Overview

• Wine microbiology
• Microbial faults
  – *Brettanomyces*
  – Lactic acid bacteria
  – Cork Taint
• Controlling microbial faults
  – Sanitation
  – Quality programs
Saccharomyces cerevisiae
Saccharomyces cerevisiae

(Piskur et al 2006)
Does Not Always Work
The Wine Fermentation

**Yeast**
- Metschnikowia sp.
- Pichia sp.
- Candida sp.
- Kluveromyces sp.
- Hanseniaspora sp.
- Saccharomyces

**Bacteria**
- acetic acid bacteria
- lactic acid bacteria

**Molds**
- Botrytis & others

**Time**

**OD**

**EtOH**

**Sugar**

**NC State University**

**Cooperative Extension**

Empowering People • Providing Solutions
Three common microbial contaminants

- Brettanomyces
- Lactobacilli
- Cork taint
Brettanomyces
Brettanomyces bruxellensis

4-ethylphenol produced in absence of platable population

Am J. Enol Vitic 54:294-300
Brettanomyces bruxellensis

Isolated by Dr. Clausen in 1904

The brewing industry just started using yeast

Provides missing element of traditional beers
Friend or Foe?

For Now

Brett
4 - Ethylguaiacol (4-EG): ~175 ppb
4 - Ethylphenol (4-EP): 600 - 800 ppb
Diacetyl: 2-4 ppm
Geraniol: 0.5 – 1 ppm
Trichloroanisole (TCA): very low ppb
## Typical odors

<table>
<thead>
<tr>
<th>Compound</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-ethyl phenol</td>
<td>Band-aid, burnt plastic</td>
</tr>
<tr>
<td>4-ethyl guaiacol</td>
<td>Smoky, Spicy, Clove</td>
</tr>
<tr>
<td>4-ethyl catechol</td>
<td>Sweaty, Horsey</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>Rancid, Cheesy, Vomit</td>
</tr>
<tr>
<td><strong>Combined</strong></td>
<td>Barnyard, mouldy</td>
</tr>
</tbody>
</table>
Sensory threshold

- 4-ethylphenol 0.23 mg/L
- 4-ethylguaiacol 0.047 mg/L
- Perception is dependant on the type of wine
- Can mask varietal character
- May degrade/reduce some fruity aromas
- Metallic finish
- May also produce acetic acid
Production of volatile phenols

Hydroxycinnamic acids → Vinyl derivatives → Ethyl derivatives

- Cinnamate decarboxylase
- Vinyl phenol reductase

**Plant derived**
- p-coumaric acid
- p-ferluic acid
- Caffeic acid

**Saccharomyces**
- Wild yeast
- Lactic acid bacteria

**Brettanomyces**
- Pichia
- Candida
- Lactic acid bacteria

- 4-vinylphenol → 4-vinylguaiacol → 4-ethylphenol
- vinylcatechol → ethylcatechol → 4-ethylguaiacol
Things to remember

- A low levels can enhance varietal flavors
- If you think you have it under control you will probably not be able to keep it that way
- Phenolic precursors from the grapes are metabolized into ethylphenols so levels will very with variety
Where do you find Brett?
Geographic distribution

(Conterno et al. 2006)
Locally

- Present at low levels on grapes
  - Damaged grapes may provide nutrients
- Found in fruit flies
- Present throughout the winery
Growth during winemaking

- More prevalent as a problem in reds
  - More phenolic compounds
  - Longer aging
- May be found in white wines
- May be in dry wines
- Likes to have oxygen but does not need much
Growth during winemaking

![Graph showing bacterial growth during winemaking](image)

- **End of the AF**
- **MLF**
- **End of the MLF: sulfur dioxide addition**

<table>
<thead>
<tr>
<th></th>
<th>Medium of AF</th>
<th>End of the AF</th>
<th>End of the MLF (before SO₂ addition)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hanseniaspora uvarum</strong></td>
<td>100%</td>
<td>&lt;5%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td><strong>Candida cantarelli</strong></td>
<td>&lt;5%</td>
<td>10%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td><strong>Debaryomyces hansenii</strong></td>
<td>&lt;5%</td>
<td>5%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td><strong>Brettanomyces bruxellensis</strong></td>
<td>&lt;5%</td>
<td>85%</td>
<td>100%</td>
</tr>
</tbody>
</table>

(Renouf et al 2006)
Growth during winemaking

- Brett can even survive up to 8 millimeters into the barrel staves
- Can consume sugars present from the wood
- Can survive in a viable nonculturable state
Things to remember

• Strain variation may account for differences in between wines
  – We are looking for useful strains
• Present through out the winery
• Able to persist throughout fermentation
  – Low numbers do not mean it is a problem
  – Predominate yeast after alcoholic fermentation
  – May enter viable nonculturable state
Best to prevent Brett from being a problem

Minimizing off flavors
**Prevention-In the vineyard**

- Remove damaged grapes
- Use SO$_2$ at harvest
- Off-flavors generally have lower sensory thresholds in lighter wines so pay even more attention to good winemaking practices
**Prevention-In the winery**

- Proper sanitation
- Long maceration
  - More substrate to produce off-flavors
- Red wine more prone to Brettiness
  - Higher substrate
  - Longer aging
  - White wines: lower pH, higher SO$_2$
**Prevention—In the winery**

- pH below 3.6
- Try to keep temperatures lower
- SO$_2$ usage (use 0.8 ppm molecular SO$_2$)
  - Make sure to use at picking and or crushing
  - Use after MLF
- Keep alcohol levels above 13%
- Keep residual sugars below 0.2 g/L
  - Even dry wines can support growth
Prevention-In the winery

• Measure must nitrogen level and add just enough nitrogen to have a strong fermentation
  – Too much can leave nutrient which Brett can use

• If leaving on lees make sure to control other parameters

• Keep containers topped up
  – Limits amount of oxygen available
Prevention - In the winery

- Highest risk of spoilage from end of MLF through aging
  - Use an active MLF strain
- Use clean wines to top-up
  - Either filter or add DMDC (velcorin)
- Beware of cheap used barrels
  - Don’t use infected barrels
- Segregate infected barrels
Wine prevention—In the winery

- Watch cross contamination
  - Use plastic pipets to sample from barrels and change pipets after each barrel

- Best barrel cleaning
  - Cold rinse, then 70°C then steam at low pressure for 10 minutes
  - Barrels cannot be sterilized even with SO₂ or Ozone

- Monitor before bottling
  - Check pH, SO₂, alcohol, watch oxygen levels
  - May need to filter or add DMDC
Lactobacillus spp.
The problems

- May cause stuck fermentations
- Higher VA-produces acetic acid and lactic acid
- “Tourne”
- Acrolein
- Geranium
- Mousiness
- Biogenic amines
- Diacetyl
- Ropiness
The problems

- May cause stuck fermentations
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- Mousiness
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- Diacetyl
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Fermentation rates: Long lag

- Usually suggest presence of a toxin or deficient population of healthy yeasts
Fermentation rates: sluggish throughout

- Often due to nutrient deficiency
  - Diammonium phosphate (DAP)
    - Added at 200 mg per liter (0.03 ounces/Gal)
    - Not all at once three times throughout fermentation
- Poor strain tolerance to stress
- Inhibitory compounds
Fermentation rates: Becoming sluggish

- Toxins produced by molds
- Mild temperature shock
- Moderate deficiency in compounds needed to survive at higher ethanol concentrations
- Too high an inoculum
Fermentation rates: abrupt arrest

- Usually due to traumatic shock
  - Temperature increase or decrease
  - Higher ethanol concentration makes it more sensitive
- May be due to some malolactic strains
The other taints

- Tourne-rare breakdown of tartaric acid
  - Loss acidity color becomes brown
- Acrolin-bitterness
- Mousiness- mouse cage
- Biogenic amine formation
- Ropiness
On Your Table

4 - Ethylguaiacol (4-EG): \(~175\ \text{ppb}\)
4 - Ethylphenol (4-EP): \(600 - 800\ \text{ppb}\)
Diacetyl: \(2-4\ \text{ppm}\)
Geraniol: \(0.5 - 1\ \text{ppm}\)
Trichloroanisole (TCA): \textit{very low ppb}\)
Geranium taint

- Most likely to occur in sweet wines
Geranium taint

- Sorbate typically used at 100 to 200 mg/L
Geranium taint

Size Bar = 3um

Courtesy of Jeff Broadbent and the Utah State University electron microscope facility.
Geranium taint

- Sorbate typically used at 100 to 200 mg/L
- Keep SO$_2$ levels up
Diacetyl production

- Produced by lactic acid bacteria during growth
- Use citrate
- Threshold
  - 0.2 mg/L in Chardonnay
  - 2.8 mg/L in Cabernet
Diacetyl control

- Inoculate for ML
- Don’t use citrate
- Keep pH low
Controlling Lactic acid bacteria

- Keep pH low <3.5
- Keep SO$_2$ levels to 50 to 75 mg/mL
- Addition of lysozyme 250 mg/mL
- Keep temp low during cold soak <59°F
- Conduct ML
- Sanitation
Cork taint

2,4,6-trichloroanisole
On Your Table

4 - Ethylguaiacol (4-EG): ~175 ppb
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Cork taint

\[
\text{O} \quad \text{CH}_3
\]

\[
\text{Cl} \quad \text{Cl} \\
\rightarrow \\
\text{Cl}
\]

2,4,6-trichloroanisole
Cork taint

Perception threshold

4 ng/L

2,4,6-trichloroanisole
Cork taint
Cork taint
Cork taint
Cork taint
Cork taint control

• Limit use of chlorine based sanitizers
• Limit use of chlorophenol based insecticides and fungicides
• Control humidity (high humidity encourages mold growth)
• Have good ventilation
Controlling microbial issues
Winery Sanitation

- Sanitation = Disinfection
- Cleaning
- Sterilization
Winery Sanitation

Sanitation is an attempt to reduce the number of spoilage microorganisms on equipment surfaces.
Winery Sanitation

Cleaning is an attempt to physically and chemically remove food for microorganisms and to eliminate hospitable environments for their growth.
Winery Sanitation

Soil is a material in the wrong place such as dirt dust and organic material- tartrate deposits
Five Steps in a Sanitation Program

• Rinse to remove large debris
• Apply cleaning compound - remove soil
• Rinse to remove dispersed soil
• Sanitizing - kill microorganisms
• Monitoring
Cleaning

• Think of the soil characteristics

• Solubility
  – Water (salts, sugars, starches)
  – Acid soluble (oxidized iron, zinc carbonates, calcium oxalates, hard water scale)
  – Alkaline soluble (Fatty acids, proteins, other organic deposits)
Methods of Sanitization

• Heat
  – Time
  – Temperature

• Chemical
  – Time
  – Temperature
  – Concentration
  – pH
  – Organic matter
Methods of Monitoring Sanitization

• Sensory
  – Does the surface look clean
  – Does it feel clean
  – Does it smell clean

• Microbial counts
  – Swab 4x4 in area for defined period of time
  – Direct contact-press plate against surface

• Luciferase bioluminescence
Methods of Monitoring Sanitization

<table>
<thead>
<tr>
<th>Equipment and Description</th>
<th>Procedure Number</th>
<th>Frequency</th>
<th>Times</th>
<th>Notes</th>
<th>Employee Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crusher (line 2) Rinse</td>
<td>9.01</td>
<td>After every lot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td>9.02</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitize</td>
<td>9.03</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Destemmer (line 2) Rinse</td>
<td>9.01</td>
<td>After every lot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td>9.02</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sanitize</td>
<td>9.03</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Press (#2) Rinse</td>
<td>9.01</td>
<td>After every load</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean</td>
<td>9.05</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sanitize</td>
<td>9.06</td>
<td>Every 8 hours</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assigned Employee: ___________________ Approval of Supervisor: ___________________

Figure 9.3. An example of a cleaning and sanitizing schedule.
Sanitation

• Develop a plan
• Have written protocols
• Place someone in charge
Quality control

• Conduct a Hazard Analysis
• Identify the critical control points
• Establish critical limits for control measures
• Establish procedures for monitoring
• Establish corrective action to be taken when monitoring indicates there is a deviation from a critical limit
Quality control

- Establish effective record keeping procedures that document the Quality control system
- Establish procedures for verification of the Quality control system
Controlling microbial issues

• Use quality fruit
• Pay attention to acid levels
• Use SO$_2$
• Watch cross contamination
• Keep barrels topped up
• Develop a sanitation plan
• Develop a quality plan
Controlling microbial issues

• Use quality fruit
• Pay attention to acid levels
• **Use SO\textsubscript{2}**
• Watch cross contamination
• Keep barrels topped up
• Develop a sanitation plan
• Develop a quality plan
Trevor Phister
Phone: 919-513-1644
Email: trevor_phister@ncsu.edu
How do we know Brett is in the wine?
Classical *Brett* Enumeration

Other yeasts such as may grow on cycloheximide
Gas Chromatography

Detects ethylphenol

Some strain of Brett do not produce 4-ethylphenol

Detecting metabolic end product. If you can measure it then it is probably already too late.
• Need high levels of cells
  – Brett does not always reach 1000 cells/mL
• Brett may be difficult to identify due to variable morphology
• Are the cells living or dead?
  – Use stain
PCR

- Rapid (3-4 hours)
- Specific (sometimes to specific)
- Detect and enumerate before Brett is a problem
- Costly
- Need lots of training
  - Performed at service laboratories