ML School
Take control of your MLF and of your wine quality

1500 winemakers from around the world completed the ML school with success
Introduction to MLF and biodiversity

Maret du Toit

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HOW TO MAKE WINE

1. Sort through grapes, leaving anything that is not a grape including rocks, sticks, expectations, etc.
2. Crush + Destem
3. Collect "must" (any juice + skins) in a bucket or other large holding device
4. Add wine yeast
5. Add nutrients
6. Add acid
7. Ferment an fermenting bucket for a couple weeks. Keep closed (except when you do step 8)
8. Twice daily punch down "cap" with a plastic flower (fernly new)
9. Use a hydrometer to test levels of sugar vs alcohol
10. After a couple weeks (give or take)
11. Press down
12. Collect juice from press into a glass carboy*
13. Add tannin (optional)
14. Add oak chips for flavor to your mother's barrel
15. Let it sit for months to months + months + months
16. Rack again and again (move from one carboy to the other) until clear
17. Taste until it tastes good
18. Add Camden tablet (stops fermentation)
19. Bottle
20. Cork
21. Wait for years...
22. Drink
Microbiology of wine – your perspectives

• What has changed in the last decade with regard to wine microbiology?

• What is the impact of yeasts on MLF?

• What malolactic bacteria and practices is preferred?

• How do you control your fermentations to ensure success?

• What was your biggest challenges in 2016?
What is novel currently in wine microbiology?

**From grape berries to wine: population dynamics of cultivable yeasts associated to “Nero di Troia” autochthonous grape cultivar**
Carmela Garofalo, Mariana Tristezza, Francesco Grieço, Giuseppe Spano, Vittorio Capozzi

**Dynamic changes in microbiota and mycobiota during spontaneous ‘Vino Santo Trentino’ fermentation**
Irene Stefanini, Davide Albanese, Agostino Cavazza, Elisa Francisci, Carla De Filippo, Claudio Donati, and Duccio Cavallaro

**Microbial terroir and food innovation: The case of yeast biodiversity in wine**
Vittorio Capozzi, Carmela Garofalo, Maria Assunta Chiriatti, Francesco Grieço, Giuseppe Spano

**Wine microbiome, a dynamic world of microbial interactions**
Youzhong Liu, Sandrine Rousseaux, Raphaëlle Tourdot-Maréchal, Mohand Sadoudi, Régis Gougeon, Philippe Schmitt-Kopplin, Hervé Alexandre

**Unraveling the Diversity of Grapevine Microbiome**
Cátia Pinto, Diogo Pinho, Susana Sousa, Miguel Pinheiro, Conceição Egas, Ana C. Gomes

**The Vineyard Yeast Microbiome, a Mixed Model Microbial Map**
Mathabatha Evodia Setati, Daniel Jacobson, Ursula-Claire Andong, Florian Bauer

**Unraveling the Enzymatic Basis of Wine “Flavorome”: A Phylo-Functional Study of Wine Related Yeast Species**
Ignacio Belda, Javier Ruiz, Ana Alaixruoy-Izquierdo, Eva Navas, Domingo Marquina, and Antonio Santos

**Grape berry bacterial microbiota: Impact of the ripening process and the farming system**
Guilherme Martins, Cécile Mirot-Sertier, Béatrice Lauga, Olivier Claisse, Aline Lonvaud-Funel, Guy Soulas, Isabelle Masneuf-Pomarède
Factors that influence natural grape flora

• Grapes are a source of natural yeasts and bacteria in wine production
• Variety and proportion is affected by different factors
  – Grape ripeness and integrity
  – Viticultural practices
    • Leaf removal strategies
  – Agricultural practices
    • Organic/biodynamic
    • Conventional
    • Integrated production systems
Impact of pruning on yeast diversity

- Both treatments show similar major yeasts
  - Shaded display more diversity
  - Exposed shows high levels of *Rhodotorula* and *Cryptococcus* spp.
Wine is much more diverse than believed previously!!

Bockulich et al. 2012 Botrytized wine
Portuguese wine appellations - diversity

Pinto et al. 2015  IM - must; SF – start of alcoholic; EF – end of alcoholic
Why *O. oeni*?

Grape

- Lactobacillus plantarum
- Lactobacillus mali
- Lactobacillus kefiri
- Lactobacillus lindneri
- Lactobacillus brevis
- Lactobacillus buchneri
- Lactobacillus kunkeei
- Lactococcus lactis
- Enterococcus faecium
- Enterococcus avium
- Enterococcus durans
- Enterococcus hermanniensis
- Leuconostoc mesenteroides
- Pediococcus parvulus
- Pediococcus damnosus

*Oenococcus oeni*

Must

- L. plantarum
- L. sanfrancisensis
- L. casei
- P. parvulus
- L. hilgardii
- *O. oeni* 0-10%

AF

- L. plantarum
- P. parvulus
- L. hilgardii
- *O. oeni* 80-100%

MLF

- (Lactobacilli)
- (pediococci)
- *O. oeni*

Aging

- Lactobacilli
- Pediococci
- (O. oeni)

*O. oeni* is best-adapted species for wine
Understanding the biodiversity

16 strains:
- 4/ Burgundi white wine
- 4/ Burgundi red wine
- 4/ Cider
- 4/ O. kitaharae

pH tolerance
Cider > B white > B red > O. kitaharae

Ethanol tolerance
B red = B white > cider > O. kitaharae
Importance of MLF

• Deacidification of wine
  – Decrease in malic acid (1-3 g/L)
  – pH increase of 0.1-0.3

• Microbial stability

• Improvement of aroma and flavour profile
  – more complex, better structured
  – creamier and fuller palate (ethyl lactate)
  – more butteriness (diacetyl)
  – reduced vegetative aromas
  – enhanced fruity notes (esters)
What factors impact them?

- pH
- Sulfur dioxide
- Ethanol
- Temperature of wine
- Interaction with yeast
- Malic acid
- Nutrients
- Fungicide residues
- Inhibitors that will influence growth
  - Phenolic acids
  - Lysozyme
Impact of malic acid

### Factors that impact MLF

<table>
<thead>
<tr>
<th>Initial level of malic acid (g/L)</th>
<th>Easy</th>
<th>Moderate</th>
<th>Difficult</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - 4</td>
<td></td>
<td>4 - 5 or</td>
<td>5 - 7 or</td>
<td>&gt;7 or &lt;0.5</td>
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<tr>
<td>or 1 - 2</td>
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<td>0.5 - 1</td>
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</tbody>
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Chardonnay – 12.5%v/v  pH - 3.25  Malic acid - 2.6g/L Temp. - 16°C

- Duration of MLF increases with malic acid content.
- Speed of malic acid degradation increases with malic acid content.
Impact of lactic acid

Chardonnay – 12.5% v/v  pH - 3.25  Temp.= 16°C

• Addition of 1.5 g/L highly increases the time to achieve MLF.

• Addition of 3g/L induces a high loss of viability which leads to stuck MLF.
Time of inoculation

• After alcoholic fermentation
  – Advantages:
    • Easy to control MLF as alcoholic fermentation is finished
  – Risks:
    • Stuck MLF due to high [alcohol]
    • Nutrients depleted

• Simultaneous or Co-inoculation
  – Advantages:
    • No impact of alcohol
    • Enough nutrients available
    • MLF finish earlier and wine can be stabilized after alcoholic fermentation
  – Risks:
    • Stuck alcoholic due to acetic acid
Co-inoculation overcome difficult wine conditions?

Comparison of the malic acid degradation between sequential and co-inoculation in Cabernet Sauvignon 2009

Wine parameters:
- pH: 3.46
- Volatile acidity: 0.14 g/L
- Total acidity: 7.36 g/L
- Malic acid: 1.63 g/L
- Lactic acid: 0.02 g/L
- Ethanol: 13.92 g/L
Yeast – bacteria interactions

• Negative impact:
  – Ethanol
  – SO$_2$
  – Medium chain fatty acids
  – Antibacterial metabolites
  – Depletion of nutrients

• Positive impact:
  – Yeast autolysis
    • Release nitrogenous compounds such as amino acids, peptides
  – Yeast mannoproteins
    • Adsorb medium chain fatty acids
**O. oeni** characteristics

- Tolerates wine pH 2.8-4.0
- Tolerant up 16% ethanol
- Conduct MLF at low temperatures
- Survive 50 mg/L of total SO$_2$
- Minimal increase in VA
- Enhance aroma profile of wine
- Produces no off-flavours
L. plantarum characteristics

- Prefers wine pH >3.5
- Survive up 12% ethanol
- Grow best at 20°C temperatures
- Survive 40 mg/L of total SO$_2$
- Produces no acetic acid
- Enhance aroma profile of wine
- Produces bacteriocins
Can we use MLF to control spoilage?

- Reduce contribution of natural LAB
- Reduce risk of biogenic amines
What is MLF science doing?
What is your need for MLF?
THANK YOU