Use of non-\textit{Saccharomyces} yeasts to reduce alcohol content of red wines

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Outline

• Introduction
• Research Objectives
• Screening for Crabtree Negative Yeasts
• Microvinification Experiments
• Pilot-Scale Winemaking Experiments (2016, 2017)
• Conclusions and Future Work
• Questions?
Introduction

• Increased temperatures, hangtime = Increased [Sugar]

• Increased [Sugar] → Increased [Ethanol]

• Problems with high alcohol wines:
  • Increased perception of ‘hotness’\(^1\)
  • Decreased aroma, flavor intensity\(^1\)
  • Marketing problems\(^2\)
Introduction

Some solutions already exist:

• Earlier harvest
• Amelioration
• Filtration, reverse osmosis

→ Compromise quality\(^3\)

Microbial methods: Crabtree negative non-\textit{Saccharomyces} yeasts\(^2,4\)
Introduction

Crabtree (-) *non-Saccharomyces* yeasts:

- Naturally present on grape berries
- Respiro-fermentative (Oxidative) metabolism
- With oxygen, respire sugars directly to CO₂ → Bypass ethanol production
- Can’t metabolize all sugar → Must be inoculated with *S. cerevisiae*
Research Objectives

- Identify native Crabtree (-) NS yeasts
- Decrease ethanol
- Reduce off-aromas and flavors
- Develop inoculation regime with *S. cerevisiae* to produce dry wines

Recreated from Gonzalez et al. 2013
Screening for Crabtree (-) Yeasts

Grape Juice from Concentrate:
- 310 g/L Glu+Fru
- 270 mg/L YAN, pH 3.58

(+) Oxygen
(0.025 mL O₂/mLmin)

(-) Oxygen

Inoculate with NS Yeast
(10⁵ CFU/mL)

Filter Sterilize

Transfer to 100 mL to dilution bottle (day 6)

Inoculate with S. cerevisiae D254
Screening for Crabtree (-) Yeasts

18 non-Saccharomyces yeasts from WA

- Candida californica
- Metschnikowia chrysoperlae
- Candida oleophila
- Metschnikowia pulcherrima
- Candida railenensis
- Meyerozyma carribica
- Cryptococcus adeliensis
- Meyerozyma guillermondii
- Cryptococcus saitoana
- Pichia fermentans
- Curvibasidium pallidacorallinum
- Pichia kluyveri
- Hanseniaspora uvarum
- Pichia membranifaciens
- Issatchenkia orientalis
- Wickerhamomyces anomalus
- Kluyveromyces marxianus
- Yamadazyma mexicana
Screening for Crabtree (-) Yeasts

When aerated:

- NS yeasts reduced ethanol content...
  ...increased acetic acid (>1.5 g/L)
- Five yeasts reduced alcohol + less acetic acid

  *Mt. chrysoperlae* P40D002
  *Mt. pulcherrima* P01A016
  *My. guillermondii* P40D002
  *P. kluyveri* P01C002
  *P. membranifaciens* P43C010

- High acetic acid = Too much aeration?\(^5\)
Microvinification Experiments

Five Yeasts Isolated From WA vineyards

Reconstitute Merlot juice

pH 3.62, 24.3°Brix

Filter- sterilize

Whatman Bugstopper™

Inoculate NS Yeast

3 Days

Inoculate S. cerevisiae D254

Fermentation Air-Lock

Three Strains from Industry:

Mt. pulcherrima NS-MP

Mt. fructicola NS-MF

Torulaspora delbrueckii NS-TD
## Microvinification Experiments

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Ethanol (% v/v)</th>
<th>Volatile Acidity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. chrysoperlae P40A002</td>
<td>13.3 e</td>
<td>0.51 b</td>
</tr>
<tr>
<td><em>Mt. pulcherrima</em> P01A016</td>
<td>11.7 a</td>
<td>0.23 a</td>
</tr>
<tr>
<td><em>Mt. pulcherrima</em> NS-MP</td>
<td>12.1 b</td>
<td>0.25 a</td>
</tr>
<tr>
<td><em>Mt. fructicola</em> NS-MF</td>
<td>12.1 bc</td>
<td>0.26 a</td>
</tr>
<tr>
<td><em>My. guillermondii</em> P40D002</td>
<td>12.3 c</td>
<td>0.24 a</td>
</tr>
<tr>
<td><em>P. kluyveri</em> P01C002</td>
<td>13.3 e</td>
<td>0.33 a</td>
</tr>
<tr>
<td><em>P. membranifaciens</em> P43C010</td>
<td>13.0 e</td>
<td>0.25 a</td>
</tr>
<tr>
<td><em>T. delbrueckii</em> NS-TD</td>
<td>13.4 e</td>
<td>0.62 c</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> D254</td>
<td>13.7 f</td>
<td>0.60 bc</td>
</tr>
</tbody>
</table>

2.0 % v/v less Alcohol!

All wines dry (≤ 2 g/L reducing sugars). Means within a column with different letters (a-f) significantly different (p ≤ 0.05) by Tukey’s HSD.
Pilot-scale Merlot Wine Production 2016 and 2017

WSU IAREC (Prosser, WA)

Hand-harvested Merlot Grapes

Crush/Destem 37.5 kg
+ 24 g/hL DAP, 20 ppm SO$_2$

Inoculate NS Yeast

Inoculate $S.~cerevisiae$ Enoferm Syrah +24 g/hL Fermaid K

Open Fermenter

Closed Fermenter

3 Days
2016 Pilot-Scale Winemaking

- Initial Must: 25.4°Brix, pH 3.50, 4.23 g/L TA

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Ethanol (% v/v)</th>
<th>Titratable Acidity (g/L)</th>
<th>Glycerol (g/L)</th>
<th>Succinic Acid (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mt. pulcherrima</em> P01A016</td>
<td>13.8 a</td>
<td>6.09 b</td>
<td>10.1 a</td>
<td>1.91 a</td>
</tr>
<tr>
<td><em>Mt. pulcherrima</em> NS-MP</td>
<td>13.9 a</td>
<td>6.09 b</td>
<td>10.1 a</td>
<td>1.74 a</td>
</tr>
<tr>
<td><em>My. guillermondii</em> P40D002</td>
<td>15.0 b</td>
<td>6.81 c</td>
<td>10.1 a</td>
<td>1.82 a</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> Eno. Syrah</td>
<td>14.9 b</td>
<td>5.56 a</td>
<td>10.0 a</td>
<td>1.75 a</td>
</tr>
<tr>
<td>Uninoculated*</td>
<td>14.9 b</td>
<td>5.61 a</td>
<td>10.1 a</td>
<td>1.73 a</td>
</tr>
</tbody>
</table>

All wines were dry (≤ 2 g/L reducing sugars). Means within a column with different letters (a-c) significantly different (p ≤ 0.05) by Tukey’s HSD.

*Treatment was left uninoculated for three days prior to *S. cerevisiae* addition.*
Quantification of Volatiles by HS-SPME-GC/MS

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Volatile Acidity (g/L)</th>
<th>Ethyl Acetate (mg/L)</th>
<th>Total Higher Alcohols (mg/L)</th>
<th>Total Esters (mg/L)</th>
<th>Total Fatty Acids (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. pulcherrima P01A016</td>
<td>0.35 b</td>
<td>73.1 a</td>
<td>244 b</td>
<td>2.51 a</td>
<td>6.76 a</td>
</tr>
<tr>
<td>Mt. pulcherrima NS-MP</td>
<td>0.33 a</td>
<td>64.1 a</td>
<td>263 b</td>
<td>2.50 a</td>
<td>7.94 a</td>
</tr>
<tr>
<td>My. guillermondii P40D002</td>
<td>0.40 c</td>
<td>148 b</td>
<td>267 b</td>
<td>2.73 a</td>
<td>7.29 a</td>
</tr>
<tr>
<td>S. cerevisiae Eno. Syrah</td>
<td>0.37 b</td>
<td>52.3 a</td>
<td>179 a</td>
<td>2.83 a</td>
<td>7.95 a</td>
</tr>
</tbody>
</table>

Means within a column with different letters (a-c) significantly different (p ≤ 0.05) by Tukey’s HSD.

*Treatment was left uninoculated for three days prior to *S. cerevisiae* addition.
### 2017 Pilot-Scale Merlot Winemaking

- Initial Must: 25.5°Brix, pH 3.54, 5.94 g/L TA

<table>
<thead>
<tr>
<th>Species/Strain</th>
<th>Ethanol (% v/v)</th>
<th>Titratable Acidity (g/L)</th>
<th>Volatile Acidity (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mt. pulcherrima P01A016</td>
<td>14.4 a</td>
<td>7.23 b</td>
<td>0.60 a</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> Eno. Syrah Open*</td>
<td>15.2 b</td>
<td>6.80 a</td>
<td>0.60 a</td>
</tr>
<tr>
<td><em>S. cerevisiae</em> Eno. Syrah Closed*</td>
<td>15.3 b</td>
<td>6.70 a</td>
<td>0.63 a</td>
</tr>
</tbody>
</table>

All wines were dry (≤ 2 g/L reducing sugars). Means within a column with different letters (a or b) significantly different (p ≤ 0.05) by Tukey’s HSD.

*S. cerevisiae* treatments fermented either “Closed” or “Open” for 3 days prior to closing.
Conclusions

- *Mt. pulcherrima* reduced ethanol by 0.9-1.0% v/v

- Increased higher alcohols, titratable acidity

- Limited production of negative metabolites

  → VA, ethyl acetate, fatty acids

Can improve wine quality, especially when sourcing from hot growing regions!
Future Work

• Sensory Qualities, Perception?

• Optimize Winemaking Protocol
  → Inoculum size?
  → Temperature?
  → Aeration Method?

• Antagonistic/Synergistic effects of other microorganisms?
Thank you for your attention!

Any Questions?
References


