Using non-Saccharomyces yeasts during alcoholic fermentation: taking Advantage of Yeast Biodiversity

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Winerys Unlimited, 2012. Richmond, VA
Non-Saccharomyces in Winemaking

Native Microflora

- Pichia
- Metchnikowia
- Kloechera
- Kluyveromyces
- Candida
- Zygosaccharomyces
- Torulaspora
- Cryptococcus
- Brettanomyces
- Hanseniaspora
Non-Saccharomyces on Grapes

Flowering  Nouaison  Harvest

Dominant species
- Cryptococcus
- Candida
- Pichia
And others
- Torulaspora delbrueckii

Total yeasts/grape

Beginning of AF

50% non-Saccharomyces
50% S. cerevisiae

Decrease in the non-Saccharomyces population

End of AF

> 99% S. cerevisiae
Dynamics and Diversity

Dynamics of yeast populations established by real time PCR, from cold soaking until barrel settling for FML (Zott, 2009)

0 hours = addition of a commercial yeast.
### Taking Advantage of Biodiversity

Many ecological studies bring light to the role of non-*Saccharomyces* yeasts

Specific metabolisms: different technological interests

#### Torulaspora delbrueckii: A non-*Saccharomyces* with no organoleptic defects and compatible with *S. cerevisiae* species

<table>
<thead>
<tr>
<th>Espèce utilisée en association avec <em>S. cerevisiae</em></th>
<th>But</th>
<th>Références</th>
</tr>
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<tbody>
<tr>
<td>Candida cantarellii</td>
<td>Augmentation teneur en glycérol</td>
<td>Toro et Vazquez (2002)</td>
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<tr>
<td>Candida pulcherrima</td>
<td>Modulation aromatique</td>
<td>Jolly et al. (2003); Zohre et Erten (2002)</td>
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<tr>
<td>Candida stellata</td>
<td>Augmentation teneur en glycérol</td>
<td>Ciani et Ferraro (1995, 1999); Ferraro et al. (2000)</td>
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<tr>
<td></td>
<td>Modulation aromatique</td>
<td>Soden et al. (2000)</td>
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<tr>
<td>Debaryomyces vanrijii</td>
<td>Augmentation teneur en géraniol</td>
<td>Garcia et al. (2002)</td>
</tr>
<tr>
<td>Hanseniaspora guilliermondii</td>
<td>Modulation aromatique</td>
<td>Zironi et al. (1993)</td>
</tr>
<tr>
<td>Hanseniaspora uvarum <em>(Kloeckera apiculata)</em></td>
<td>Modulation aromatique</td>
<td>Ciani et al. (2006); Herreraiz et al. (1990); Mendoza et al. (2007); Moreira (2005); Moreira et al. (2008); Zironi et al. (1993); Zohre et Erten (2002)</td>
</tr>
<tr>
<td>Issatchenkaorientalis</td>
<td>Réduction teneur en acide malique</td>
<td>Kim et al. (2008)</td>
</tr>
<tr>
<td>Kluyveromyces thermotolerans</td>
<td>Réduction production acide acétique</td>
<td>Ciani et al. (2005); Mora et al. (1990)</td>
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<tr>
<td></td>
<td>Augmentation acidité totale</td>
<td>Kapsopoulou et al. (2007)</td>
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<tr>
<td>Pichia fermentans</td>
<td>Modulation aromatique</td>
<td>Clemente-Jimenez et al. (2005)</td>
</tr>
<tr>
<td>Pichia kluyveri</td>
<td>Augmentation teneur en thiois volatils</td>
<td>Anfang et al. (2009)</td>
</tr>
<tr>
<td>Pichia anomala</td>
<td>Modulation aromatique</td>
<td>Kurita et al. (2008)</td>
</tr>
<tr>
<td>Schizosaccharomyces pombe</td>
<td>Dégradation acide malique</td>
<td>Ciani (1965); Magyar et Panic (1989); Snow et Gallender (1979); Yokotsuka et al. (1993)</td>
</tr>
<tr>
<td>Torulaspora delbrueckii</td>
<td>Réduction production acide acétique</td>
<td>Bely et al. (2008); Ciani et al. (2005); Lafon-Lafourcade (1991); Salmon et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Modulation aromatique</td>
<td>Herraiz et al. (1990)</td>
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</table>

Source: P. Renault 2010
Torulaspora delbrueckii: Natural Positive Traits

- Low VA production
- No volatile phenols production (POF negative)
- Low ethanal and ethyl acetate production
Biodiversity

**Torulaspora delbrueckii**
- 6 chromosomes
- Complex aromatic profile
- Positive and significant impact on mouthfeel

**Saccharomyces cerevisiae**
- 16 chromosomes

Microscopie électronique à Balayage, grossissement X 24000
Bordeaux Imaging Center - Pôle d'Imagerie Electronique - Université Bordeaux 2.
Natural Advantages: Low VA Production

Volatile acidity (g/L acetic acid)

S. cerevisiae T. delbrueckii

⇒ Important phenotypic variability: Essential for the selection of a top performing strain
Fermentative Properties

Collection of 30 strains of Td. Only few with excellent fermentative capacity for a non-Saccharomyces but a slower growth rate and fermentation kinetics than S. cerevisiae.
Fermentative Properties

Td collection of strains: low ethanol production (7-9 % v/v) hence not a complete fermentation solution

→ Mixed yeasting necessary:
  - Ensures complete fermentation
  - Increases wine complexity and uniqueness
Mixed Yeasting

*Torulaspora delbrueckii* and *Saccharomyces cerevisiae*

→ Sequential inoculation: *S. cerevisiae* 24 – 72 hrs after *Torulapora* introduction:

**I.** 300 ppm TD n.sacch.  
Important: rehydration in 77-86F water, **without nutrient.**

**II.** 24 - 72hrs* after TD n.sacch. addition:  
*S. cerevisiae* addition at 200ppm; rehydration nutrient recommended.

Complex nutrient additions recommended

*In the case of sweet wines, our trials show that the best results are achieved when *S. cerevisiae* is inoculated 5-10 hrs after TD (at 400 ppm).*

**Rehydration temperature:**  
25-30 °C / 77-86 ° F
Yeast Nutrition Management

Yeast Nutrition Recommendations

1. Torulaspora
   (+ ½ dose of ammonium source of low YAN)

2. 24hrs (in red wines) – 72hrs (in white and rose wines) after: S. cerevisiae. **Rehyration nutrient** recommended

3. 24hrs after: ½ dose or entire dose of **complex nutrient**
   (A thiamine addition is important to ensure the implantation and the activity of S. cerevisiae)

Bisson et Butzke, 2000
SO2 effect at 10 and 25 ppm SO2 in a synthetic medium at 75F

→ The higher the SO2 concentration the longer the lag phase.
→ Alpha is resistant (good viability) and can start fermenting in musts with high [SO2]. There is a variability in Torulaspora strains!
Sauvignon Blanc
Pessac Leognan 2010

Must Analyses:

Sugar (g/l): 216
Pot. Alcohol (% v/v): 12.77
TA (g/l): 6.04
MA (g/l): 3.5
pH: 3.25
Free SO2 (mg/l): 12
Active SO2 (mg/l): 0.62
Total SO2 (mg/l): 51
YAN (mg/l): 179 (adjusted to 218 mg/L
1/3 through fermentation)
Turbidity (NTU): 190

Trial tanks:

T : Control Zymaflore X5
E1 : Zymaflore Alpha + X5
Sauvignon Blanc
Pessac Leognan 2010

1/3 of AF: nitrogen addition

Fingerprint of yeasts

Density

0h 12h 24h 36h 48h 60h 72h 84h 96h 108h 120h 132h 144h 156h 168h 180h 192h 204h 216h 228h 240h 252h 264h

0h 10h 20h 30h 40h 50h 60h 70h 80h 90h 100h 110h

Témoin Essai 1

Temps depuis
Sauvignon Blanc

Pessac Leognan 2010

More complex aromatic perception (synergistic effects between flavors) and more aromatic volume.
### Sauvignon Blanc

**Pessac Leognan 2010**

<table>
<thead>
<tr>
<th>Metric</th>
<th>X5</th>
<th>Alpha + X5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RS (g/l)</strong></td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Alc (% v/v)</strong></td>
<td>13</td>
<td>13.05</td>
</tr>
<tr>
<td><strong>TA (g/l H2SO4)</strong></td>
<td>4.45</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Malic acid (g/l)</strong></td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>3.23</td>
<td>3.22</td>
</tr>
<tr>
<td><strong>Free SO2 (mg/l)</strong></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Active SO2 (mg/l)</strong></td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>VA (g/L H2SO4)</strong></td>
<td>0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Increased aromatic intensity due to higher expression of thiols**

[Graph showing increased aromatic index (+50%) for Alpha + X5 compared to X5]
Sauvignon Blanc
Pessac Leognan 2010

- Descriptive analysis (22 member tasting panel) (ISVV, Bordeaux)

Wine Alpha/X5:

Significant difference:

+ More Complexity
+ More Fruitiness
+ More Thiols
- Less Vegetal

Wine Alpha/X5:

Significant difference:

+ More Volume
**Rosé Merlot 2010**

- Lafazym CL 30 ppm
- Turbidity: 80 NTU
- AF Temperature: 61-68 °F
- Initial YAN: 112 ppm
- Nitrogen correction: 200 ppm Thiazote after X16 addition + 200 ppm Nutristart after 1/3 Fermentation

<table>
<thead>
<tr>
<th></th>
<th>X16 (200 ppm)</th>
<th>Alpha (300 ppm) + X16 (200ppm) (72hrs after Alpha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol % vol.</td>
<td>12,5</td>
<td>12,5</td>
</tr>
<tr>
<td>TA g/L</td>
<td>5,2</td>
<td>5,1</td>
</tr>
<tr>
<td>VA g/L H2SO4</td>
<td>0,13</td>
<td>0,17</td>
</tr>
<tr>
<td>AF duration (days)</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>
Rosé Merlot 2010
Entre deux mers

Zymaflore X16

X16

Alpha 72h. X16
Rosé Merlot 2010
Entre deux mers
Rosé Merlot 2010
Entre deux mers

![Bar chart showing wine test results for various conditions and durations. The chart includes categories such as PE, APE, AI, AH, C4C2, C6C2, C8C2, C10C2, with X16, Alpha X16 48h, and Alpha X16 72h as markers.

Legend:
- X16
- Alpha X16 48h
- Alpha X16 72h

The chart represents the test results for Entre deux mers wine with different conditions and durations, focusing on the X16 and Alpha X16 48h and 72h markers.

Entre deux mers wine test results for 2010 show a marked variation in pH levels across different conditions and durations. The chart highlights the significant role of pH in wine quality and stability.
# Sauternes 2010

<table>
<thead>
<tr>
<th></th>
<th>Zymaflore ST</th>
<th>Zymaflore Alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol % Vol.</td>
<td>14,2</td>
<td>13,93</td>
</tr>
<tr>
<td>Sugars g/L</td>
<td>145</td>
<td>148</td>
</tr>
<tr>
<td>TA g/L H2SO4</td>
<td>3,88</td>
<td>3,81</td>
</tr>
<tr>
<td>VA g/L H2SO4</td>
<td>1</td>
<td>0,73</td>
</tr>
<tr>
<td>pH</td>
<td>3,85</td>
<td>3,83</td>
</tr>
<tr>
<td>Free SO2 mg/L</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>Total SO2 mg/L</td>
<td>183</td>
<td>179</td>
</tr>
</tbody>
</table>

![Graph showing AV and Density over time](image-url)

**AV and Density over time**

- **AV and Density over time**
- **AV over time (g/L H2SO4)**
- **Density (g/L)**

27% VA reduction
Sauternes 2010

Zymaflore Alpha wine perceived as more complex, fresher and fruitier compared to control wine.
Sequential Inoculation Protocol

Schematic Representation

Influence of Zymaflore Alpha

Fermentation duration

S.cerevisiae addition timing after Zymaflore Alpha

Microbial load

24h  48h  72h

Reds  Whites  Roses

Microbial load
We are making the best Bonarda in all Argentina! We have added 300ppm Alpha to our high end Bonarda on the Thursday. After 3 days of cold soak at theoretically 46F, we have added RX60 after having raised the temperature. We have a control with the exact same grapes and same protocol (same Optizym enzyme, etc.), inoculated with RX60 only.

The tasting is impressive:
- Nose: very floral (rose, peony), fresh strawberries (impressive), blackberry but still very fresh.
- Mouth: an incredible volume, much more than in the control.

The control is at 11.5 Beaume and the Alpha tank is at 7.5 Beaume. It is much rounder. Hard to believe that both tanks come from the same grapes!
Torulaspora delbrueckii: summary

- **Mouthfeel and volume** increase.
- Higher aromatic **complexity** on all varieties – differentiated organoleptic profile.
- **VA decrease**, especially in high Brix grapes and sweet wines.
- Recreates conditions of a **native/ecological fermentation**, in a more controlled way and without negative aromatic impact by indigenous microflora.
Thank You for your Attention!

Let’s taste some Virginia wines made with TD

For more information: charlotte.gourraud@laffort.com and booth #925!