Mastering Malolactic Fermentation
Lake Michigan College
November 9th, 2020
Chr. Hansen is a bio-science company since 1874 ... 
- Making culture is our core activity
Understanding the life of *Oenococcus oeni*

*Oenococcus oeni* can survive and perform malolactic fermentation (MLF) under very stressful conditions - as wine.

- The main stress factors for *O. oeni* in wine are:
  - SO$_2$, pH, alcohol and temperature
  - These stress factors are synergistic
  - They enhance each other’s effect
While this often gave acceptable results, it also carries a high risk of spoilage/contamination from unwanted microbes.

In late 20th century, commercial yeast and MLF cultures that can be added to wine were introduced, allowing greater control over the microbial population in the wine.
Manage Your Fermentations with Chr. Hansen VINIFLORA®

COMPANY
› Fermentation experts
› Highest QC methods
› Sustainability
› Global reach
› Innovation bio-tech
› VINIFLORA® for wine

PRE-ALCOHOLIC FERMENTATION
› VINIFLORA® yeast range
› Non-Saccharomyces yeast for
  › BioProtection
  › Mouthfeel
  › Differentiation
  › Acid Balance

ALCOHOLIC FERMENTATION
› VINIFLORA® yeast range
› Saccharomyces yeast for
  › Fruit forward wines
  › Consistency
  › Low nutrient demands
  › Bacteria synergies

MALOLACTIC FERMENTATION
› VINIFLORA® bacteria range
› Lactic acid bacteria for
  › Stability
  › Sensory
  › Efficiency
  › Food Safety
Manage Your Fermentations with Chr. Hansen VINIFLORA®

- Time (in days then weeks)
  - Pre-alcoholic fermentation
  - Alcoholic fermentation
  - Malolactic fermentation

- Population
  - Non-Saccharomyces spp.
  - Saccharomyces spp.
  - Oenococcus oeni
# VINIFLORA® Bacteria Range for Winemakers

<table>
<thead>
<tr>
<th>Product</th>
<th>Wine Color</th>
<th>Temperature</th>
<th>Alcohol</th>
<th>Minimum pH</th>
<th>Total SO₂</th>
<th>Summary</th>
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<td>Viniflora® CH35 The broadest spectrum</td>
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Always check temperature, alcohol, pH and total SO₂ before inoculation. Bacteria are living organisms and wine is a harsh environment for them.
What is Malolactic Fermentation (MLF)?

- Conversion of L-malic acid to L-lactic acid by lactic acid bacteria
- It is an intra-cellular process carried out by the malolactic enzyme
- It is a decarboxylation, and yields CO$_2$ and Energy in the form of ATP
- Traditionally takes place after alcoholic fermentation
Malolactic fermentation (MLF)

L-malic $\rightarrow$ L-lactic + CO$_2$

 › Enzymatic conversion of L-malic acid to L-lactic acid

 › The bacteria is performing MLF to survive and grow

⇒ All this happens in the very harsh environment of wine! This requires some very robust bacteria

FROM THE WINES PERSPECTIVE

 › Softens the acidic profile (pH increases)

 › Affects the aromatic properties of the wine

 › Enhances the microbial stability of the wine
Spontaneous ML fermentation

- Is carried out by **indigenous bacteria**
  - < pH 3.5: *Oenococcus oeni* only
  - > pH 3.5: *O. oeni*, *Pediococci* and *Lactobacilli* → fast

- **Risky business** → Very little control over microflora in wine
  - Variable speed of MLF
  - VA production
  - Biogenic amines (e.g. Histamine)
  - Might happen in the bottles (CO2!!)
  - Opportunity for spoilage microbes to establish
    - *Brettanomyces sp.*, *Acetobacter*, *undesirable LAB species*
    - Unwanted sensory flavours

[Further information: http://genome.jgi.doe.gov/pedpe/pedpe.home.html](http://genome.jgi.doe.gov/pedpe/pedpe.home.html)
Many faults may be avoided by a suitable management of MLF

Most of the defects recorded in the market are linked to a deviation in MLF, or else could be solved by a suitable management of MLF.

QUALITY ISSUES (RANKING OF QUOTE FREQUENCY BY PRODUCERS, TRADERS & BOTTLERS)

Defects that can be linked to MLF deviation or can be solved with a suitable management of MLF

« I get problems of quality in 20% of the wines I purchase »
A trader from Italy
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Nutritional Considerations for Malolactic Fermentation

Why do you need nutritional supplements for malolactic fermentation?

- Lack of nutrients can cause sluggish or even stall malolactic fermentations.
- Wine finished with alcoholic fermentation can often be deficient or void of nutrients due to yeast utilization.
- Certain *Saccharomyces* yeast strains take longer to lyse, entrapping nutrients within their cells that malolactic bacteria might otherwise be able to use.
- Unlike *Saccharomyces* yeast used in winemaking, *O. oeni* cannot store nor synthesize all essential amino acids.
When should you use nutrients for your MLF?

- Nutrients are recommended whenever you have challenging conditions:
  - High Alcohol
  - High SO2
  - Very Low pH
  - Temperatures on the extreme ends of the fermentation range
  - Highly clarified wines can lack adequate nutrition for MLF
  - Certain varietals have historically been difficult to put through MLF
  - Your own experience is vital!
    - You know your grapes and your conditions. Previous vintages can predict how the current one will behave.
    - Some hybrid varieties can pose interesting stylistic issues with high malic acid content
Wine as stressful environment

### Conditional for MLF - pH
- **Very Difficult**: pH < 3.0
- **Difficult**: 3.0-3.2
- **Favourable**: 3.2-3.7
- **Very favourable**: >3.7

### Conditional for MLF - Total SO₂
- **Very difficult**: > 45 ppm
- **Difficult**: 30 – 45 ppm
- **Less favourable**: 15 – 30 ppm
- **Favourable**: < 15 ppm

### Conditional for MLF - Temperature
- **Very Difficult**: <59°F
- **Not so favourable**: 59-66°F
- **Favourable**: 66-77°F
- **No MLF**: >77°F

### Conditional for MLF - Ethanol
- **Favourable**: < 13%
- **Difficult**: 13 – 15%
- **Very difficult**: 15-17%
At Chr. Hansen, we screen for the next robust bacteria

1. Higher robustness to extreme and changing conditions
2. Enhance flavor attributes and explore new ones
3. BioProtection
4. Improve capacity of production and quality

Where do we get the strains from?

➔ Exclusively from NATURE
➔ Isolated from grape, must, wine samples and winery environment
  ➔ Chr. Hansen discovery platform
  ➔ Project collaborations with universities and research centers
  ➔ External culture collections
Viniflora® - high scale production of natural ingredients

Chr-Hansen has 6 plants devoted to culture production (DK, Germany, France, US)
Viniflora® - highly standardized and process stabilized cultures

Production

Frozen/Freeze-dried

Storage

Transport

Use

Two ways to keep the culture stable

Freezing Process

Frozen culture pellets

Freeze-drying process

Freeze-dried culture pellets
Viniflora® - a careful storage

• Chr. Hansen has the largest -55°C freezer capacity in Europe
• Frozen cultures are shipped to dairies, wineries and meat industries all around the globe
Viniflora® - a dedicated shipment

**Production**

**Frozen/Freeze-dried**

**Storage**

**Transport**

**Use**

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**Freeze dried cultures**
- Shipment at ambient temperature for a maximum of 7 days.
- Our FD cultures are shipped with ice pack to maintain a cool temperature.

**FroZen cultures**
- Shipment with dry-ice to maintain a temperature of -45°C in the product during the whole transportation time.
- The quantity of dry-ice is adjusted to the shipment duration.
Viniflora® - direct inoculation for all users

**Production**

- Freezers at -45°C are necessary for FroZen™ products;
- Freeze-dried product can be stored at -18°C for a period of up to 2 years

**Frozen/Freeze-dried**

Always use products immediately to keep viability at its highest level.

**Storage**

**Transport**

**Use**

One simple gesture. Follow the inoculation guidelines provided into the inoculation protocol.

FroZen™ have a very short lag phase once inoculated.
Direct inoculation cultures

Convenience
› Viniflora products are ready to use!

Consistency through Top Quality:
› Each batch of Viniflora bacteria is tested before released through a proprietary and unique test named “MACC Test” (Malic Acid Conversion Test) resulting in:
   › Receiving in only bacteria adapted to wine conditions
   › High number of active cells to achieve the malolactic fermentation
   › Viable cells in wine, can survive once inoculated in the environment

Process Control Benefits:
› Faster and safer fermentations with finished wines ready early, saving on energy costs.
› Improve food safety without producing biogenic amines
Quality Control – of each batch

› Cell viability (Flowcytometry and cell count on plates)

› MACC activity test (unique for Chr. Hansen)
  › Accelerated malic acid conversion test, so we ensure that cells are not only viable but also active when it comes to conversion of malic acid

› Contaminant analysis (clean products)
  › Non-Lactic Acid Bacteria (NLAB) (< 5000 cfu/g)
  › Lactobacilli (< 5000 cfu/g)
  › Yeasts and Molds (< 10 cfu/g)
  › Acetobacter (< 100 cfu/g)
  › Enterobacteriaceae (< 100 cfu/g)
  › Enterococci (< 1000 cfu/g)
  › Total Bacillus (< 100 cfu/g)
  › Listeria monocytogenes (absent in 25 g)
  › Salmonella spp. (absent in 25 g)
What is the Magic Number?

› 1,000,000 (1E=06 cfu/ml) is the number of *Oenococcus oeni* cells needed per ml wine to start a malolactic fermentation (MLF)

› For example, you need 24,983,700,000,000 cells in a 6,660 gallon tank

› Viniflora® products are ready for direct inoculation – no rehydration or acclimatization needed

› Not all commercial products are the same in quality and cell numbers

› Viniflora® are alive and active – CH monitors this activity through their exclusive, proprietary test, named MACC (Malic Acid Conversion Capacity)
Magic Number $1 \times 10^6$ CFU/mL

› For MLF to occur the cell count of *O. oeni* needs to be above $10^6$ cfu/mL

From 2006, Australian Cabernet - pH 3.43, EtOH 13.3%

› The VINIFLORA® range secures the inoculation of > Magic number every time
   *(If used according to recommendations)*
When possible, get co-inoculation benefits!

- Co-inoculation is a technique which is growing in popularity
  - Involves running both AF and MLF concurrently, but needs to be properly managed

### Impact on wineries
- **Save time**
  - Reduce time to market/respect deadlines
  - Improve tank management

- **Save costs**
  - Save heating energy
  - Reduce carbon footprint

- **Control better**
  - Avoid sluggish or stuck ferment
  - Adapt *O. oeni* to high ethanol concentration

- **Enhance fruitiness**

### Impact on wines
- Keep the initial quality potential
- Avoid spoilage microorganisms and BA
- Diacetyl produced by bacteria is partly degraded by yeast → **fruitiness enhanced**
Choosing the right timing

› It is important to choose the right timing when inoculating Viniflora® O. oeni strains

› When having a wine that is very stressful for O.oeni, co-inoculation can be a good idea

› Important to remember:

› Co-inoculation is not about the bacteria, but about the application!!

› Early co-inoculation - approx. 24 Hours after the yeast
  › (pH < 3.4, Malic acid > 2 g/L, temp.< 77°F)

› Late co-inoculation - during the alcoholic fermentation (ρ 1000)
  › (More flexible, but still low pH and temp <77°F)
The choice of inoculation time depends on winery conditions and on winemaker objectives.
Parameters to consider before doing co-inoculation: pH, Malic acid, Ethanol (ethanol potential), Temperature and AF history of wine

Firstly, select the Viniflora® bacteria that fits the wine conditions

<table>
<thead>
<tr>
<th>Alc. (% v/v)</th>
<th>pH</th>
<th>Inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>High – alc. &gt; 14%</td>
<td>pH &lt; 3.4</td>
<td>Early co-inoculation</td>
</tr>
<tr>
<td>High – alc. &gt; 14%</td>
<td>pH 3.4 – 3.7</td>
<td>Late co-inoculation</td>
</tr>
<tr>
<td>High – alc. &gt; 14%</td>
<td>pH &gt; 3.7</td>
<td>Sequential</td>
</tr>
<tr>
<td>Low – alc. &lt; 14%</td>
<td>pH &lt; 3.4</td>
<td>Early or late co-inoculation</td>
</tr>
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<td>Low – alc. &lt; 14%</td>
<td>pH 3.4 – 3.7</td>
<td>Late co-inoculation or Sequential</td>
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<tr>
<td>Low – alc. &lt; 14%</td>
<td>pH &gt; 3.7</td>
<td>Sequential</td>
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Temp. always < 77 °F
Select the **Viniflora®** strain that fits your wine

It is very important to remember that *O. oeni* strains are different.

Within the Viniflora™ range, the *O. oeni* strains are selected according to different strengths.

The range covers most wine conditions.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Temperature (°F) Max:77°F</th>
<th>Alcohol % (v/v)</th>
<th>pH</th>
<th>SO₂ (ppm)</th>
<th>Flavor</th>
<th>Fermentation Speed</th>
<th>Wine making parameters</th>
<th>Cultures benefits</th>
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<tr>
<td>Viniflora® Oenos</td>
<td>≥ 63</td>
<td>≤ 14</td>
<td>≥ 3.2</td>
<td>≤ 40</td>
<td><img src="#" alt="Blue" /></td>
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<td>Viniflora® Oenos 2.0</td>
<td>≥ 59</td>
<td>≤ 14</td>
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<tr>
<td>Viniflora® CH11</td>
<td>≥ 57</td>
<td>≤ 15</td>
<td>≥ 3.0</td>
<td>≤ 35</td>
<td><img src="#" alt="Gray" /></td>
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<td>Viniflora® CH16</td>
<td>≥ 63</td>
<td>≤ 16</td>
<td>≥ 3.4</td>
<td>≤ 40</td>
<td><img src="#" alt="Red" /></td>
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<td>≤ 14</td>
<td>≥ 3.1</td>
<td>≤ 45</td>
<td><img src="#" alt="Green" /></td>
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Viniflora® CH11

› Oenococcus oeni isolated from cool-climate wines (→ low pH)
  › Fast, reliable malolactic fermentation in low-pH and low-temperature wine

› Used also in moderate pH wines for high-speed malolactic fermentation

› Low to medium amounts of diacetyl, keeping the wines lean and elegant

› VINIFLORA® CH11 can be used for early co-inoculation, late co-inoculation as well as sequential inoculation.

Figure 1. Malolactic fermentation with VINIFLORA® CH11 in Chardonnay (pH 3.0 and temperature 16 °C). Sequential inoculation

Figure 2. Late co-inoculation of VINIFLORA® CH11 in Merlot, France (2013), pH 3.4
Viniflora® CH35

› *Oenococcus oeni* strain selected to perform malolactic fermentation in wines with up to 45 ppm SO2.

› Extremely robust - often used in wines that have difficulties undergoing malolactic fermentation (high SO2 concentration combined with low pH and low temperature).

› High amounts of diacetyl fore reds and classic Chardonnay style with powerful body

› As the buttery aroma fits very well with barrel-aged white and red wines, VINIFLORA® CH35 is the perfect choice for malolactic fermentation in barrels.

› As CH35 performs well at low temperature and low pH, the strain fits cool-climate wines with a rich and powerful character

› Can be used in both red, rosé and white varieties

**Figure 1. Diacetyl production of CH35, compared to CH11, CINE™ and a control without MLF at 16°C**
Viniflora® CiNe™

- Malolactic fermentation without the buttery characteristic
- Citrate-negative *Oenococcus oeni* selected for malolactic fermentation without producing diacetyl
- Unable to convert citric acid to diacetyl, as it lacks the required enzymes to take up and utilize citric acid, making it citrate negative
- Launched to meet an increased demand for balanced and fruity wines.
- Widely used in elegant styles of white wine where structure, balance and crispness are in focus.
- Also popular for rose winemaking, where it is used to create a balanced and stable rose, without sacrificing the fresh and fruity characters of a good rose.

**Figure 1:** Inoculation of VINIFLORA® CINE™ and VINIFLORA® OENOS™ in Pinot blanc, pH 3.4, alc. 13%v/v and TSO2 10ppm showing malic acid degradation and absence of citric acid consumption with CINE™

**Figure 2:** Diacetyl production by VINIFLORA® CINE™ compared to VINIFLORA® CH35, VINIFLORA® CH11 and a control without malolactic fermentation
Contact Information:
Bryan Forbes
Technical Sales Representative for
Michigan, Ohio, Indiana & Illinois
Bforbes@gusmerenterprises.com
Improving food & health
Keep it great! With Direct Inoculation **VINIFLORA®**
- High CFU and activity in every pack

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