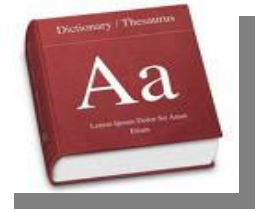
CHR HANSEN

Improving food & health

Co-inoculation and wine

Chr. Hansen Fermentation Management
Services & Products

A definition of ‘co-inoculation’



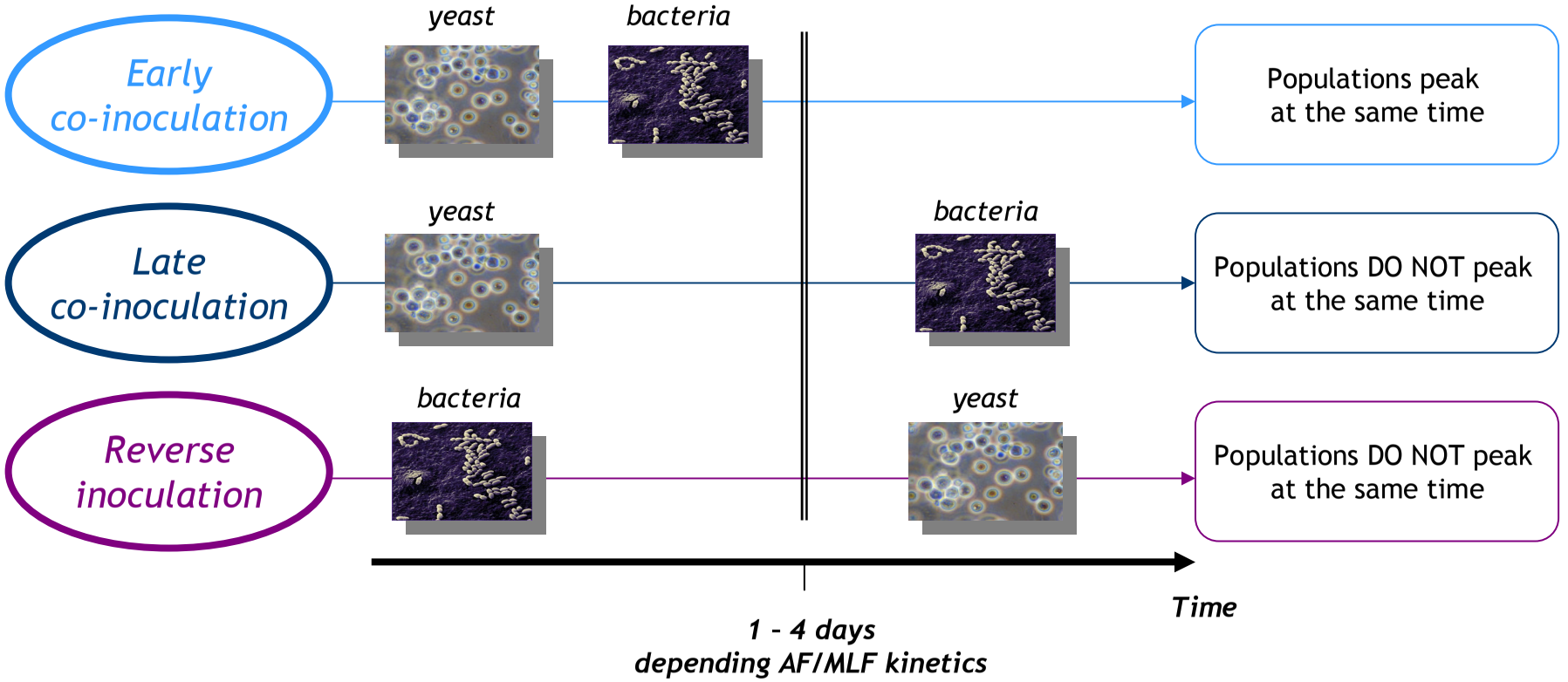
- ‘Co-inoculation’ is the term used in winemaking when…
  - yeasts (used to manage alcoholic fermentations - AF) and
  - malolactic bacteria (used to manage malolactic fermentations - MLF)

- … are inoculated in a different way than the classical and conventional protocol known as ‘sequential inoculation’ where:
  - yeasts are first used for AF management
  - and then -when AF is finished- bacteria for MLF management are inoculated into the wine

- Therefore during ‘co-inoculation’ populations of both active yeasts and malolactic bacteria co-exist in musts. Each population of microorganism achieving its fermentation.

3 realities behind ‘co-inoculation’

- Depending malolactic bacteria timing of inoculation, 3 different types of ‘co-inoculation’ have been identified



Co-inoculation: current knowledge

- Commonly practiced in some countries:
  - Australia, France, Spain on red and/or rosé wines
  - France, Germany and South Africa on white wines

- Experiments have started in the 80´s and since 2005 are booming

- Therefore current knowledge is sufficient enough today to:
  - Get an overview of co-inoculation **benefits**
  - Get a clear picture of co-inoculation **risks**
  - Get the elements to define the **'comfort zone'** where co-inoculation is possible and risks are limited
  - Select the **appropriate protocol** (early, late, reverse)
  - Follow the two fermentations through the right parameters to **monitor** the co-inoculation and succeed

Co-inoculation benefits

see the 'case study' part to get quantitative impact

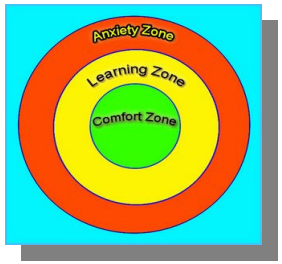
	IMPACT on WINERIES	*IMPACT on WINES*
 **Save time**	• Reduce time to market/ respect deadlines • Improve tank management	
 **Save energy**	• Save costs of heating • Reduce Carbon footprint	
 **Control indigenous population**	• Avoid sluggish or stuck ferment • Avoid downgrading	• Keep the initial quality potential of the wine • Avoid spoilage microorganisms (Brett. & others) • Diacetyl produced by bacteria is degraded by yeasts => fruitiness is kept when needed + wine is stabilized
 **Save fruitiness**		

Co-inoculation risks

	IMPACT on WINERIES	*IMPACT on WINES*
Increase of Volatile Acidity	• Cost of downgrading • Additional costs to treat wines / manage AF & MLF	• Potential increase of acetic acid giving vinegar character
 **Stuck fermentations**	• Stuck AF due to bacterial antagonist compounds/ competition for nutrients • Stuck MLF due to SO_2 production by yeasts and/or long chain fatty acids	• Spoilage microorganisms (Brett. & others)
 **Absence of classical MLF flavors**		• Absence / reduction of buttery, creamy flavors coming from MLF fermentation

Co-inoculation benefits/risks management

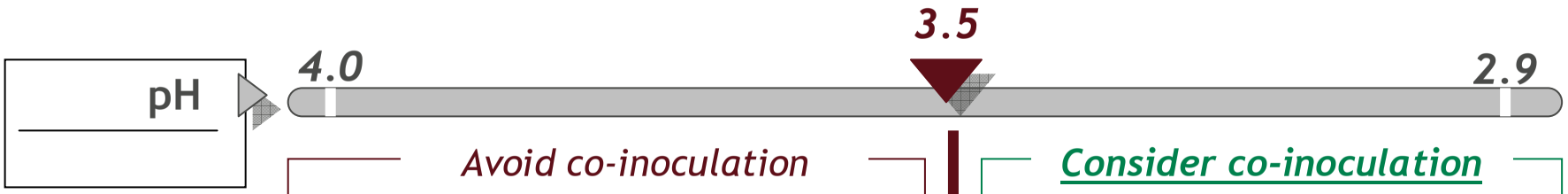
Experiments, trials and scientific literature
help to understand *Oenococcus oeni* metabolism in must & wine
and therefore a frame work to get safe co-inoculation



A ‘comfort zone’ where co-inoculation benefits can be reached
with minimum risks has been defined

The ‘comfort zone’ for co-inoculation: pH<3.5

(with Oenococcus oeni DVS starter cultures)



Avoid co-inoculation

O. oeni prefers sugars over Malic Acid

Consider co-inoculation

O. oeni prefers Malic Acid over sugars

Metabolism of *Oenococcus oeni* in grape juice/must with pH>3.5

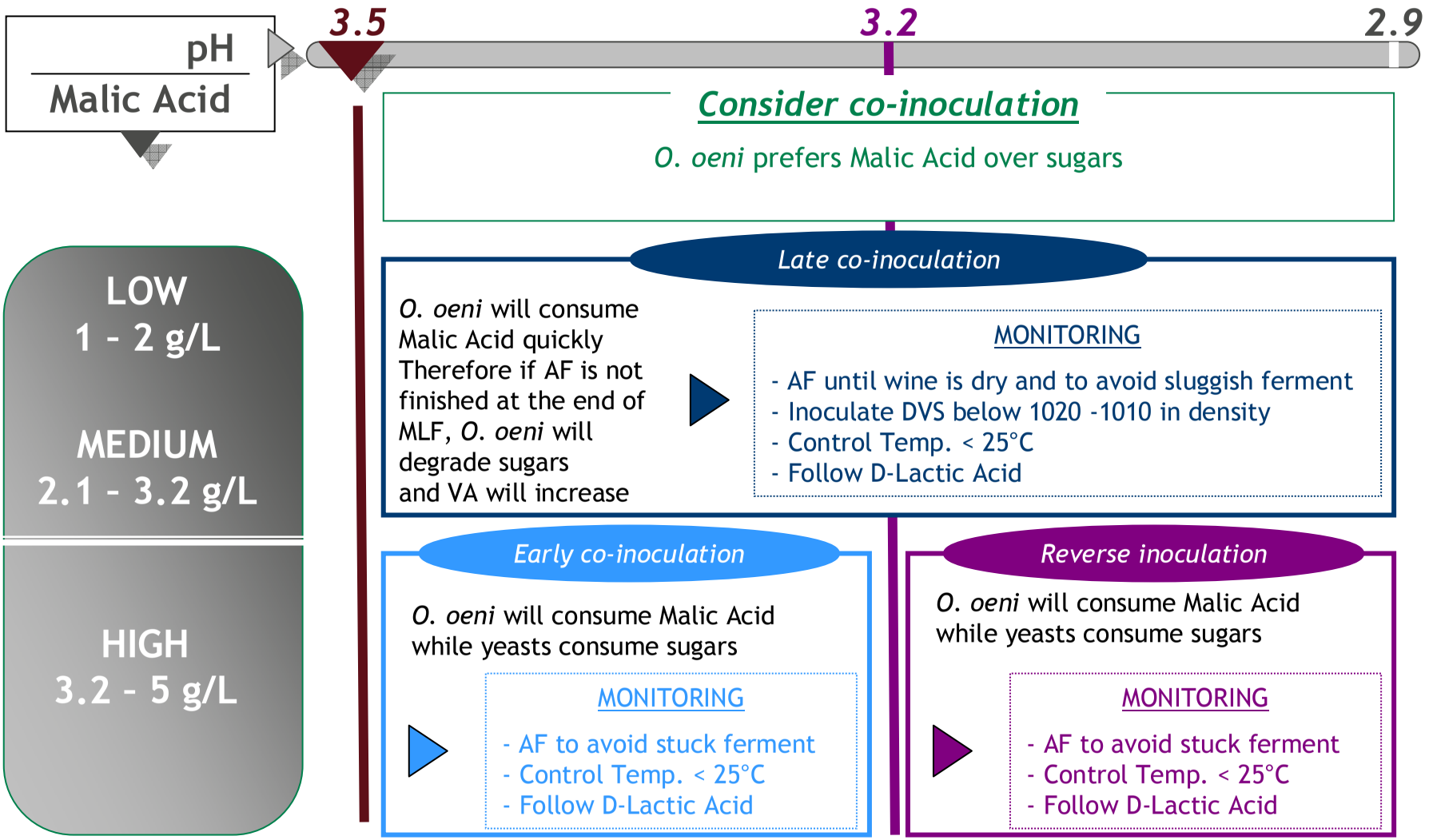
Glucose/fructose	→D-lactic acid acetic acid (VA) ethanol CO_2
Fructose	→Mannitol
L-malic acid	→L-lactic acid CO_2

Metabolism of *Oenococcus oeni* in grape juice/must with pH<3.5

Same Metabolism than in wine

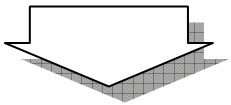
L-malic acid	→L-lactic acid CO_2
Citric acid	→Diacetyl 2,3-butanediol acetoin

Decision tool and ‘co-inoculation’ protocol



Co-inoculation: Go/ No Go checklist

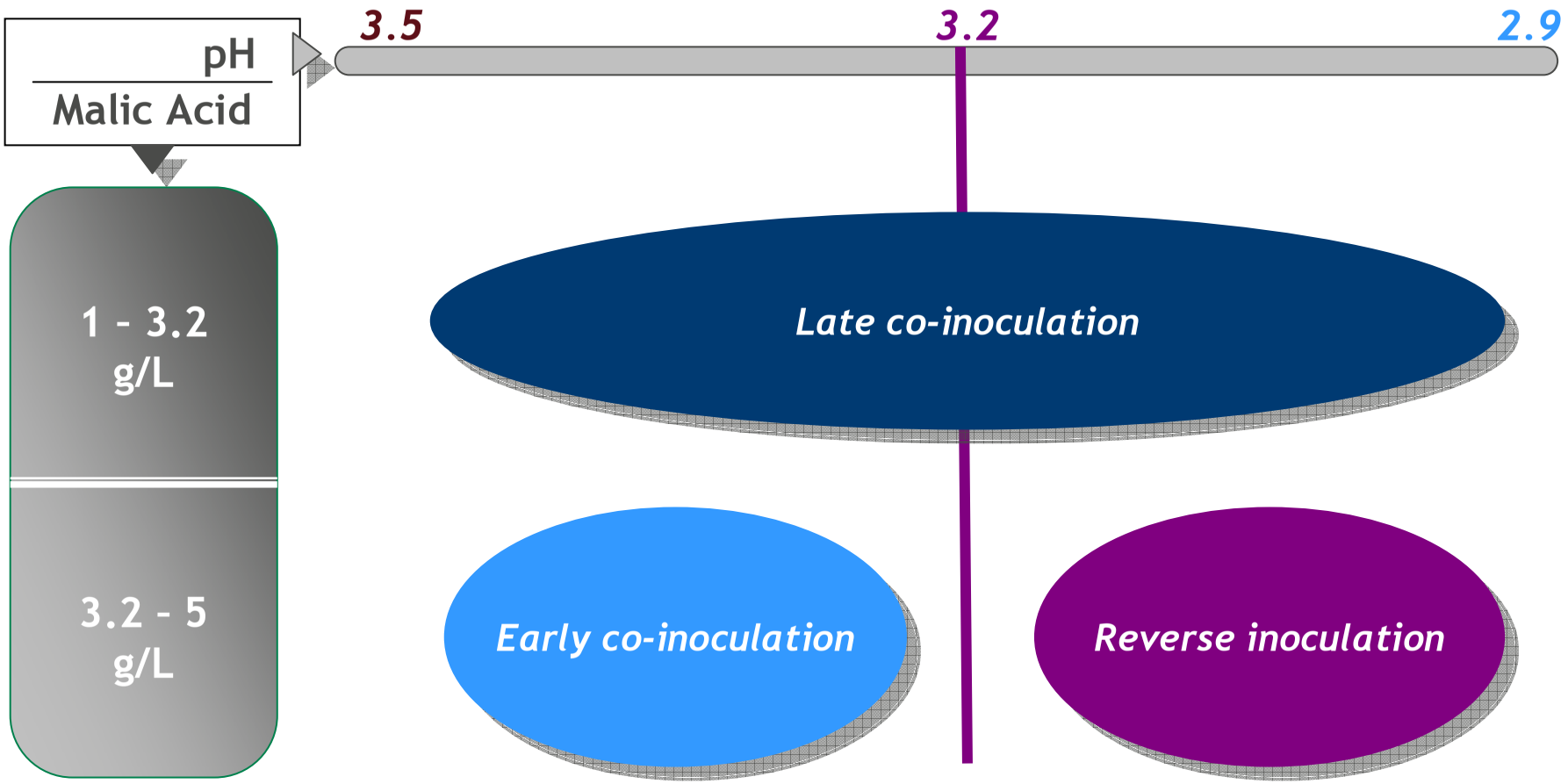
Healthy grapes Clean equipments & cellar	Hygiene is always a key factor success	YES / NO
Temperature controlled tanks	Heating and cooling are necessary to get safe co-inoculation	YES / NO
History of good AF	Stuck of sluggish AF could lead to deviations If unknown start with at least one sequential	YES / NO
Total SO_2 < 20 ppm @ DVS inoculation time	To ensure a good survival after inoculation, SO_2 level has to be as close as possible to 0 ppm	YES / NO
pH< 3.5	Above 3.5, *O. oeni* will degrade sugars	YES / NO
Temperature < 25°C	Optimum DVS conditions are in the range 20-25°C	YES / NO
Yeast strain MLF compatible (produce low levels of SO_2) Yeast strain producing low VA	Yeast and DVS have to be compatible and both of them have to produce low amount of VA Preferably use Viniflora® yeasts and DVS	YES / NO YES / NO



1 'NO' or more? => Prefer sequential inoculation

Only 'YES'? => Select now the appropriate co-inoculation protocol

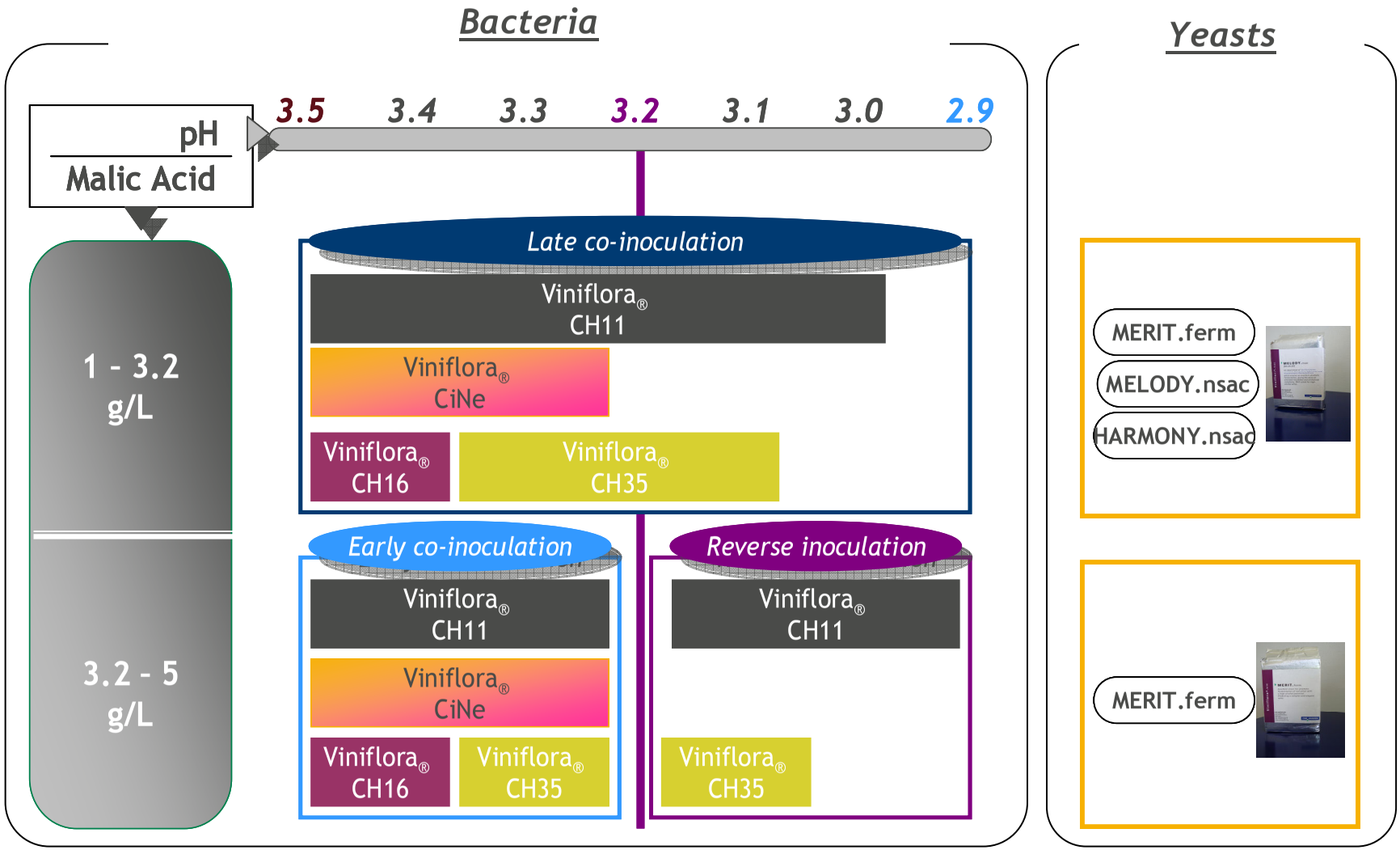
Co-inoculation: relevant protocol checklist



Parameters to follow for a good monitoring

- Alcoholic fermentation
  - Density (specific gravity)
    - Falling down from 1100 => 990
    - Using a densimeter
  - Temperature
    - Co-inoculation T< 25°C
    - Using thermometer allowed in food production
  - Time
    - In days
- Malolactic fermentation
  - Population after inoculation in cfu/ml
    - Magic number: 10^6 cfu/ml
    - Using PCR
  - L-Malic Acid
    - Using standard methods
  - D-Lactic acid production
    - Using Enzymatic kit
  - Temperature
    - Co-inoculation T< 25°C
    - Using thermometer allowed in food production

Viniflora® range of products and co-inoculation



Conclusion

- Co-inoculation in winemaking is still in the learning phase
- However trials conducted all around the world for several years give enough information to define clear routines for successful co-inoculations
- 'Co-inoculation' represent 3 types of protocol:
  - Reverse inoculation, early co-inoculation and late co-inoculation
- Using pH and Malic Acid content of wines it is possible to define:
  - A 'comfort zone' where risks are minimum: pH < 3.5
  - Decision tools to select the most suitable protocol to use depending initial malic acid concentration
- Both AF and MLF have to be carefully monitored to reduce risks (main one is an increase of VA related to sugar consumption by inoculated ML bacteria)
- Chr. Hansen provides:
  - a full range of yeasts and malolactic cultures suitable for co-inoculation
  - Services (advice, decision tools, follow-up) to manage co-inoculation in winemaking and get its full benefits:
    - time & energy savings,
    - microbiological stability,
    - better bio-safety (no biogenic amines production during MLF…)

Examples of co-inoculation

4 case studies

Case 1: Red wine from Tempranillo, Spain

Wine parameters

pH: 3.5

Initial Malic Acid: 2.8 g/L

Protocol

Late co-inoculation

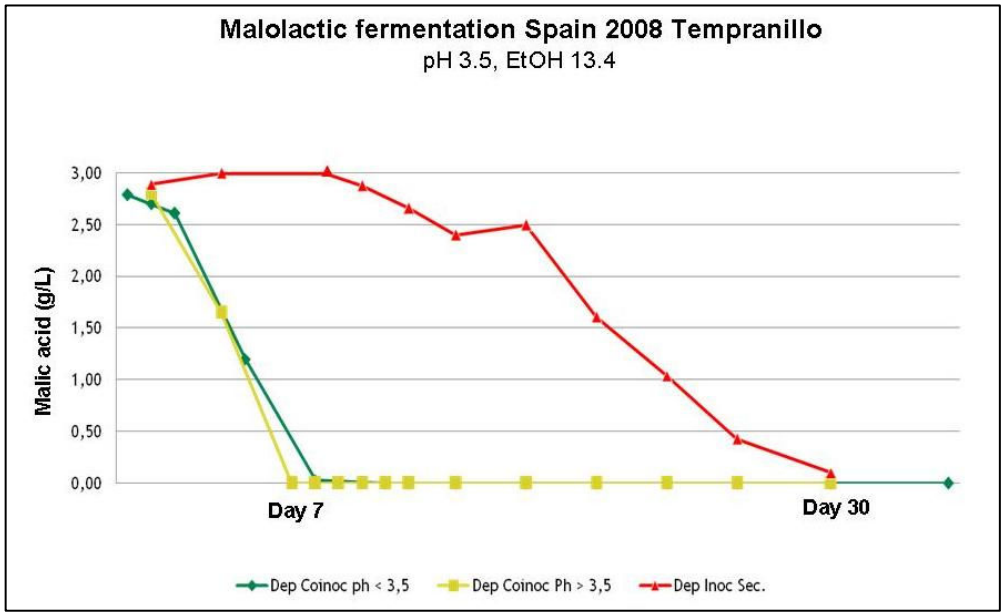
Products

MERIT.ferm

MELODY.nsac

HARMONY.nsac

Viniflora® CH16



The rate of malolactic fermentation compared with co-inoculation with freeze dried Viniflora® (pH 3.5 green and pH 3.8 yellow) and sequential inoculation (pH 3.5).

Alcoholic fermentation was completed on day 7, so in this case the co-inoculated MLF completed at the same time as AF. The sequential was inoculated on day 7, and completed on day 30, hence, a saving of 23 days.

Calculate the Return On Investment corresponding with Vinisav-e

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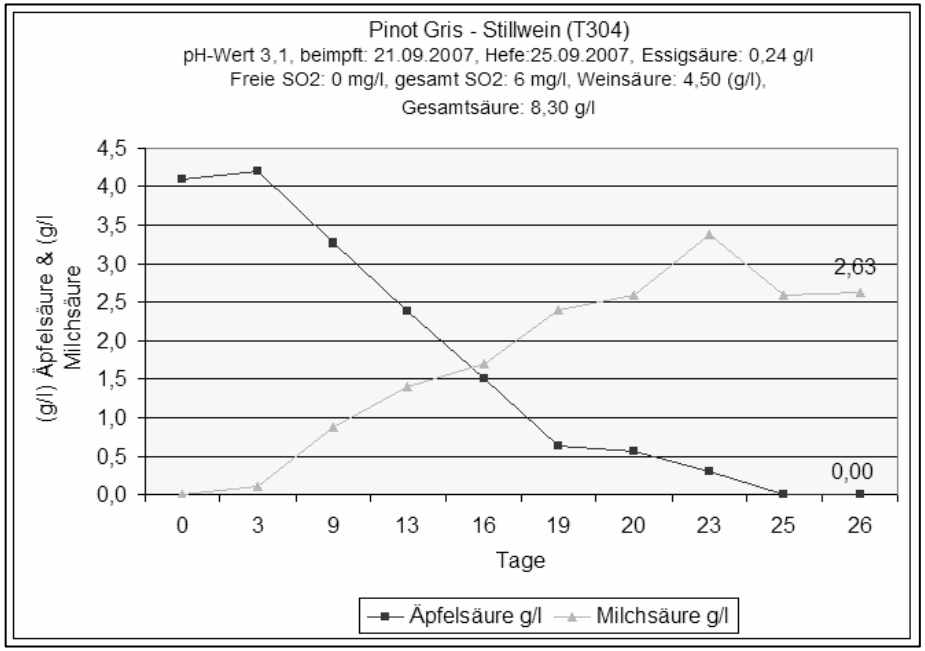
Case 2: white wine Pinot Gris, Germany

Wine parameters	Protocol	Products
pH: 3.1 Initial Malic Acid: 4.2 g/L	*Reverse inoculation*	MERIT.ferm Viniflora® CH11



Chr. Hansen had significant success with co-inoculating Viniflora® CH11 (FroZen™ version) cultures in Sparkling base wines and low pH white wines, notoriously difficult to induce MLF.

In Germany, the malolactic culture was inoculated 4 days before the yeast was inoculated, representing a 'reverse' inoculation. After 4 days, 0.5 g/L of malic acid was already converted to lactic acid, before the onset of AF. Malolactic fermentation was completed 25 days after inoculation.

Important: Total SO_2 in the juice was less than 20 ppm.

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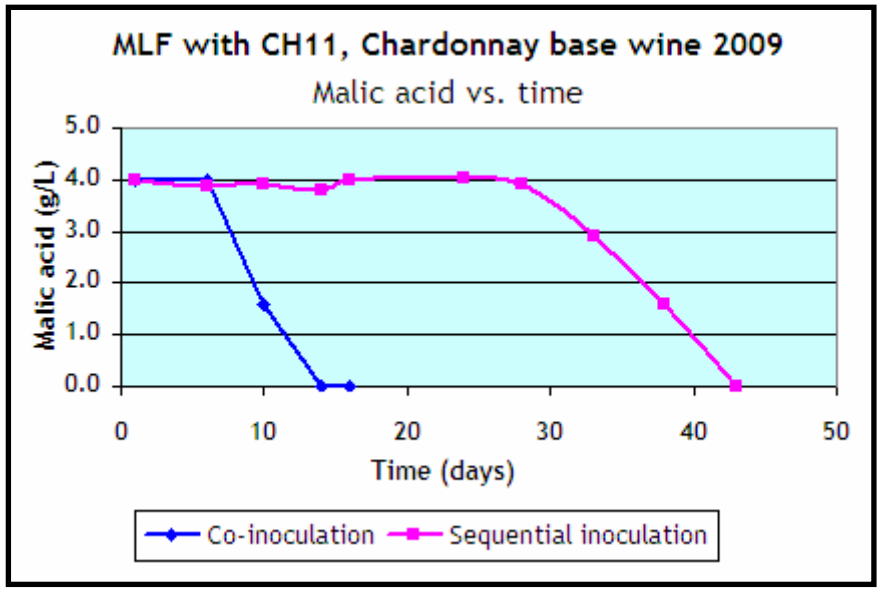
Case 3: Chardonnay sparkling base, South Africa

Wine parameters	Protocol	Products
pH: 3.2 Initial Malic Acid: 4.0 g/L	*Early co-inoculation*	MERIT.ferm Viniflora® CH11



In South Africa, Chardonnay was inoculated with Viniflora® CH11 (FroZen™ version) 24h after yeast inoculation and the MLF completed 14 days after AF.

Normally, the winery depends on spontaneous MLF that can take between 2-4 months.

The sequential inoculated CH11 completed more than a month after inoculation.

Important: Total SO_2 in the juice was less than 20 ppm.

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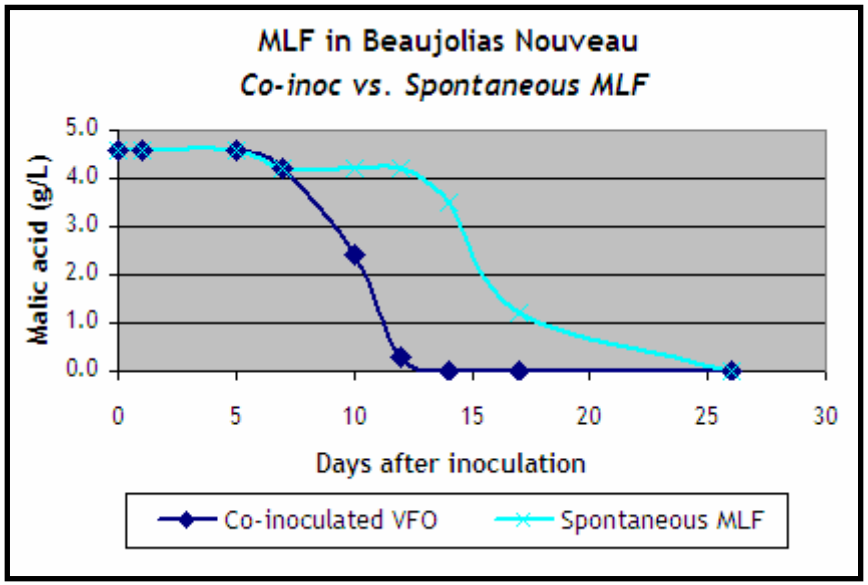
Case 4: red wine Gamay, France

Wine parameters	Protocol	Products
pH: 3.4 Initial Malic Acid: 3.0 g/L	*Early co-inoculation*	MERIT.ferm Viniflora® Oenos®



In Southern Burgundy region of France a very fast process is required in order to produce Beaujolais Nouveau. Early co-inoculation suits this perfectly.

After an initial thermo-vinification stage, Viniflora® Oenos (FroZen™ version) is inoculated into the fermenting must, 24h after the yeast is added at pressing. The MLF completed 14 days after AF.

Both fermentations being complete in two weeks meets both market requirements and appellation rules

Calculate the Return On Investment corresponding with Vinisav-e

Thank you for your attention!

Get the latest update about fermentations management on our web site:

www.chr-hansen.com/wine