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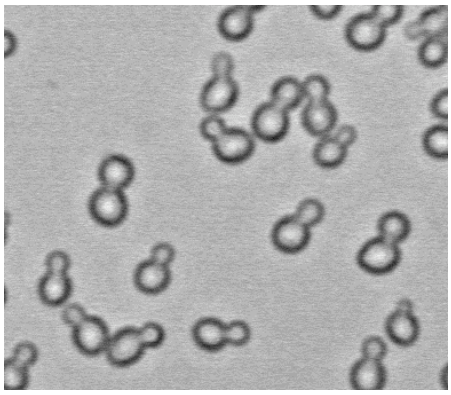
Brewer's Yeast Cell Biology 101:



Saccharomyces cerevisiae (budding/brewer's/bread yeast)

Chemical composition of yeast:

75% water
23-24% organic matter
- 50-60% proteins, peptones, and amino acids
- 30-40% carbohydrates (glycogen, cellulose, etc.)
- 2-5% lipids and fats (glycerol, lecithin, sterols, etc.)
- >0.1% vitamins and other molecules

1-2% minerals
- 50% phosphorous
- 30% potassium
- 6% magnesium
- 4% calcium
- 1.5% sodium
- 1% silica
- 0.5% iron
- 0.5% sulfates

What are the characteristics of healthy yeast?
- Should appear yellow/brown in solution or on plate. Glossy colonies are most likely bacteria. Black yeast are dead yeast.
- Budded vs. unbudded (depletion of nutrients, stationary phase)
- Smell (yeast should smell like beer)
- Compare yeast to *E.coli, Brett, Lactobacillus, Aspergilius* (plates/ microscope)

Growing yeast:
Unlike a laboratory where we can make media of a defined composition (2% glucose, 2% malt extract, 2% yeast extract, 1% bacto-peptone, vitamins, pH 5), wort does not have a detailed ingredient list. The wort is derived from a vegetative source which is inherently variable (degree of malting, adjunct sugars, hops, etc.). Although we have control of the temperature, time, solid-liquid ratios, and gravity, we don't have a strong grasp on the actual nutrient content of the wort. With that said, a wort (not an extract starter) contains a sufficient amount of nutrients for healthy yeast growth.

Basic nutrient requirements:
- Sugar (glycolysis, ATP production)
- Free nitrogen and phosphate
- Vitamins (coenymes for synthesis of metabolites)
- lipids
- Inorganic ions (Na^{+}, K^{+}, Mg^{+2}, $Ca^{+2,}$, Zn^{+2}- essential for proper protein function in cells and regulation of cellular osmolarity)

Starter culture media composition:
Compared to a wort created from a full mash, malt extract based started will have far less free amino nitrogen and vitamins. As a result, it's recommended that you add addition yeast nutrients (i.e. yeast hulls, di-ammonium phosphate, zinc, etc.) to your starter.

Commercial yeast nutrient supplements:

- Di-ammonium Phosphate - This is strictly a nitrogen supplement that can take the place of a lack of free amino nitrogen (FAN).
- Yeast Hulls/extract - This is essentially dead yeast, the carcasses of which act as agglomeration sites and contain some useful residual lipids.
- Yeast Nutrient or Energizer - The name can vary, but the intent is a mixture of di-ammonium phosphate, yeast hulls, biotin and vitamins. These mixtures are a more complete dietary supplement for the yeast and what I recommend.
- Servomyces - This product from Lallemand is similar to yeast hulls but differs by having a useful amount of rapidly assimilable zinc, which is an essential enzyme co-factor for yeast health. This product falls within the provisions of the Rheinheitsgebot.

Basic starter recipe:
Typically boil ½ cup of DME in 2 cups of water (1.040 OG). Might want to add a bit more water to account for evaporation that will occur during boil. Pinch of ½ tsp Di-ammonium Phosphate, 5 grams of Yeast Nutrient/extract, few granules of $ZnCl_2$ (optional, 0.2mg/liter), and one hop pellet. If you add way too much zinc, it will be toxic to the cells. Boil for 10 minutes. Cool in sterile flask. Pitch yeast. Aerate by shaking. Grow for 2-3 days at room temperature. pH ≤ 5.3. Making twice as much wort will yield twice as much yeast and greatly improve fermentation. If you're going to brew a high gravity beer, pitch more yeast. Higher gravity is also correlated with higher osmolarity. It's important to acclimate the yeast in high gravity starter so that they are not shocked when you pitch them into your high gravity wort.

Starter Cultures: Aerobic or Anaerobic?
Yeast are going to try to synthesize membrane fatty acids, sterols, and carbohydrate reserves (glycogen and trehalose) when grown in nutrient rich media. These processes are executed with more ease in the presence of oxygen. Yeast that have to work harder to make these molecules are stressed and therefore more likely to produce unfavorable compounds.

Storage of Yeast:
1. Splits from starter cultures (put in sterile canning jar)
2. Slants or agar plates
3. Frozen 30% glycerol stocks

Pitching Rate and Repitching:
"Despite the rapid rate of yeast growth, a relatively large yeast starter or slurry of yeast is required for optimal beer production. Siebel Institute recommends one-sixth of the batch-size, one-tenth if you continuously aerate or agitate your starter. 1/10 of 5 Gallon is ½ gallon. Use of lower pitching rates such as those typically used by home brewers - 1/20 to 1/100 of the batch size - yield slightly higher terminal gravities because of an alteration in the metabolism of sugars in wort. Generally the order of sugar consumption is glucose (10% wort sugar), sucrose (2% wort sugar), maltose (40% sugar), then maltotriose (10% sugar). When underpitched, later fermented sugars are not metabolized. The end result is a sweeter beer with terminal gravities above 1.010. Theoretically, then, optimal fermentation does not involve significant yeast growth but rather more yeast metabolism of the wort and fermentation to alcohol."

from "Yeast Culturing Practices for Small-Scale Brewers," by Karl King.

In a lab we could yeast can be repitched 15-20 times before seeing an appreciable amount of genetic drift. At home, yeast should not be pitch more than 5 times, because of possible bacterial and wild yeast contaminations. Pitching yeast from the bottom of the fermenter?

How to make plates, slants, and starters?
Yeast on plates in a refrigerator (4^0 C) will stay good for 3-6 months.
Basic recipe is wort + agar.

Recipe:
550mL water (added more than 500mL to account for evaporation during boil)
10g agar (2% wt/vol or 2g/100mL)
10g dry malt extract
10g dextrose/glucose
5g Yeast Hulls/extract
½ tsp (5 grams) di-ammonium Phosphate
This is enough media to pour 20-25 plate. Cost is about $22-25 or $1 per plate.
Boil for 15 minutes
Meanwhile, sterilize jars, lids, and screw caps for 15 minutes in boiling water.
Fill jars with agar wort and seal. Leave screw cap loose to allow air to escape.
Boil for 45-60 minutes.
Remove with jar grabber
Remove lid with knife.
Rotate and decant agar wort from untouch lip of jar into sterile Petri dishes

Cost of reagents/equipment:
Agar (2 oz., $9 at MoreBeer)
2000mL baffled Erlenmeyer Flask ($20)
Metal Inoculation Needle ($4 at MoreBeer)
Petri Dishes (x25) ($10-19)
Magnetic Stir plate (~$120 at MoreBeer or cheaper through Ebay)
Diammonium phosphate (DAP) ($2 for 2 oz. MoreBeer)
Go-Ferm Protect (essentially yeast extract) 100 grams for $6
Fermaid K (yeast extract, lipids, DAP, vitamins) 80 grams for $6

Brewing with non-cerevisiae strains.
Brettanomyces Masters project (http://brettanomyces.wordpress.com/)
"Wild Brews" book
How to grow Lactobacillus and Pediococcus for sour beers:

Rogosa broth:
15g sodium acetate
10g dextrose/glucose
5g yeast extract
5g arabinose
10g tryptone
6g monopotassium phosphate
5g sucrose
add 0.05% Tween-80 post sterilization of media
0.12g manganese chloride
2g ammonium iron citrate
0.51g magnesium sulfate
0.66g ammonium sulfate
0.03g ferrous chloride
1.5g citric acid
adjust to pH 5.4