Mastering Malolactic Fermentation

July 2021

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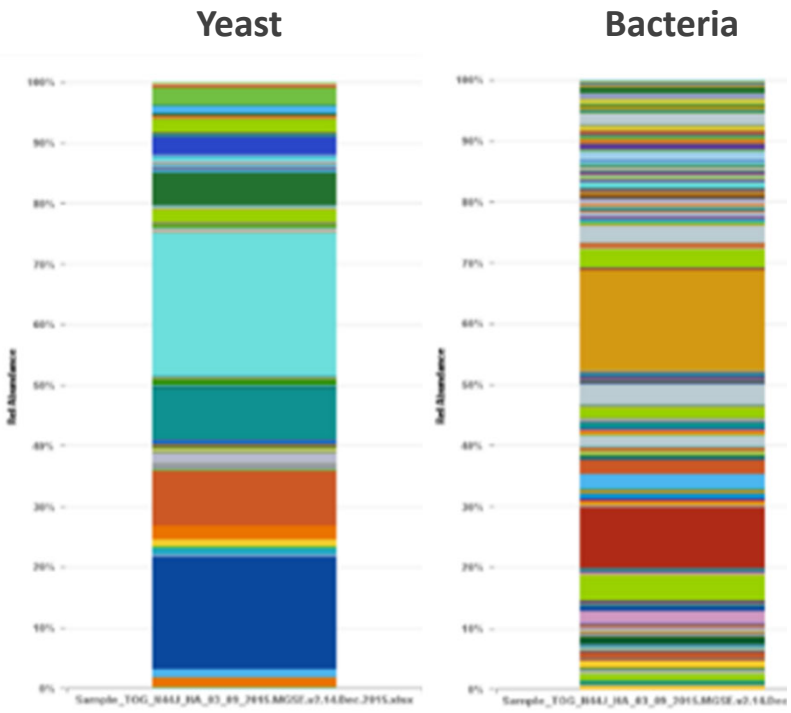
Managing fermentation



In red grape must

Environmental yeast to carry out the alcoholic fermentation

Environmental bacteria to carry out the malolactic fermentation

- 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

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Manage your fermentations with Chr. Hansen VINIFLORA®

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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

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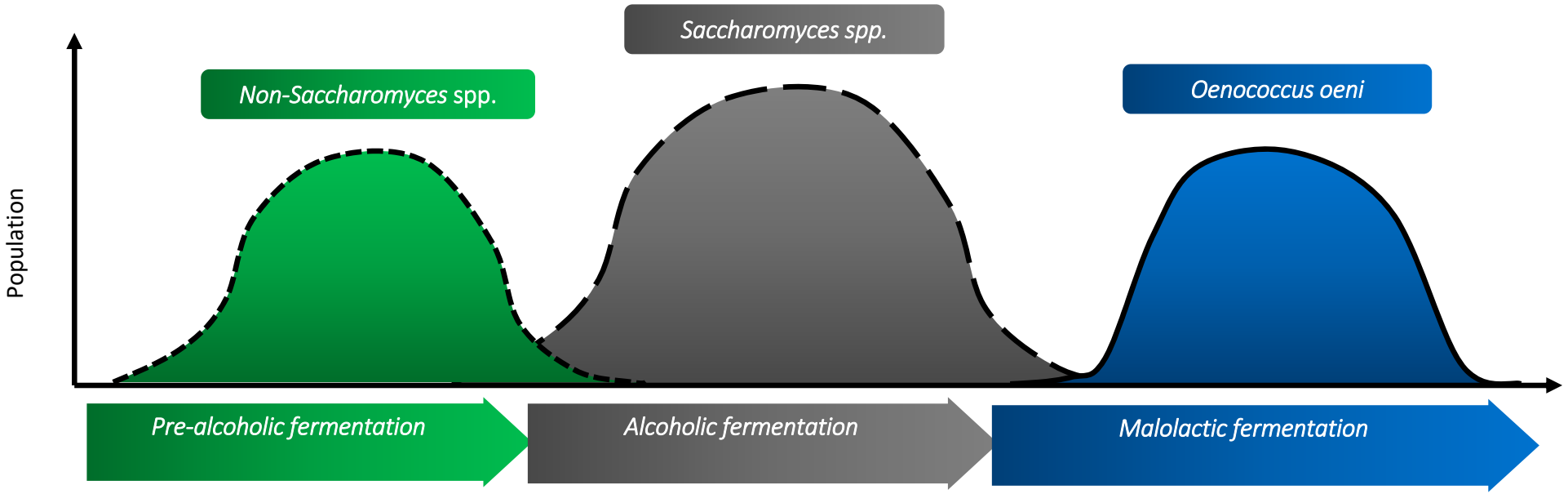
Classical population sequence in winemaking



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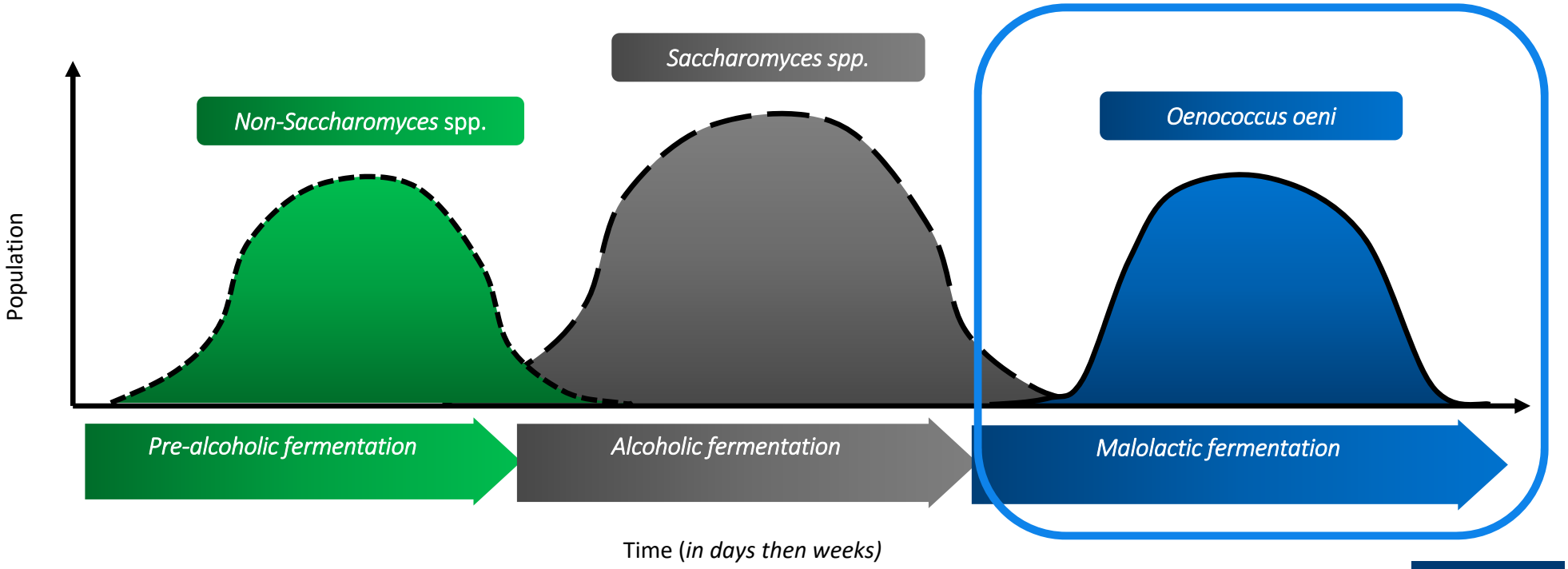

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Keep it great! With VINIFLORA®

Product	Wine Color	Temperature	Alcohol	Minimum pH	Total SO_2	Summary
Viniflora® CH35 The broadest spectrum	Red, selected white	59-77 °F	14% v/v	3.1	45 ppm	› Secure malolactic fermentation › High SO_2 tolerance › Promotes diacetyl
Viniflora® CiNe™ The fruit keeper	Red, rosé, white	63-77 °F	14% v/v	3.2	30 ppm	› Fruit driven › Clean and modern
Viniflora® CH11 The fast track	Red, rosé, white	57-77 °F	15% v/v	3.0	35 ppm	› Low pH › Low temperature › Fast malolactic fermentation
Viniflora® Oenos The classic	Red	63-77 °F	14% v/v	3.2	40 ppm	› Most wines
Viniflora® Oenos 2.0 The new classic	Red, rosé, white	59-77 °F	15% v/v	3.2	40 ppm	› Most wines › Faster Faster malolactic fermentation › Barrel aging
Viniflora® CH16 The ethanol resistant	Red	63-77 °F	16% v/v	3.4	40 ppm	› High alcohol › High maturity

Always check temperature, alcohol, pH and total SO_2 before inoculation
Bacteria are living organisms and wine is a harsh environment for them

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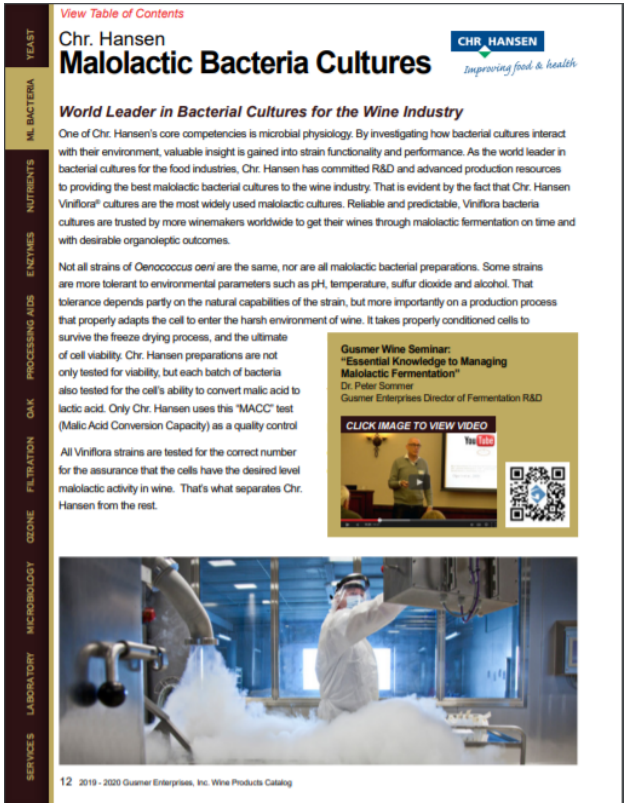
VINIFLORA® resources in North America



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YEAST | ML BACTERIA | NUTRIENTS | ENZYMES | PROCESSING AIDS | OAK | FILTRATION | OZONE | MICROBIOLOGY | LABORATORY | SERVICES

Chr. Hansen

Malolactic Bacteria Cultures

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World Leader in Bacterial Cultures for the Wine Industry

One of Chr. Hansen's core competencies is microbial physiology. By investigating how bacterial cultures interact with their environment, valuable insight is gained into strain functionality and performance. As the world leader in bacterial cultures for the food industries, Chr. Hansen has committed R&D and advanced production resources to providing the best malolactic bacterial cultures to the wine industry. That is evident by the fact that Chr. Hansen Viniflora® cultures are the most widely used malolactic cultures. Reliable and predictable, Viniflora bacteria cultures are trusted by more winemakers worldwide to get their wines through malolactic fermentation on time and with desirable organoleptic outcomes.

Not all strains of *Oenococcus oeni* are the same, nor are all malolactic bacterial preparations. Some strains are more tolerant to environmental parameters such as pH, temperature, sulfur dioxide and alcohol. That tolerance depends partly on the natural capabilities of the strain, but more importantly on a production process that properly adapts the cell to enter the harsh environment of wine. It takes properly conditioned cells to survive the freeze drying process, and the ultimate of cell viability. Chr. Hansen preparations are not only tested for viability, but each batch of bacteria also tested for the cell's ability to convert malic acid to lactic acid. Only Chr. Hansen uses this "MACC" test (Malic Acid Conversion Capacity) as a quality control

All Viniflora strains are tested for the correct number for the assurance that the cells have the desired level malolactic activity in wine. That's what separates Chr. Hansen from the rest.

Gusmer Wine Seminar: "Essential Knowledge to Managing Malolactic Fermentation"
Dr. Peter Sommer
Gusmer Enterprises Director of Fermentation R&D

CLICK IMAGE TO VIEW VIDEO

12 2019 - 2020 Gusmer Enterprises, Inc. Wine Products Catalog

Viniflora® bacteria range for winemakers

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Viniflora® CH35 The broadest spectrum	Red, selected white	59-77 °F	14% v/v	3.1	45 ppm	› Secure malolactic fermentation › High SO_2 tolerance › Promotes diacetyl
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Presenter Bio

- **Duncan Hamm** is currently Regional Technical Manager for the Asia-Pacific region, based out of Chr. Hansen Australia.
- From 1 August however, he will relocate to the Chr. Hansen head office in Denmark, where he will work as **Senior Application Specialist**.
- Duncan initially studied chemistry at the University of Waikato in NZ, then worked for ten years in the dairy industry, including five years with Chr. Hansen.
- After completing a graduate diploma in Viticulture & Enology at Lincoln University (NZ), added to some valuable hands-on cellar experience, Duncan re-joined Chr. Hansen as a commercial oenologist in 2008.
- Duncan is very much looking forward to working again across the wide world of Chr. Hansen's wine activities, which of course includes North America and the great team at Gusmer Enterprises.

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Malolactic fermentation (MLF)

L-malic ⇒ L-lactic + CO_2

- Enzymatic conversion of L-malic acid to L-lactic acid
- The bacteria is performing MLF to survive and grow

FROM THE WINE'S PERSPECTIVE

- Softens the acidic profile (pH increases)
- Affects the aromatic properties of the wine
- Enhances the microbial stability of the wine

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

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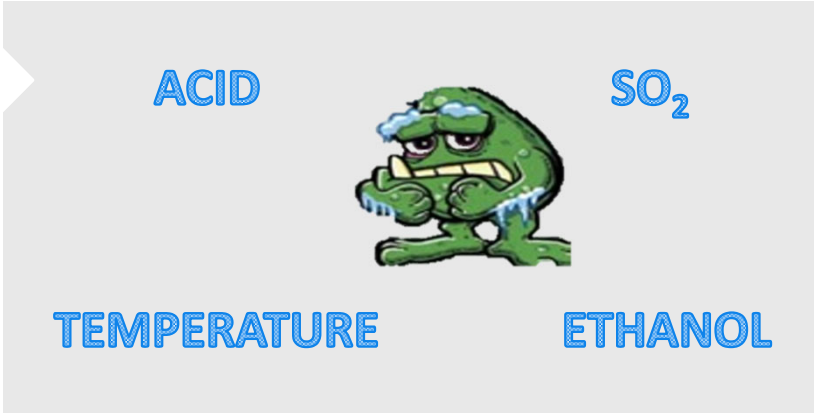
Understanding the life of *Oenococcus oeni*

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

The predominant stress factors for *O. oeni* in wine are **SO_2**, **pH**, **alcohol** and **temperature**…..

….these stress factors are synergistic, meaning that they enhance each other's effect



Other stress factors sometimes seen in wine are **Low Nitrogen** or the presence of **Medium-Chain Fatty-Acids**

A **high concentration of Malic Acid** is not necessarily a stress factor….wine pH is more important

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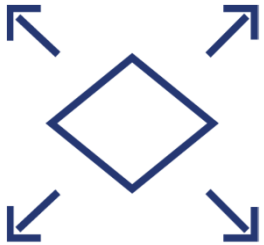
Wine as stressful environment

Condition for MLF	pH
Very Difficult	< 3.0
Difficult	3.0-3.2
Favourable	3.2-3.7
Very favourable	>3.7

pH



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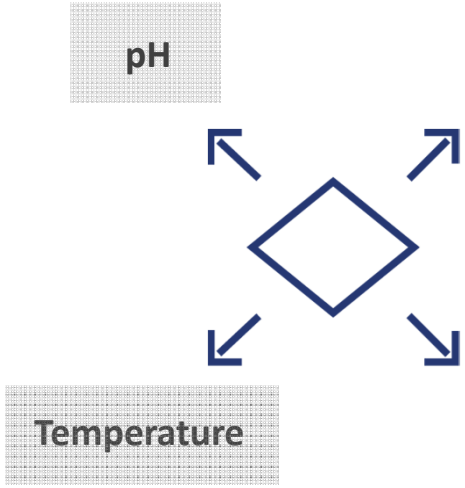
Wine as stressful environment

Condition for MLF	pH
Very Difficult	< 3.0
Difficult	3.0-3.2
Favourable	3.2-3.7
Very favourable	>3.7

Condition for MLF	Temperature
Very Difficult	<59°F
Not so favourable	59-66°F
Favourable	66-77°F
No MLF	>77°F



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Condition for MLF	Temperature
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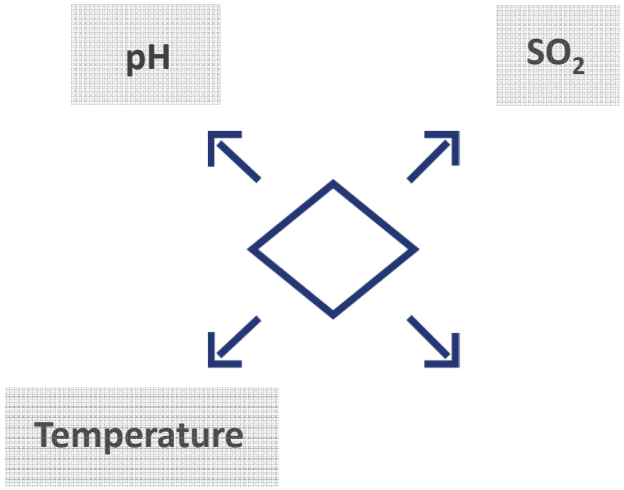
Not so favourable	59-66°F
Favourable	66-77°F
No MLF	>77°F



Condition for MLF	Total SO_2
Very difficult	> 45 ppm
Difficult	30 – 45 ppm
Less favourable	15 – 30 ppm
Favourable	< 15 ppm

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Wine as stressful environment

Condition for MLF	pH
Very Difficult	< 3.0
Difficult	3.0-3.2
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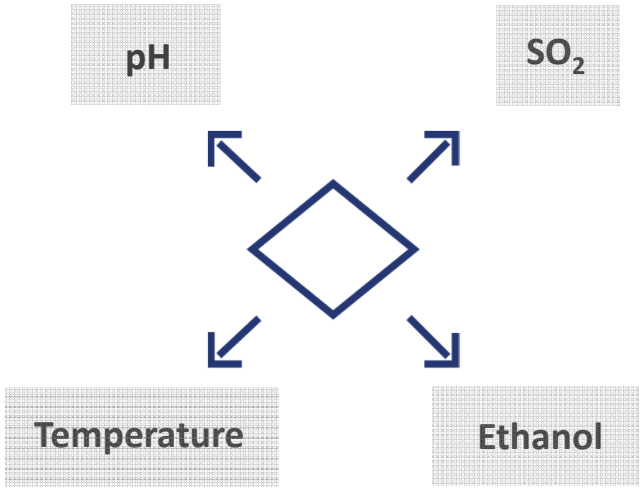
Condition for MLF	Temperature
Very Difficult	<59°F
Not so favourable	59-66°F
Favourable	66-77°F
No MLF	>77°F



Condition for MLF	Total SO_2
Very difficult	> 45 ppm
Difficult	30 – 45 ppm
Less favourable	15 – 30 ppm
Favourable	< 15 ppm

Condition for MLF	Ethanol
Favourable	< 13%
Difficult	13 – 15%
Very difficult	15-17%

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MLF - getting a population of MLB into a wine

- There are six main approaches to get the necessary bacteria into a wine to be put through MLF:

  - Spontaneous flora
  - Cross-seeding
  - Build-up cultures
  - Pre-activation cultures
  - Quick reactivation
  - Direct inoculation

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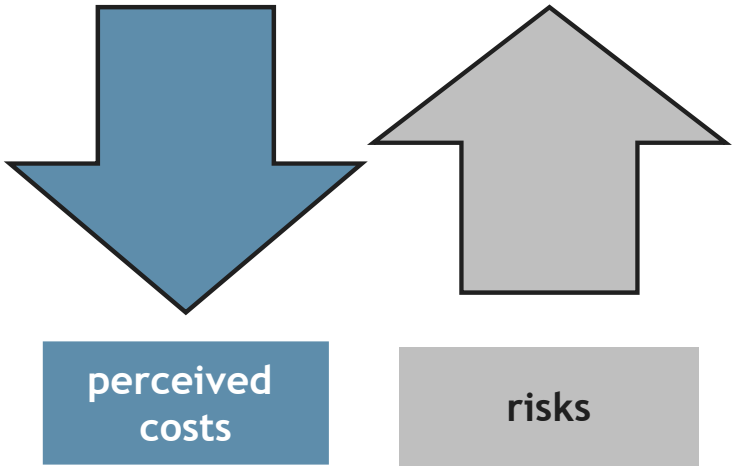
⇨ **Involve inoculating with a commercial product**



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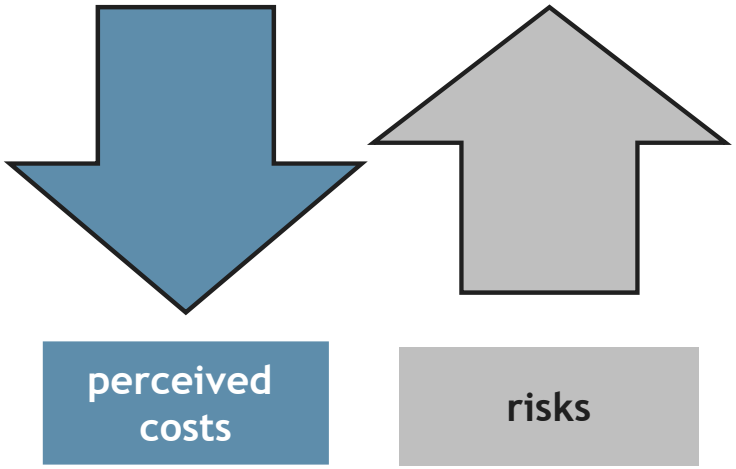

**Chr. Hansen only produces Direct-Inoculation MLF cultures based on a high number of already adapted cells*

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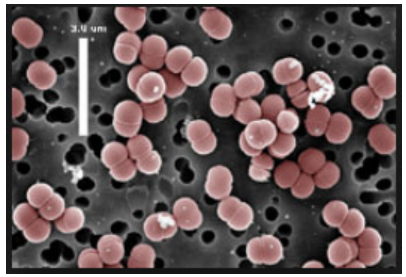
Spontaneous ML fermentation

- The most traditional method whereby MLF is carried out by adventitious bacteria
- Still routinely used in many regions
- The culture is itself 'free'
  - < pH 3.5: *Oenococcus oeni* only
  - > pH 3.5: *O. oeni*, *Pediococci* and *Lactobacilli* → fast

Carries risk → very little control over microflora in wine

- Variable speed of MLF
- Opportunity for spoilage microbes to establish
  - *Brettanomyces sp., Acetobacter, undesirable LAB species*

http://allpix.club/pages/l/lactobacillus-bacteria/



http://genome.jgi.doe.gov/pedpe/pedpe.home.ht

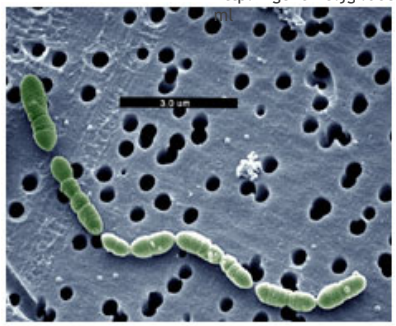


Photo: Jeff Broadbent, Utah State University

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So where do the Chr. Hansen MLF cultures and strains originate?

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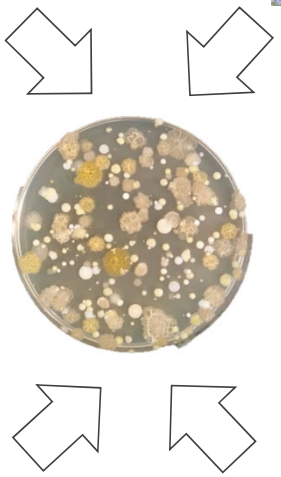
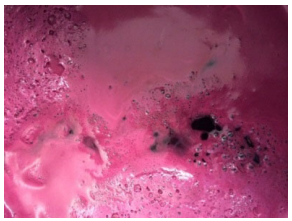
At Chr. Hansen, we screen for the next robust bacteria

1. Robust in extreme and changing conditions
2. Enhance flavor attributes and explore new characteristics
3. Biological protection
4. Improve quality and efficiency of production



Where do we get the strains from?

- Exclusively from NATURE
- Isolated from grape, must, wine and winery environment
  - Chr. Hansen discovery platform
  - Project collaborations with universities and research centers
  - External culture collections



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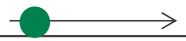
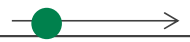
Select the VINIFLORA® strain that fits your wine

	Wine making parameters				Cultures benefits	
Culture	Temperature (°F) **Max :77°F**	Alcohol %(v/v)	pH	SO_2 (ppm)	Flavor Buttery to fruity	Fermentation Speed Slow to fast
Viniflora® Oenos	≥ 63	≤ 14	≥ 3.2	≤ 40		
Viniflora® Oenos 2.0	≥ 59	≤ 14	≥ 3.2	≤ 40		
Viniflora® CH11	**≥ 57**	≤ 15	**≥ 3.0**	≤ 35		
Viniflora® CH16	≥ 63	**≤ 16**	≥ 3.4	≤ 40		
Viniflora® CH35	≥ 59	≤ 14	≥ 3.1	**≤ 45**		
Viniflora® CiNe™	≥ 63	≤ 14	≥ 3.2	≤ 30		

- 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 the vast majority of global wine conditions

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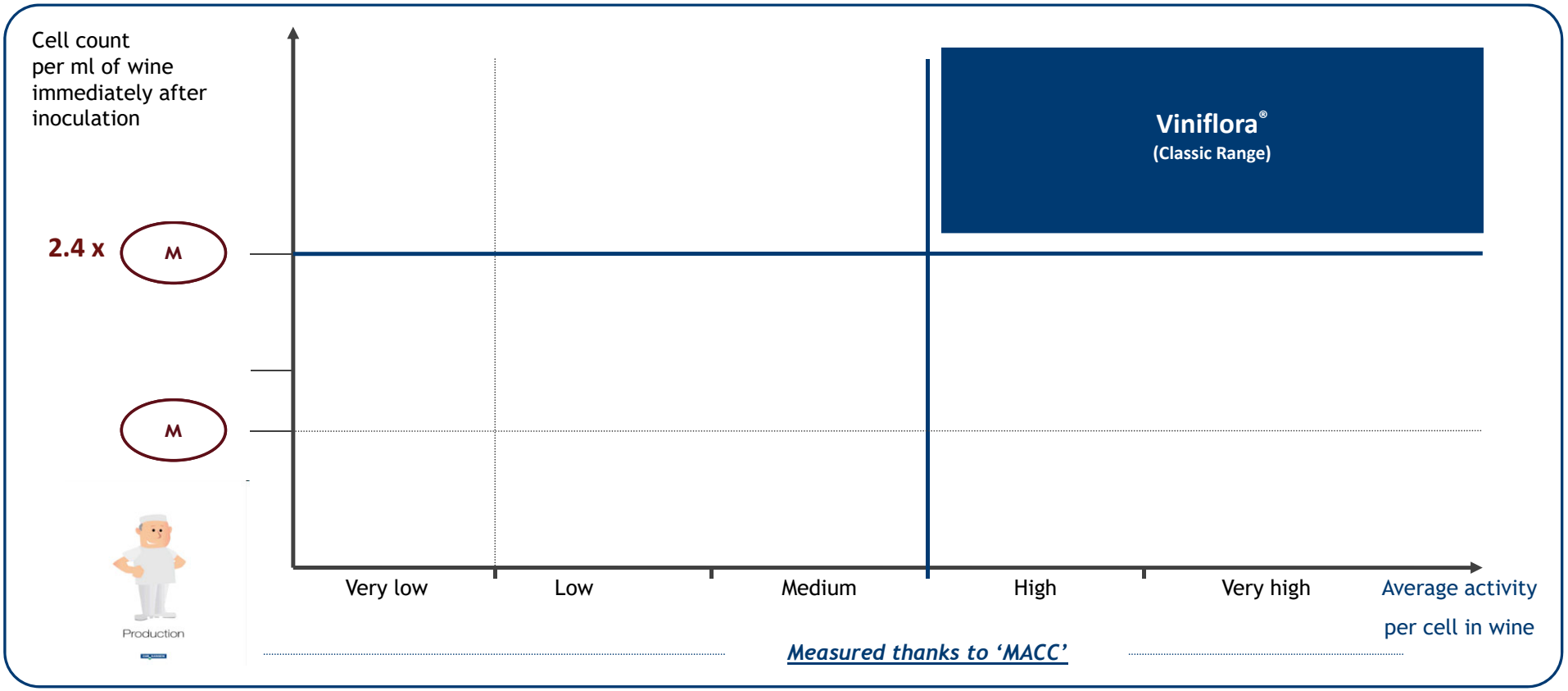
VINIFLORA® are different from other MLF Bacteria

Thanks to their MACC* test & their high concentration of viable cells



M = the 'magic number' or 10^6 CFU/ml – the critical population required for MLF to happen in wine

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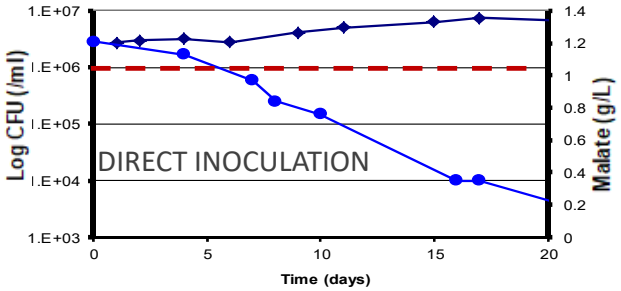
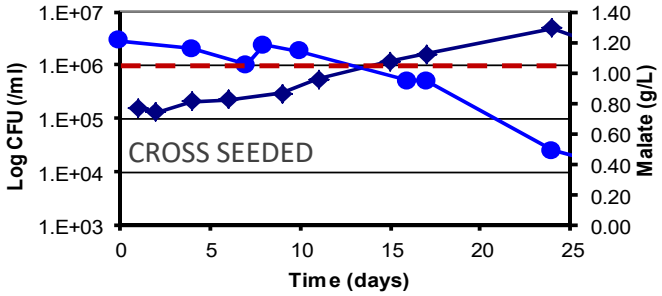
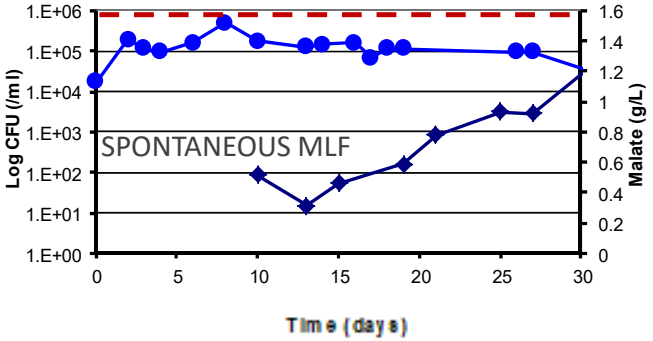
Magic Number 1x10⁶ CFU/mL

Cabernet Sauvignon, Australia 2006 - pH 3.43, EtOH 13.3%







- Direct inoculated wine has >10^6 CFU/ml upon culture addition and completes MLF in 20 days (<0.2g/L MA)
- Cross-seeded starts at 10^5 CFU/ml so has a 13-day lag before it hits the magic number and MLF commences
- After 30 days spontaneous MLF treatment still only at 10^4 CFU/ml

Key to graphs:

- *Malic Acid (g/l)*
- *O. oeni cell count (CFU/ml)*
- *The 'Magic Number'*

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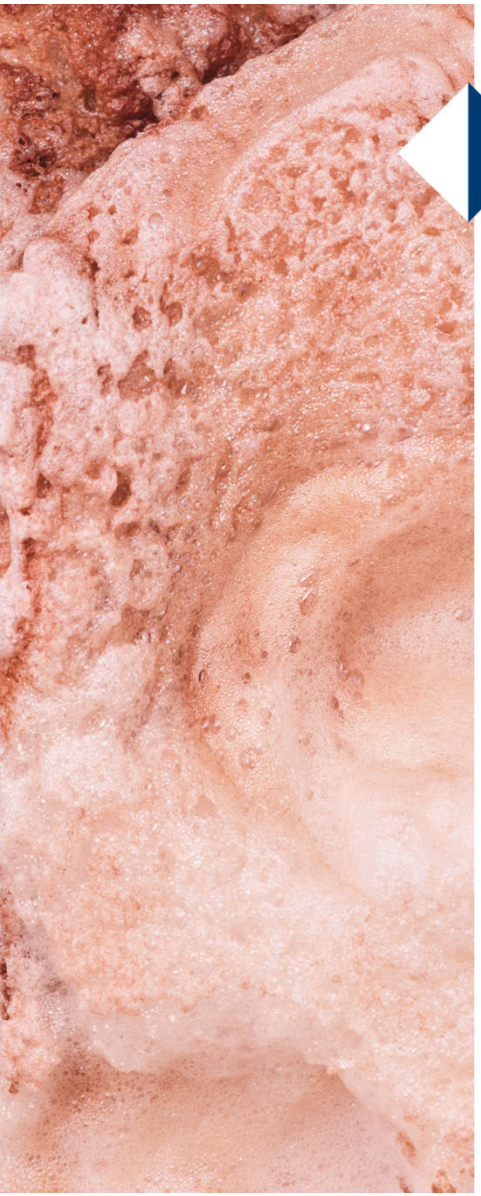
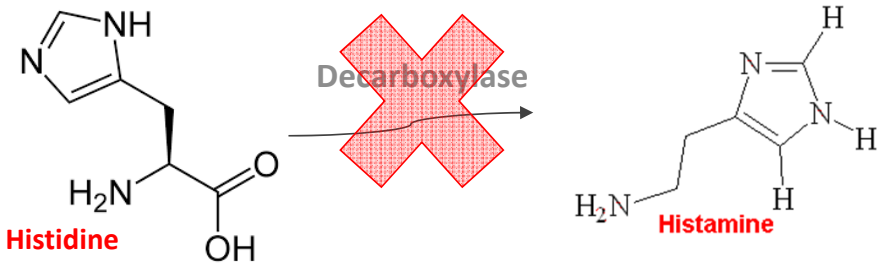


Quality Control

- All strains sold by Chr. Hansen go through an extensive Quality control
- Starting with checking strains for anti-biotic resistance and bio-genic amine production – this happens before selection of new strains
- The most relevant bio-genic amine is **Histamine**, which is produced from **Histidine** (amino acid) via an enzymatic pathway
- The bacteria in the VINIFLORA® range <u>do not have this pathway</u>



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VINIFLORA® is not only a product, it is also an ”insurance” for your HACCP & FSMA process

Product Documentation


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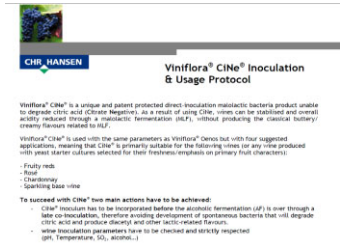
Viniflora ® LS CH35

Product Information

Inoculation Protocol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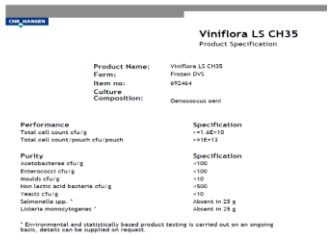
Viniflora® CINe® Inoculation & Usage Protocol

Specifications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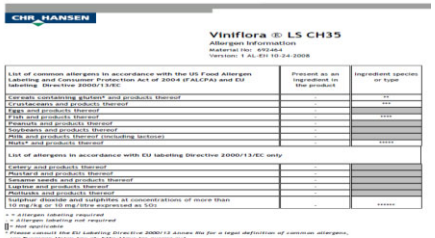
Viniflora LS CH35

Product Specification

Allergen information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Viniflora ® LS CH35

Allergen Information

Safety Data Sheet


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FICHA DE SEGURANÇA

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SICHERHEITSDATENBLATT

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Certificate of Analysis

Same document as specifications but issued per batch number

Non-GM statement


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Viniflora ® LS CH35

GMO Statement

All cultures are validated for organic wines

All cultures are biogenic amines negatives

Other quality and safety certificates are available upon request…

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Inoculation protocols

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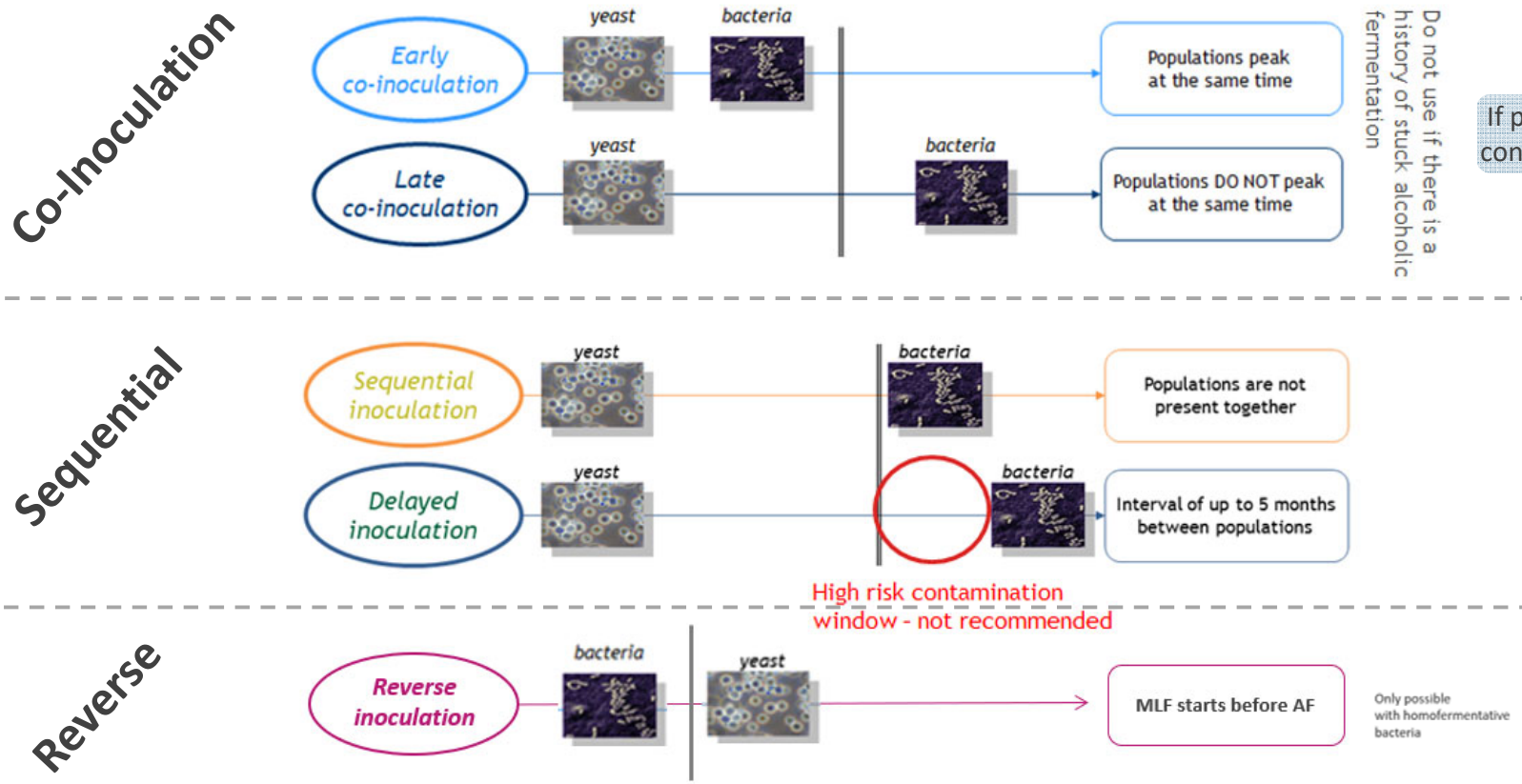
The choice of inoculation time depends on winery conditions and on winemaker objectives



If pH is lower than 3.5 → consider early co- inoculation

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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

	Benefits to cellar	Impact on final wine
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**	Keep the initial quality potential **Avoid spoilage microorganisms and BA**
Enhance fruitiness		Diacetyl produced by bacteria is partly degraded by yeast → **fruitiness enhanced**

Stuck fermentation and Troubleshooting

1. First step: check the basic parameters (pH, alcohol, SO_2 and temperature)
   - Often the issue can be solved with a more appropriate strain
2. It could be the nitrogen (MLF requires 60ppm of Amino Nitrogen)
   - Re-inoculate with Malo nutrients (typically provides small peptides)
   - MLF nutrients will also bind MCFA's, produced by certain yeasts when stressed, which are inhibitory to *O. oeni*
3. The cell count…..CFU/mL
   - Good to see how many *O. oeni* cells there are in the wine
   - Investigate inoculation method (NB! Cross seeding or stretching)
4. Phenolics can be an issue with some red varieties…..blending or fining with PVPP

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Winemaking conditions vary greatly

Malolactic fermentation depends on the thriving of different inoculated bacteria. Some wine environments can be too harsh for bacteria, forcing winemakers to spend time and resources on controlling the parameters before starting bacteria inoculation.

CONDITIONS INFLUENCING MLF

pH	< 3.2	3.2 – 3.5	3.5 – 3.7	> 3.7[1]
Temperature (°C)	< 15	15 – 19	19 – 25	> 25
Malic acid concentration (g/l)	< 1	1 – 2.5	2.5 – 5	> 5
Total SO_2 (ppm)	> 45	15 – 30	< 15	
Free amino nitrogen (FAN mg/l)[2]	< 60	60 – 80	> 80	

- Very favorable
- Favorable
- Difficult
- Very difficult
- No MLF

1 Hard to avoid spontaneous malolactic fermentation
2 FAN preferred or PAN/YAN

EASY ASSESSMENT TOOL

It can be complicated to judge the level of difficulty based on all the parameters.

To help you make timely decisions regarding your fermentation, Chr. Hansen offers support through our North American distributor – Gusmer.

Check out Gusmer's website to find your local representative - www.gusmerwine.com

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Keep it great! With VINIFLORA®

Product	Wine Color	Temperature	Alcohol	Minimum pH	Total SO_2	Summary
Viniflora® CH35 The broadest spectrum	Red, selected white	59-77 °F	14% v/v	3.1	45 ppm	› Secure malolactic fermentation › High SO_2 tolerance › Promotes diacetyl
Viniflora® CiNe™ The fruit keeper	Red, rosé, white	63-77 °F	14% v/v	3.2	30 ppm	› Fruit driven › Clean and modern
Viniflora® CH11 The fast track	Red, rosé, white	57-77 °F	15% v/v	3.0	35 ppm	› Low pH › Low temperature › Fast malolactic fermentation
Viniflora® Oenos The classic	Red	63-77 °F	14% v/v	3.2	40 ppm	› Most wines
Viniflora® Oenos 2.0 The new classic	Red, rosé, white	59-77 °F	15% v/v	3.2	40 ppm	› Most wines › Faster Faster malolactic fermentation › Barrel aging
Viniflora® CH16 The ethanol resistant	Red	63-77 °F	16% v/v	3.4	40 ppm	› High alcohol › High maturity

Always check temperature, alcohol, pH and total SO_2 before inoculation
Bacteria are living organisms and wine is a harsh environment for them

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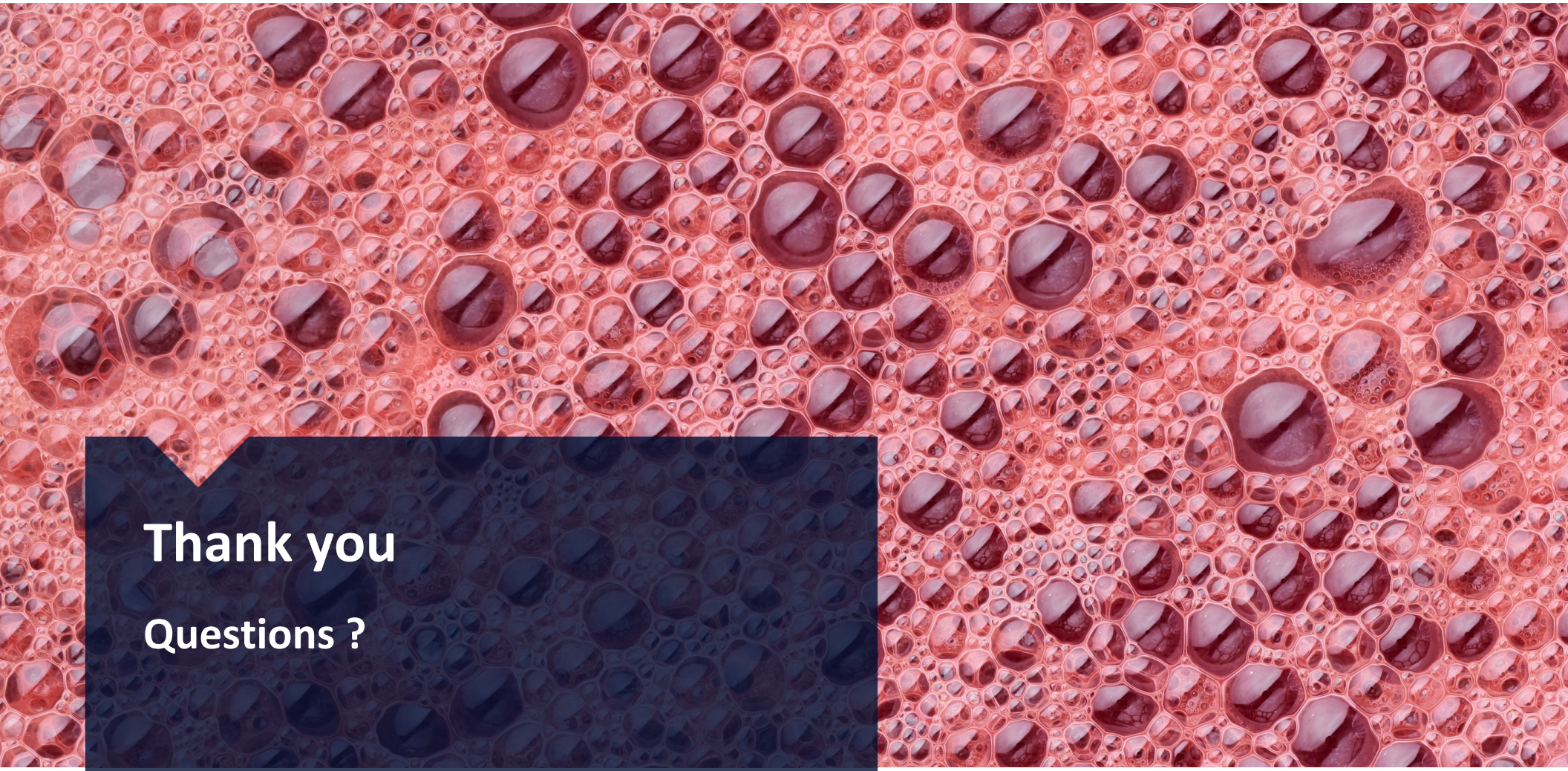

Thank you

Questions ?

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