supplemented with nutrients at high temperatures (85°F). These adverse conditions may induce mutation or alter their performance in wort.

Freezing- Yeast can be frozen if a cryoprotectant is added. Glycerin and sucrose are commonly used and should be added to exponentially growing yeast at a final concentration of 5-15% (5-15 g/100 ml). Yeast stored at ultra cold temperatures (in liquid nitrogen or -112 °F) are stable for almost indefinitely (at least over 5 years) with over 99% of the cells surviving freezing. Freezing at higher temperatures (6.4 °F) yields shorter shelf-lives and less viability. It is important that once you freeze your yeast that, it does not thaw. This requires a really good quality non-frostfree freezer which maintains temperatures at or near 6.4 °F. Some homebrewers place their tubes of in is some denatured alcohol which has a supercooling effect and helps stabilize the temperature. Others imbed the tubes in ice. If you're going to use this method freeze small aliquots (1-5 ml), then just thaw your yeastsicle and pitch it into a starter.

Homebrewers are faced with a variety of options on maintaining their yeast (summarized in Table below). The method of choice depends solely on the needs of the individual and their equipment. We are fortunate that there is an ever increasing number of inexpensive commercial sources of yeast so long-term

storage by the homebrewer is not the necessity it once was. No matter what source of yeast or how it is stored, further propagation along with adequate aeration and fermentation at the correct temperature are sure to improve the quality of the beers you make at home.

Table 6. Summary of methods for yeast storage.

Method	Shelf-life (years)	Advantages /Disadvantages
Liquid media	0.5	Convenient but low viability and stability, questionable purity
Agar plate	0.2-1	Pure cultures but unreliable shelf-lives.
Agar slant	1-2	Easy, reliable, but moderate shelf-life
Agar stab	2-4	Easy, reliable, good shelf-life, but messy.
Dried	3-6	Inconvenient, requires purification.
Frozen	>5	Need special freezer or liquid nitrogen

REFERENCES

1. D.R. Berry and C. Brown, "Physiology of yeast growth" in *Yeast Biotechnology* (Allen & Umwin, Boston, Massachusetts, 1987).
2. DIFCO Manual (DIFCO Laboratories Inc., Detroit, Michigan, 1984).
3. W.A. Hardwick, "Beer" in *Biotechnology Vol. 5* (Verlag Chemie, Weinheim, Germany, 1983)
4. J. R. Helbert, "Beer" in Prescott & Dunn's Industrial Microbiology (AVI Publishing Co., Westport, Connecticut, 1983).
5. B. Kirsop, "Maintenance of yeast cultures" in *Yeast Biotechnology* (Allen & Umwin, Boston, Massachusetts, 1987).
6. B. E. Kirsop, "Maintenance of Yeasts" in Maintenance of Microorganisms (Academic Press London, 1984).
7. E. O. Morris, "Yeast Growth" from some unknown yeast textbook.
8. C. Pederson, "Alcoholic Beverages" in *Microbiology of Food Fermentation* (AVI Publishing Co., Westport, Connecticut, 1979)
9. *Microbiological Methods* (Siebel Institute of Technology, Chicago, Illinois, 1994).
10. *The Practical Brewer* (Master Brewers Association of the Americas, Madison, Wisconsin, 1977).
11. C. Rainbow, "Brewer's Yeasts" in *The Yeasts* (Academic Press London, 1970).
12. M. Raines, *Advanced Yeast Culturing Kit Instruction Booklet* (Brewers Resource, Camarillo, California, 1992).
13. M. Raines, "Yeast Freezing" *Zymurgy* **15** (4) pp. 1992.
14. M. Raines, *Wort Aeration Instruction Booklet* (Brewers Resource, Camarillo, California, 1994).
15. M. Raines, "Laboratory Methods" in *Course Manual* (American Craftbrewers Academy, Torrance, California, 1995)
16. *Recommended Methods of Analysis Part 2* (Institute of Brewers, London, 1991).
17. D. Ryder, "The Fermentation Cycle" from Siebel Institute of Technology 1993 Yeast Culturing Class Handout, Portland, OR.
18. J. P. van der Walt and D. Yarrow, "Methods for isolation, maintenance, classification and identification of yeasts" in *The Yeasts a taxonomic study* (Elsevier Science Publishing Co., New York, New York 1984).
19. Patrick Weix, "Frequently asked Question about Yeast" *Zymurgy* **17** (3) pp. , 1994