Enology Research and Extension at Oregon State University

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Enology Extension Specialist
Department of Food Science and Technology
Overview

• Introduction
• Research
  – Previous studies
• New research projects
  – Future direction
• Other enology research in the Dept
• Enology extension
• Questions
Winemaking

Grapes

Crushed/Destemmed

Must

Alcoholic fermentation
Alcoholic Fermentation

Grape sugars $\rightarrow$ Yeast $\rightarrow$ Ethanol + CO$_2$

• Natural vs. Induced
  – *Saccharomyces cerevisiae*

• Flavor contribution
  – Higher alcohols, esters, aldehydes, acids, volatile sulfur compounds
Winemaking

Grapes

Crushed/Destemmed

Must

Alcoholic fermentation

Malolactic fermentation
Malolactic Fermentation (MLF)

- **O. oeni**
  - Malic acid $\rightarrow$ Lactic acid
    - Bacterial enzymatic conversion
- **Decreased acidity**
  - Particularly important in wines produced in cool climates
- **Contribute to wine flavor**
Winemaking

Grapes
Crushed/Destemmed
Must
Alcoholic fermentation
Malolactic fermentation
Ageing
Bottling
Problem Malolactic Fermentations

• Malolactic bacteria (MLB) inhibited in wine by:
  – Ethanol
  – Low pH
  – Lack of nutrients
  – Sulfur dioxide (SO₂)

• Growth influenced by presence of wine yeast
Problem Malolactic Fermentations

Malolactic bacteria die-off after inoculation

Representation of Growth of Yeast and MLB during Alcoholic Fermentation

Viable cells (cfu/mL)

Time

1.0E+06

Yeast

MLB
Inhibitory Substances

Production of inhibitory substances by the yeast:

- Antibacterial compounds
  - Ethanol
  - Medium-chain fatty acids
  - Proteins/peptides
- Sulfur dioxide (SO₂)
Sulfur Dioxide in Wine

- SO$_2$
  - Antimicrobial and antioxidant agent
  - Bacteria more sensitive to SO$_2$ than *Saccharomyces*
  - Exists in various pH dependant forms
  - Can exist in a free or bound form
    - Bound SO$_2$ less inhibitory to malolactic bacteria
- Produced in varying amounts by yeast strains
Objectives

• Investigate the mechanisms by which *Saccharomyces* inhibits *Oenococcus oeni* during alcoholic fermentation

• Determine whether the nutrient content of musts impacts production of antibacterial compounds by wine yeast
Benefits to Winemaker

- Better understanding of metabolic interactions between *Saccharomyces* and *Oenococcus*
  - Encourage or prevent MLF when desired
  - Determine optimal time for bacterial inoculation
  - Select compatible yeast and bacterial strains
Alcoholic Fermentation

- Viable cells
- Glucose & fructose
- Free and total SO$_2$

Sampled weekly

Synthetic grape juice

➤ High and Low Nitrogen
**Alcoholic Fermentation**
- Viable cells
- Glucose & fructose
- Free and total SO₂

Sterile filtration

100 mL

**Malolactic fermentation**
- Viable cells
- Malic acid

Sampled weekly
Maximum production of SO₂ by *S. cerevisiae* during alcoholic fermentation under high and low nitrogen conditions

![Graph showing maximum production of SO₂ by different strains under low nitrogen conditions.](image)

- **St George**
- **EC1118**
- **RUBY.ferm**
- **UCD 522**
- **V1116**
- **UCLM S325**

**Legend:**
- Low Nitrogen
Maximum production of SO₂ by *S. cerevisiae* during alcoholic fermentation under high and low nitrogen conditions

- **High nitrogen**
- **Low Nitrogen**

<table>
<thead>
<tr>
<th>Strain</th>
<th>High Nitrogen</th>
<th>Low Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>St George</td>
<td>ab</td>
<td>a</td>
</tr>
<tr>
<td>EC1118</td>
<td>cd</td>
<td>ab</td>
</tr>
<tr>
<td>RUBY.ferm</td>
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<td>b</td>
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<td>g</td>
<td>e</td>
</tr>
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<td>f</td>
<td>c</td>
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</table>
SO$_2$ Production

- Yeast strains produce varying amounts of SO$_2$ during alcoholic fermentation
- Higher production of SO$_2$ during fermentation under high nitrogen conditions
What effect does this SO$_2$ have on the MLF?
Degradation of malic acid by *O. oeni* in samples fermented for 0 days by *S. cerevisiae* UCD 522 under high and low nitrogen conditions.
Degradation of malic acid by *O. oeni* in samples fermented for 2 days by *S. cerevisiae* UCD 522 under high and low nitrogen conditions.
Degradation of malic acid by *O. oeni* in samples fermented for 9 days by *S. cerevisiae* UCD 522 under high and low nitrogen conditions.

**Day 9**

- **High N**
  - Malic acid level: 21 mg/L SO$_2$

- **Low N**
  - Malic acid level: 7 mg/L SO$_2$
Degradation of malic acid by *O. oeni* in samples fermented for 16 days by *S. cerevisiae* UCD 522 under high and low nitrogen conditions.

Day 16

- High N: 23 mg/L SO₂
- Low N: 9 mg/L SO₂

### Effect of SO₂ production by *Saccharomyces* on MLF

<table>
<thead>
<tr>
<th>Yeast Strain</th>
<th>Nitrogen</th>
<th>Maximum SO₂ (mg/L)</th>
<th>Inhibit MLF</th>
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<tr>
<td>Saint Georges S101</td>
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<td>Low</td>
<td>6.30&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>EC1118</td>
<td>High</td>
<td>18.6&lt;sup&gt;cd&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>Low</td>
<td>10.1&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>RUBY.ferm</td>
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<tr>
<td></td>
<td>Low</td>
<td>11.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<tr>
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<td></td>
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<td>V-1116</td>
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Mean values with different letters are significantly different at p ≤ 0.05
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Mean values with different letters are significantly different at p ≤ 0.05
Conclusions

- High SO$_2$ producing yeast inhibited MLF while low SO$_2$ producing yeast did not
- Nitrogen influenced SO$_2$ production which in turn influenced MLF
- SO$_2$ concentration and inhibition of MLF did not always correlate

♦ Alternative inhibitory mechanism
  ⇒ Antibacterial protein/peptides?
Alcoholic Fermentation
- Viable cells
- Glucose & fructose
- Free and total SO$_2$

Sterile filtration
100 mL

Synthetic grape juice

Malolactic fermentation
- Viable cells
- Malic acid
Alcoholic Fermentation
- Viable cells
- Glucose & fructose
- Free and total SO$_2$

Malolactic fermentation
- Viable cells
- Malic acid

Sterile filtration

100 mL

Synthetic grape juice

Protease or nutrient addition
Degradation of malic acid by *O. oeni* in wine fermented for 14 days by *S. cerevisiae* RUBY.ferm
Degradation of malic acid by *O. oeni* in wine fermented for 14 days by *S. cerevisiae* RUBY.ferm
Degradation of malic acid by *O. oeni* in wine fermented for 14 days by *S. cerevisiae* RUBY.ferm
The Search Continues

- Inhibition relieved by protease indicating role of protein/peptide
- Inhibitory fraction identified through growth studies
- Compound present in the > 3 kDa fraction of wine but not the > 5kDa fraction
- Inhibition only in RUBY.ferm wine not Saint Georges wine

Further isolation/characterization
SDS PAGE gel of wine fermented by *S. cerevisiae*

- MW standard
- RUBY.ferm
- Saint Georges

SDS PAGE gel of wine fermented by *S. cerevisiae*
SDS PAGE Results

- Two common bands identified in RUBY.ferm and Saint Georges S101 wine
- One band present only in RUBY.ferm wine
  - Approximately 5 kDa

→ Antibacterial peptide?
Overall Conclusions

- Inhibition of *O. oeni* by *Saccharomyces* strain dependant
- Yeast produce varying amounts of SO$_2$
  - Elevated production during fermentation under high nitrogen conditions
- Bacterial inhibition was related to SO$_2$ production for some but not all strains
- Inhibition may be due to protein/peptide
- Isolated peptide approximately 5 kDa
Future Research

- Characterization of antibacterial peptide
  - Amino acid sequence
  - Develop probes/screening tools
  - Mode of action

- Enological consequences
  - When is it produced and by which yeast strains
  - Concentration influence
  - Susceptibility of other LAB species and strains
New Projects

- Impact of viticultural practices on wine quality
  - Collaborative project with Horticulture Dept
  - Micro-scale fermentations
  - Monitor fermentation parameters and phenolic composition of wine
  - Sensory analysis
  - Multiple year and vineyard study
Research Directions

• Continued research investigating MLF

• Impact of yeast strains on red wine quality
  – ‘Native’ yeast versus starter cultures
  – Role of Non-\textit{Saccharomyces} yeast
  – Working with flavor chemist to investigate impact of yeast on red wine flavor and aroma compounds

• Spoilage micro-organisms
  – \textit{Pediococcus}
Other Enology Research

- Dr Jim Kennedy
  - Grape and wine phenolics
  - Changes in phenolic composition during berry ripening and wine production
  - Wine texture
  - Development of analytical methods
Other Enology Research

• Dr Michael Qian
  – Wine flavor chemistry
  – Free and bound flavor precursors in grapes and wine
  – Off-flavor aroma development in wine
    • Volatile sulfur compounds
  – Analytical method development
Enology Extension

• Meeting the industry
  – Visiting wineries
  – Involvement in industry organizations
  – Working with the Oregon Wine Board
  – Symposiums and meetings

• Working closely with viticulture colleagues
Enology Extension

- Workshops
  - Filtration (February 2007)
  - Wine Texture (May 2007)
  - Wine Microbiology
  - Analysis of juice and wine
  - Sensory evaluation of wines
  - Post-fermentation management
Enology Extension

• Website
  – http://wine.oregonstate.edu
  – Currently being updated
  – Access to latest OSU research findings
  – Information about courses
  – Updated articles about various viticulture and enology topics
Thank you

Questions?