Brettanomyces:

Flavors and performance of single and multiple strain fermentations with respect to time.

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“Brett” Descriptors

- Horse Blanket
- Barnyard
- Pie Cherry
- Sweetarts/ Smarties Candy
- Smokey
- Band-aids
- Bacon
- Spice
- Cloves
- Cheesy
- Rancid
- Mousy/ Mouse Urine
- Bready
- Dirty Socks

- Clean?????
Outline of Talk

• General Brettanomyces Information

• Wyeast Experiment

• Tasting/ Discussion
General

• Isolated by Claussen 1904 as cause of 2° fermentation of English Stock Beer

• Brettano- myces = British Brewing-Fungus

• Dekkera
  – Interchangeable name for Brettanomyces
  – Sporagenous form or sexual anamorph
  – Spore form is rare in nature <1.0%
General

• Major spoilage organism in wine.
  – Responsible for $ millions annual losses in the wine industry.
  – Most research performed on Brettanomyces in wine.

• Very little research in beer.

• Common character of Lambics and Orval

• Homebrewers Gaining Popularity
  – (Burgundian Babble Belt, Mad Fermentationist, etc.)

• US Commercial Brewers
  – Russian River, Pizza Port, Jolly Pumpkin, The Shed etc.
General

Brettanomyces/ Dekkera Species Taxonomy

• B. bruxellensis
  – B. lambicus, B. intermedius
• B. anomala
• B. custersianus
• B. naardenensis
• B. nanus

Each specie very diverse with many strains
General

- Slow growing
  - Grow to large populations from very few cells
- Fastidious
- Very adaptable
- Resilient to harsh conditions
  - Low pH
  - High alcohol
  - Low nutrient
- Resistant to Cycloheximide
Morphology

• Variable Morphologies
  – “Boat” shaped (Ogival)
  – Spherical
  – Elongated (Pseudohyphae)
Metabolism

• Can use a large number of substrates for carbon source
  – Simple Sugars
    • Glucose, Fructose, Sucrose, Trehelose, Maltose, Maltotriose
  – Dextrins
    • Maltotetrose completely
    • Maltopentose & Larger dextrins partially
  – Alcohol
    • Ethanol, glycerol
  – Cellibiose (Wood)
    • Most of the problems in wine industry
“Brett” Flavor Contribution

• Volatile Phenols
  – Medicinal (Band-Aid, plastic)
  – Farmyard (Horse blanket, Wet animal, Wet leather)
  – Spicy Clove

• Acetic Acid (Vinegar)
  – $O_2$ required
  – More found in barrel/carboy aging
  – Less if added at bottling

• Isovaleric Acid (Rancid, Cheesy)
  – Leucine conversion

• Tetrahydropyridines (Mouse urine [High], Bready [Low])
  – Ethanol + Lysine ($O_2$)

• Ethyl Lactate
  – Fruit, Coconut
Volatile Phenols

- Why do they do that?
  - Survival mechanism.
  - Way for cell to replenish coenzyme NADH pool

- Reaction is dependant on enzyme activity and substrate levels
  - Not necessarily dependant on growth
    - Population
  - Enzyme activity is dependant on fermentation conditions.
    - Manipulated by $O_2$, pH, Density, Temperature
  - Level of substrates - Hydrocinnamic Acids (Penolic Acids)
    - Abundant in plants including cereal grains.
    - Anti-oxidants
    - Natural antimicrobials
    - Manipulated by Grist/ Mash profile
Volatile Phenols

Phenolic Acid

P-Coumaric Acid
Ferulic Acid
Caffeic Acid

Decarboxylation

Vinyl Phenol

4-vinyl phenol
4-vinyl guiacol
Vinyl calechol

Reduction

Ethyl Phenol

4-ethyl phenol
4-ethyl guiacol
Ethyl calechol

Common

Saccharomyces
Most Wild yeast
Lactic Acid Bacteria

Unique

Brettanomyces
Pichia spp.
Candida spp.
Few Lactic Acid Bacteria
Sanitation

• Why are we scared?
  – Don’t know a lot about it.
  – Will survive in low pH, high alcohol, low nutrient environments.
  – Very recognizable flavor profile.
  – Bio-film

• Don’t be scared!
  – Brettanomyces dies like everything else.
    • Use good sanitation techniques – no problems
    • Susceptible to heat and chemicals.
      – 155°F
    • Avoid plastic fermenters
    • No cracks in gaskets
    • Take all parts of corni apart
    • Chemicals
      – Cleaner
        » Caustic
        » Glo-san/ PBW
        » Can add chlorine if concerned with biofilm.
      – Sanitizer
        » Iodophor
        » Acid Sanitizer
        » Peracetic acid
How do we use Brett in the Brewhouse?

- Commercial Examples
  - Orval @ Bottling 5000 cells/ml.
    - No priming sugar added
    - Flavor production around 3 months.
  - Lambic Breweries
    - Natural inoculation
    - Flavor production 6 months
  - Russian River
    - Number of different beers.
      - Most 2° Brett in barrel
      - Sanctification all Brett
    - Bottles at 1.010, Ends up at 1.004
  - Jolly Pumpkin
    - 1° stainless, 2° Natural Brett in barrel
    - Not a lot of density drop from Brett

- Homebrew
  - Bergundian Babble Belt Brett Exchange
  - http://madfermentationist.blogspot.com/

- Not a lot of difference between US Commercial and Homebrewers
  - Pioneers. A lot to experiment with.
How do we use Brett in the Brewhouse?

• Problems
  – Not a lot of research on beer.
  – Very complex flavor production.
  – Lengthy fermentations/conditioning
  – Many variables involved.
  – Profile changes with CO₂
Brewing Variables

Brettanomyces Strain
Saccharomyces Strain
Timing of Incoculation
Pitch Rate
Temperature
Grist
Mash
Density
pH
O₂
Wyeast Experiment

• Goal
  – Determine the affect the timing of inoculation.
  – Determine the affect of other yeast strains on Brettanomyces flavor production

• Protocol
  – Brew standard/simple wort every month for 3 months
  – Inoculate 4.5 gal. fermentations
  – Test pH, density, and sensory each week
Recipe

General
Vol. 44 gal
IBU 10

Malt
25# Briess Pilsen DME (63%)
10# Briess Wheat DME (25%)
5# Maltose (12%)

Boil
30 min 8oz. Williamette (5.5%AA)
12 min 7 g. Tan B
10 min 20 g. Whirlflock
5 min 20 g. Wyeast Nutrient

Fermentation
OG 13.0°P
O₂ 13 ppm.
Fermentation Temperature 70F
Saccaromyces Pitch Rate 6 million cells/ ml.
Brettanomyces Pitch Rate 3 million cells/ ml.
Inoculations

• #1
  – 1° Brettanomyces (5526)

• #2
  – 1° Saccharomyces (1007, 1056, 3787)
  – 2° Brettanomyces (5526)

• #3
  – Co-inoculation Saccharomyces-Brettanomyces
  – (1007-5526, 1056-5526, 3787-5526)
Fermentation Data
Brett Only

Brett Only
\( \Delta \) Density/ Time

Brett Only
\( \Delta \) pH/ Time

Time (Days)
Density (Plato)

Time (Days)
pH

A2
B1
B2
B3
B4
Fermentation Data

Brett in 2°

\[\Delta \text{Density/ Time}\]

\[\Delta \text{pH/ Time}\]
Fermentation Data
Co-Inoculation

**Co-Inoculation**
\( \Delta \text{Density/Time} \)

**Co-Inoculation**
\( \Delta \text{pH/Time} \)

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**Graph 1:**
- Co-Inoculation
- \( \Delta \text{Density/Time} \)
- \( \text{Time (Days)} \)
- \( \text{Density (Plato)} \)
- Graph showing data points for A3, A4, B5, B6, C1, C2, C3, C4, C7 over time.

**Graph 2:**
- Co-Inoculation
- \( \Delta \text{pH/Time} \)
- \( \text{Time (Days)} \)
- \( \text{Density (Plato)} \)
- Graph showing data points for A3, A4, B5, B6, C1, C2, C3, C4, C7 over time.
Conclusions

• 2° inoculation preferred beer
  – Possible CO₂ inhibition with Co-inoculation

• Saccharomyces strain does make a difference.
  – Competition for metabolites
  – Production of Ethyl phenol pre-cursors

• Can produce nice Brett beer relatively quick.
  – Onset of Brett character in 3-5 weeks.

• TG stabilized as Brett flavors continued
  – TG 1.004-1.008
  – All Brett 60-80 days
  – Co-inoc & 2° inoc 30-50 days
  – May be different with different strain, grist, mash, OG.

• This is only one trial of 1 experiment!!

• Don’t be afraid to try this at home.
Tasting

- 6 samples
- Beware of “Palate Fatigue”
- Go back frequently to “Control”
- Samples changed with every tasting.
Tasting #1

• Control (A7) Saccharomyces Only
  – 1007 German Alt
  – 14 weeks
  – TG 2.8°P
  – pH 4.54

• Nose
  – Slight fruit, red apple, pear

• Flavor
  – Fairly neutral
  – Slight fruit
  – Slight yeast
  – Malty finish
Tasting #2

- Co-inoculation (C2)
  - 1056-5526
  - 6 weeks
  - TG 2.3
  - pH 4.67

- Nose
  - Slight Sweetart/Smarties
  - Med Smoke
  - Slight Band-aid
  - Med-High Horsey
  - Med Burnt Rubber

- Flavor
  - Med Smoke
  - Med Band-aid
  - Med Horsey
  - Slight Acetic
  - Full on palate
  - Bready

- Overall
  - Not preferred
Tasting #3

- Co-inoculation (C4)
  - 3787-5526
  - 6 weeks
  - TG 2.0
  - pH 4.21

- Nose
  - Med Acetic
  - Med Fruit - Juicy Fruit
  - Mild Horsy
  - Slight Burnt Rubber

- Flavor
  - Med Acetic
  - Med Horsy
  - Slightly Astringent

- Overall
  - OK
Tasting #4

• Brett in 2° (C6)
  – 3787 1°, 5526 2°
  – 6 weeks
  – TG 1.8
  – pH 4.19

• Nose
  – Med Acetic
  – Med Horsy

• Flavor
  – Med Acetic
  – Med Horsy
  – Nice “Lambic” Character
  – Med Clove

• Overall
  – Preferred
Tasting #5

• Brett only (A2)
  – 5526 1°
  – 14 weeks
  – TG 2.0
  – pH 4.20

• Nose
  – Mild Acetic
  – Mild Horsey
  – Mild Fruit - Juicy Fruit
  – Mild Stone fruit
  – Dirty Socks

• Flavor
  – Mild Acetic
  – Mild Horsey
  – Mild Fruit - Juicy Fruit
  – Mild Stone fruit
  – Dirty Socks

• Overall
  – A lot cleaner than expected
Tasting #6

- Co-inoculation (A4)
  - 1007-5526
  - 14 weeks
  - TG 2.0
  - pH 4.18

- Nose
  - Slight Acetic
  - Mild Fruit - Pear
  - Mild Bubblegum

- Flavor
  - Slight Acetic
  - Mild Fruit - Pear
  - Mild Bubblegum

- Overall
  - A lot cleaner than expected
Thanks

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Questions?

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