“Brewer’s make wort; Yeast make beer.”
Where are we going from here?

- **Practical Yeast Biology**
  - Yeast growth
  - Fermentation
  - Off flavors associated with yeast metabolism
  - Beer style and strain selection

- **How did we get here?**
  - Domestication of yeast
  - Where did lager yeast come from?

- **Where are we going?**
  - Understanding and harnessing indigenous (wild) fermentation.
    - Isolating and characterizing wild yeast
    - Developing new yeast strains for fermentation and “bioflavoring”
    - Mixed and indigenous fermentation
Practical Yeast Biology

Carlsberg Brewery (founded 1847) – Copenhagen, Denmark
Probably the best beer sign ever....

Downtown Copenhagen, Denmark
First, a few reminders about “yeast”…

- “Yeast” is a generic term for free-living unicellular growth phase of some fungi.
- When we say yeast, we are usually referring to baker’s/ brewer’s yeast, *Saccharomyces cerevisiae* and *Saccharomyces pastorianus*; ale yeast and lager yeast, respectively.
  - These are species of yeast; “strains” are genetically distinct individuals of the same species
- *Saccharomyces* and non-*Saccharomyces* yeasts are abundant in the environment and in wild fermentations
  - Few have properties that are desirable on their own for fermentation of beer (e.g., *Brettanomyces*, now *Dekkera*).
Yeast Growth...a brewer’s perspective

- What happens when we add yeast to wort?
  - “Lag phase”
    - Adaptation to the environment
      - Generally hours
      - Partially overcome by pitching vigorous Starter
    - Distinct from lag caused by insufficient cell viability
      - Cell number and viable cell number may differ
Yeast Growth (“Life cycle”)…a brewer’s perspective

• “Accelerating phase” (low krausen)
  • Initiation of cell proliferation
  • Generally hours to a day
    • Depends on health, viable cells, nutrition and temperature

• “Exponential phase” (high krausen)
  • Rapid proliferation
  • Generally a day to a week (strain, temperature, nutrient)
    • Higher cell number = shorter time to nutrient exhaustion
      • at optimal pitch cells divide 1-3 times – number increases 2-8 fold
      • Limiting nutrient can result in premature termination of growth

• “Deceleration phase” (late krausen)
  • Slowing proliferation
  • Can be long or short (strain, temperature, nutrient)
Yeast Growth ("Life cycle")...a brewer’s perspective

• "Stationary phase" (Quiescence)
  • Cells flocculate and precipitate
    • Very slow metabolism but still can “clean up” beer.
  • To rack to secondary or allow extended time on yeast?
    • Generally short phase for ales and longer for lagers.
    • Extended lagering may lead to off flavors due to autolysis
      • Off flavors: yeasty, umami
      • Not generally a big issue under normal homebrewing regimens.
Fermentation

Glucose $\rightarrow$ 2 ADP + 2 NAD$^+$ + 2 P$_i$ $\rightarrow$ 2 Pyruvate + 2 ATP + 2 NADH + 2 H$^+$

Maltose

Starch

Acetaldehyde + CO$_2$

NADH + H$^+$

Ethanol + NAD$^+$

Carlsberg Laboratory – (founded 1895) – Isolation of Yeast as distinct organisms, Isolation of S. carlbergensis (pastorianus), Concept of pH, Measurement of N, others
What happens when yeast is added to wort?

Cells grow and proliferate

Glucose is used

Ethanol and CO$_2$ are produced
What is the significance of controlling oxygen?

**Crabtree Effect**

- Fermentation of glucose is preferred over oxidative metabolism, even when oxygen is present. Produces CO$_2$ and ethanol rather than CO$_2$ and water.

- When glucose is depleted, ethanol is used to produce chemical energy and O$_2$ is used to produce water via oxidative metabolism.
Fermentation is complex!

Starch → Glucose

- Fructose
- Raffinose
- Maltose
- Maltotriose
- Sucrose

Glucose → Ethanol + CO₂

- Acetaldehyde
- Amino Acids + Fusel Alcohols
- Phenols
- Sulfur compounds
- Diacetyl
- Esters
- Ketones
Off-flavors associated with Yeast and Fermentation

- **Acetaldehyde** - an intermediate in alcohol production – green apple – allow time post-fermentation for clean-up
- **Acetic Acid** – an oxidation product of alcohol – harsh sour, vinegar
- **Alcohol** – the goal of fermentation – Hot – keep alcohol level appropriate for beer style
- **BandAid/Medicinal** – Phenolic compounds produced by wild yeast or Brett, can also come in with ingredients – avoid contamination, decrease temperature when growing strains that naturally produce them (Belgian, Hefewiezen, Brett)
- **Banana** (Isoamyl Acetate) – an ester – (see Estery/Fruity)
- **Diacetyl** – long lag phase, short post fermentation rest - buttery, slick mouth feel – avoid by healthy yeast and post-fermentation rest at >60F
- **Estery/Fruity** - formed by reaction of an acid and an alcohol – associated with stress and long lag phase
- **Fusel Alcohol** – long lag phase, excess amino acids, high temperature – hot, solventy – avoid by sufficient pitch, decrease temperature, reduce stress on yeast, avoid contamination
- **Soapy/Fatty** – product of yeast autolysis (see Yeasty)
- **Spicy/Clove** (Eugenol) – Phenol, desirable in Hefeweizen and some Belgians (see BandAid)
- **Sulfur compounds** – formed by many metabolic reactions - rotten eggs, burnt match, cooked vegetables – reduced by active, healthy fermentation
- **Yeasty** – product of yeast autolysis – caused by too much time on yeast post-fermentation or severely stressed yeast
Off flavors result from natural metabolism

- Stress alters the flux of metabolites through pathways and leads to off pathway reactions.
- Environmental compounds get metabolized as defense or utilization.
Fermentation Control is the struggle to maintain a balance between competing reactions.
The Elements of Fermentation Control

• Temperature
  • Every yeast strain has an optimal temperature range.
  • Although most ale yeast strains will grow well and ferment from 50-90F, ferment under 70F to avoid off flavors.
    • Ester and fusel alcohol production increase with higher temperature.
    • Cooler fermentation temperatures produce cleaner beers.
  • Lager yeast ferment cooler and cleaner.
    • Grow and ferment at lower temperatures, produce less fusel alcohols and esters (but do produce various sulfur compounds)
The Elements of Fermentation Control

• Oxygen
  • Why is oxygen important and when?
    • Needed for efficient cell wall synthesis
    • Important for overcoming the Lag phase and throughout growth phase.
    • Optimally 8-10 ppm, achievable with air but easier with pure O$_2$.
  • When is oxygen a bad thing and why?
    • Many components of beer are sensitive to oxidation
      • Greatly enhanced by environmental oxygen
      • Over-oxygenation can lead to over-growth and off flavors
    • In the presence of oxygen, yeast can use alcohol to produce energy and grow through “oxidative metabolism”.
  • Provide oxygen when cells are proliferating.
  • Avoid oxygen as cells cease proliferating.
The Elements of Fermentation Control

• Pitching Rate
  • Optimal rate allows 1-3 rounds of division for maximal cell number.
  • The rate required is determined by original gravity of wort and health of the yeast culture.
  • Over-pitching is less serious but can lead to premature flocculation, poor attenuation and, sometimes, “autolysis” of yeast cells.
  • Calculate pitch rate using a calculator such as “The Pitching Rate Calculator”:
    <http://www.mrmalty.com/calc/calc.html>
The Elements of Fermentation Control

• Nutrition
  • All necessary nutrients are present in wort at some level except zinc and oxygen.
  • Adjuncts (corn, wheat, etc) are low in some nutrients so dilute nutrients in the wort.
  • The essential nutrients can be supplemented by addition of additives that include nitrogen, minerals and vitamins.
  • Conditions that promote growth (temp, oxygen, and nutrients) can increase fusel alcohols and esters. Use at suggested dose.
Yeast Strains and Beer Styles

• Brewers select yeast strains to develop desired beer characteristics
  • For yeast-forward beers select strains that are consistent with the desired character.
    • Hefeweizen (Esters and Phenols, IAA & Eugenol)
    • Belgian Pale and Belgian Strong Ales (Esters and Phenols)
    • English and Scottish Ales (Esters)
    • Lagers/Pilsners ($\text{H}_2\text{S}$, DMS and Diacetyl)
    • Wild and Sour Beers (Phenols, Esters, Acids, Aldehydes)
Yeast Strains and Beer Styles

- Brewers can select yeast strains to emphasize beer characteristics
  - For hop forward beers select clean fermenting yeast with an appropriate level of attenuation for the style at hand.
    - Generally hops shine more clearly in beers fermented with clean fermentation.
  - For malt forward beers select strains that emphasize malt character.
    - Typical of British yeast strains; some lager strains (Octoberfest) emphasize malt
    - Some strains bridge the ale/lager divide
      - Hybrid yeast strains including those used for Kölsch and Altbier
Yeast Strains and Beer Styles

- Sometimes conditions dictate the selection of specific yeast strains
  - Cold temperatures: Lager/Pilsner yeast that do well at 50F-55F.
  - Warm temperatures: Belgian Ale yeast that emphasize esters and phenols, “off flavors” appropriate for the beer style at hand.

- Experiment with fermenting the same wort with different yeast and with using the same yeast under different fermentation conditions.
  - White labs tasting room is an outstanding venue for these exercises.
  - Each time you brew, split the batch or pull off a 1 gallon and test a different yeast strain or different conditions with the same strain.
Experimenting with multiple yeast strains

White Labs

Curt’s Labs
Yeast Storage

• Yeast storage should optimize long term viability.
• Large quantities, sufficient for pitching or for starters, are most easily stored in a cold refrigerator under either fermented, low alcohol beer or sterile degassed water.
  • Yeast retain viability for one month to many
  • Prepare a starter, if stored for more than a week.
• Small amounts sufficient for a starter can be stored on nutrient agar.
• Freezing in a non-frost free freezer in glycerine (7-15%) is best for long term preservation (-80°C is optimal, 3°F works).
  • Freeze slowly and thaw rapidly.
  • Works for small samples as well as with pitchable quantities.
• Drying yeast: not generally achievable by homebrewers.
How did we get here?
A little yeast history....

First observed and described single-celled organisms he called animicules. They were generally thought to arise by spontaneous generation during spoilage or fermentation.

First isolated yeast as a single species he called *Saccharomyces monocencis*, later called *S. carlsbergensis* and now called *S. pastorianus*.

Pasteur discovered that microbes were responsible for fermentation. He also disproved the prevailing theory of spontaneous generation.
Where did brewing strains come from?

Microbial Terroir
Wild *S. cerevisiae* can be unruly!
But poor behavior can be dealt with...

- We do not want yeast that are:
  - Pseudohyphal
    - Tendency to form biofilms
  - Sporogenic
    - Genetic instability and propensity to hybridize
  - Haploid or Diploid
    - Lower genetic stability than polyploid strains

- Undesirable characteristics can be eliminated by selection or by direct mutation.

La Valle and Wittenberg, 2001
Genetic alterations of yeast strains can be spontaneous or induced.
Lager yeast, *S. pastorianus*, is a spontaneous hybrid

*S. pastorianus* = *S. cerevisiae* + *S. eubayanus* hybrid

*S. eubayanus* gall on beech tree
Where are we going?
Yeast strain development

Yeast with spontaneous or induced genetic alterations can be screened for characteristics that are favorable for beer production. That might include flavor, alcohol tolerance, flocculation, or other desirable features.

**Spontaneous mutation**

- Natural Evolution or Domestication

**Induced mutation**

- GMO
On beyond Saccharomyces cerevisiae

*Pichia kudriavzevii*
Why venture beyond?

• … an increasing number of studies find that confining the microbial diversity limits the sensorial characteristics and reduces the complexity of the end product. These drawbacks have inspired some producers, often small-scale artisans looking for authentic and special niche products, to rediscover the spontaneous fermentation process. Moreover, … large-scale producers are now also showing an increased interest in using nonconventional (i.e., non-\textit{Saccharomyces}) yeasts in controlled fermentation processes to increase the flavor diversity, control microbial spoilage, and/or alter other key characteristics, such as the final alcohol content.
  
On beyond Saccharomyces cerevisiae

- There are many commercially available yeast and bacterial strains for fermentation and/or development of complexity.
  - Common ‘wild yeast” include:
    - *Brettanomyces* (now called *Dekkera* by taxonomists)
    - Wild *Saccharomyces* strains (some orginally identified as *Brettanomyces*)
    - Other *Saccharomycetales* and non-*Saccharomycetales* yeast.
  - Bacteria are also common in “wild” or indigenous fermentations
    - Lactic acid bacteria including *Lactobacillus*, *Pediococcus* and *Acetobacter* are sometimes used for their souring capabilities
  - Associated with acetic and lactic acid formation, generation of “barnyard”, “leather”, “smoke” or other flavors
Spontaneous fermentations

- Spontaneous fermentation is probably the easiest way to collect wild microorganisms that will make beer.
  - Collection in open “coolship fermenters” is common
  - Petri dishes can be used to collect independent airborne isolates.
  - Allowing malted grain to ferment at appropriate temperature is a good way to harness wild lactic acid bacteria (~110°C) and sometimes other resident organisms.
  - Yeast and bacteria are common residents on harvested or dropped fruit. They can be collected by fermenting juice from the fruit or from fermenting fruit on the tree.
  - Beware: There are dangers associated with collection of “environmental” microorganisms. On the other hand, those you end up with in your fermentation may be residents of your own brewery.
The complexity of spontaneous fermentations

Wine

Lambic

Cocoa
Distinguishing between yeast species – Low tech methods – Colony Morphology
Nutrient utilization and chemical sensitivity

The sensitivity or tolerance of yeast cells to various chemicals and conditions can provide insight into the genus. Sensitivity to chemicals including cycloheximide, ethanol, and ability to grow on specific sugars (i.e. maltose, galactose, etc.).

\[ S. \text{cerevisiae} \text{ is CHX sensitive. } Hanseniaspora \text{ uvarum (aka Kloeckera apiculata)} \text{ is CHX tolerant.} \]

\[ S. \text{cerevisiae} \text{ is ethanol tolerant. } Hanseniaspora \text{ uvarum} \text{ is ethanol sensitive (3-4%).} \]
Cell Morphology

The size and shape of a cell is sometimes informative with regard to genus and species, especially when other information is available.
Higher tech – PCR

Analysis of DNA by Polymerase Chain Reaction (PCR) is generally useful for identification of genus and, sometimes, species of fungi.

- Generation of a DNA fragment of a specific size is often diagnostic of the genus.
- Sometimes it is sufficient to determine species when combined with other information.
- Often determining the exact sequence of the short DNA fragment is required for species identification and sometimes for identification of genus.

PCR of gene encoding the 5S ribosomal RNA
High Tech – The Fermentation Microbiome

Current approaches involve analysis of DNA, RNA, protein and metabolites to develop a comprehensive picture of the composition and activities of the microorganisms making up the microbiome.

By analyzing the entire mixture at once and then deconvoluting the information one can determine precisely which species are present, what genes they are expressing and what biochemical reactions those organisms are responsible for.

This approach has been applied to the various human organs and orifices, the microbes making up various ecosystems, and, of course, various types of fermented foods (beer, wine, cheese, cocoa, kimchi, kombucha, etc.).
Microbiome of an American Coolship Ale

The microbiome of spontaneous fermentations is generally complex and very dynamic.
Even breweries have microbiomes....

Modern ‘omics approaches are even being applied to industrial environments to monitor the nature and source of contaminating microorganisms.

Summary

• Practical applications of beer microbiology
  • Managing microbes and fermentation conditions is critical for the production of desirable fermented beverages.

• How did we get here?
  • Currently available yeast and other microbes has benefited greatly from their spontaneous and managed domestication.

• Where are we going to now?
  • Traditional methods and high technology approaches are making it possible both to characterize and to alter the microbial landscape for the production of better and novel fermented beverages.
Thanks for Listening

I’m open for questions and/or BEER!