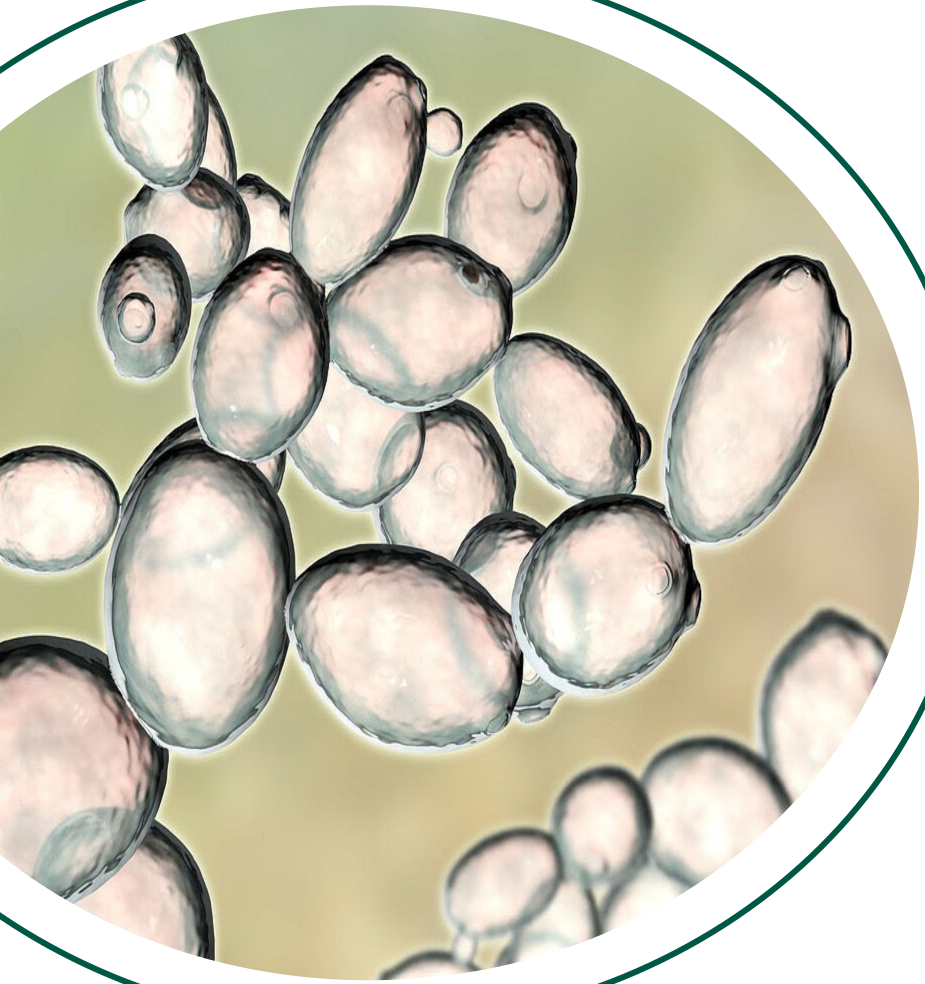


Creating natural freshness and acidity in the wine with LAFFORT®

LAFFORT

l'œnologie par nature

Dichotomy: *Saccharomyces cerevisiae* & non-*Saccharomyces* yeasts

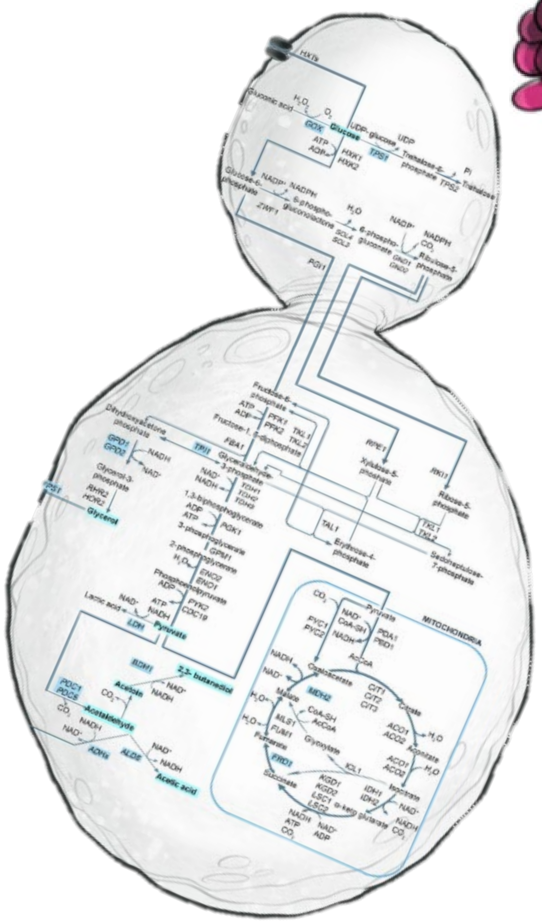
Saccharomyces cerevisiae

- **Main oenological yeast** responsible for alcoholic fermentation
- Rarely isolated from vineyards (1/1000 grapes)

Non-*Saccharomyces* yeasts

- **Heterogenous group** of yeasts predominant on the grapes
- Their presence and **metabolic activity** can affect the **physicochemical and sensory profile** of wines

POSITIVE OR NEGATIVE IMPACT

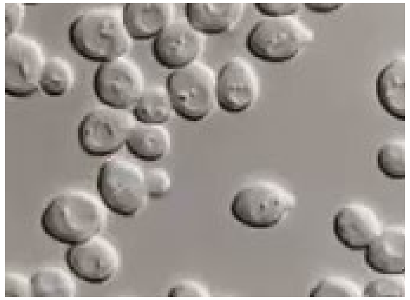


Goold et al., 2017

Non-*Saccharomyces* yeasts: "The Good, the Bad and the Ugly''

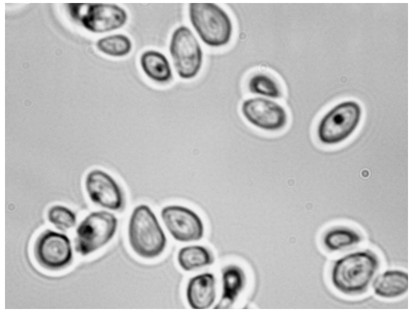
Torulaspora delbureckii

- Aromatic expression
- Bioprotection



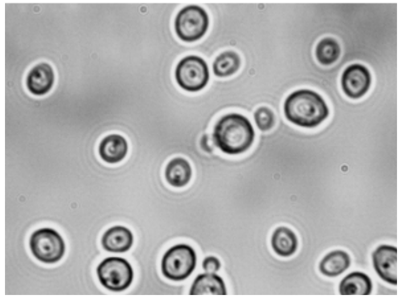
Metschnikowia pulcherrima

- Low ethanol yields
- Bioprotection



Lachancea thermotolerans

- Production of lactic acid
- Low ethanol yields

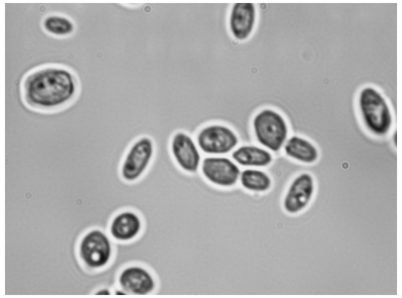


Schizosaccharomyces pombe

- Malo-ethanolic fermentation

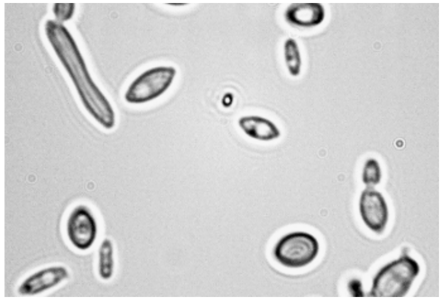
Hanseniaspora uvarum

- Main species on grapes
- Production of VA



Brettanomyces bruxellensis

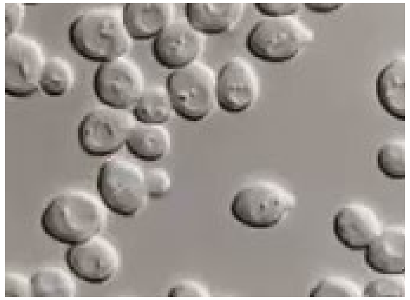
- Spoilage yeast



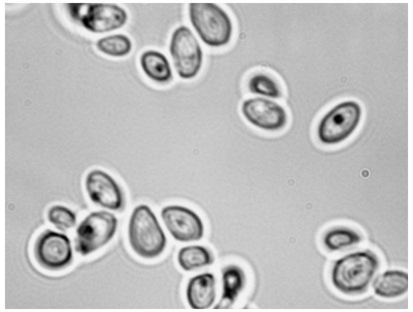
Jolly et al., 2014

Non-*Saccharomyces* yeasts: "The Good, the Bad and the Ugly''

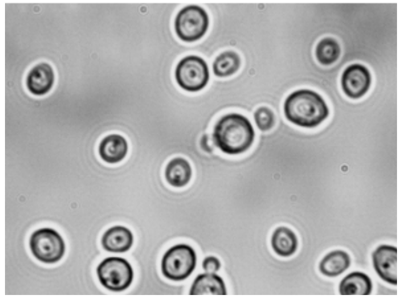
Torulaspora delbureckii
- Aromatic expression
- Bioprotection



Metschnikowia pulcherrima
- Low ethanol yields
- Bioprotection



Lachancea thermotolerans
- Production of lactic acid
- Low ethanol yields



Schizosaccharomyces pombe
- Malo-ethanolic fermentation

Hanseniaspora uvarum
- Main species on grapes
- Production of VA

Brettanomyces bruxellensis
- Spoilage yeast

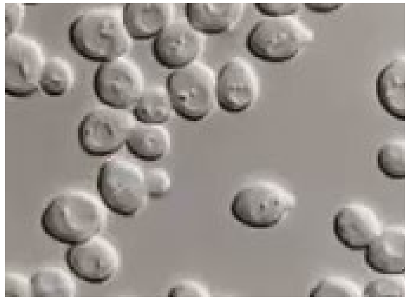
LAFFORT® PORTFOLIO

Jolly et al., 2014

Non-*Saccharomyces* yeasts: "The Good, the Bad and the Ugly''

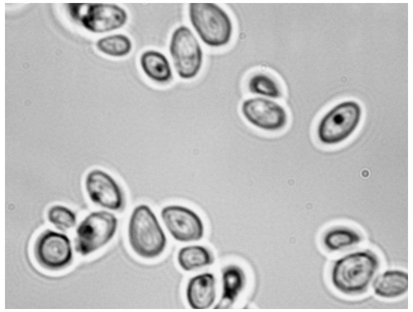
Torulaspora delbureckii

- Aromatic expression
- Bioprotection



Metschnikowia pulcherrima

- Low ethanol yields
- Bioprotection



Lachancea thermotolerans

- Production of lactic acid
- Low ethanol yields

Schizosaccharomyces pombe

- Malo-ethanolic fermentation

Hanseniaspora uvarum

- Main species on grapes
- Production of VA

Brettanomyces bruxellensis

- Spoilage yeast

LAFFORT® BIOPROTECTION

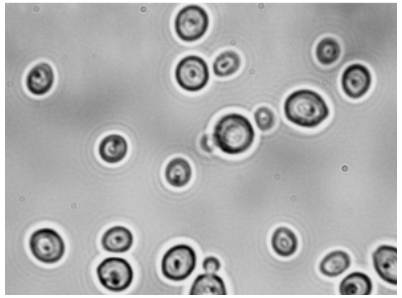
Jolly et al., 2014

Non-*Saccharomyces* yeasts: "The Good, the Bad and the Ugly''

Torulaspora delbureckii
- Aromatic expression
- Bioprotection

Metschnikowia pulcherrima
- Low ethanol yields
- Bioprotection

Lachancea thermotolerans
- Production of lactic acid
- Low ethanol yields



Schizosaccharomyces pombe
- Malo-ethanolic fermentation

Hanseniaspora uvarum
- Main species on grapes
- Production of VA

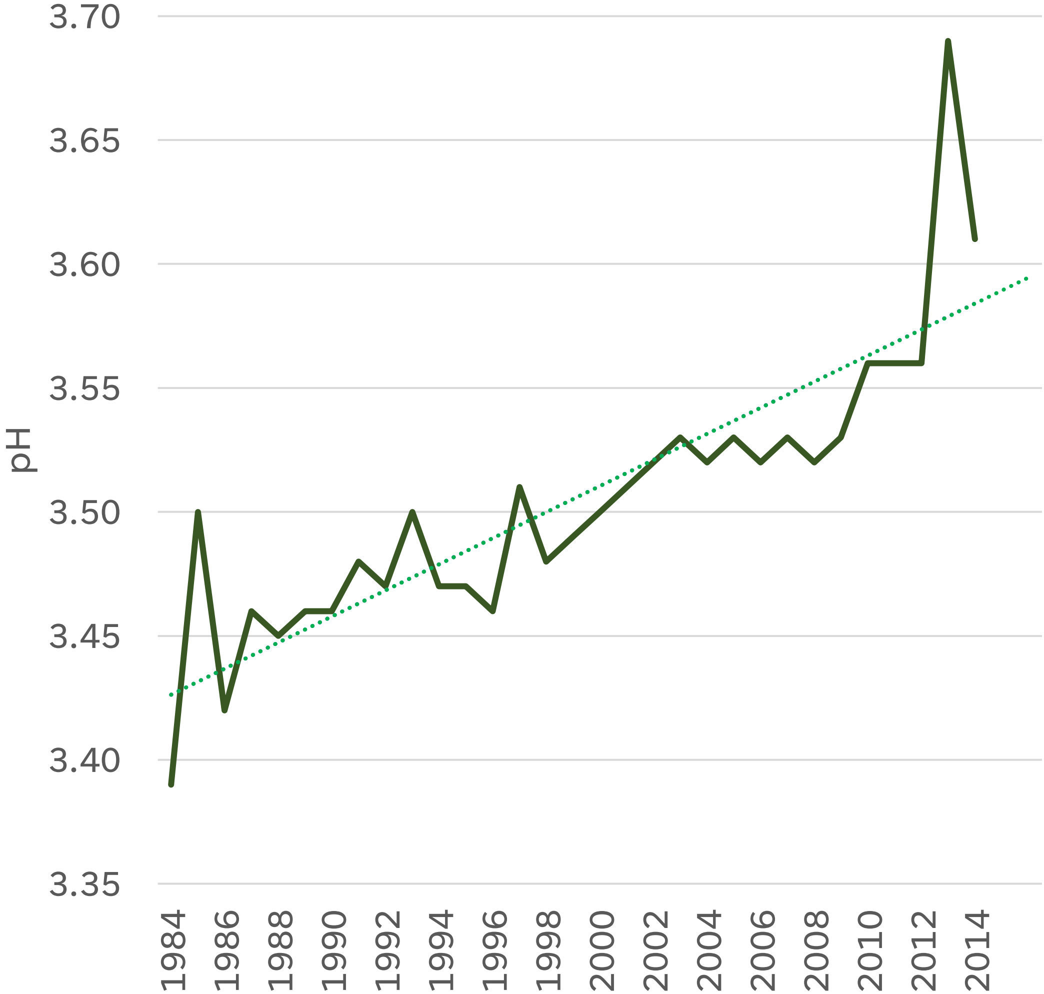
Brettanomyces bruxellensis
- Spoilage yeast

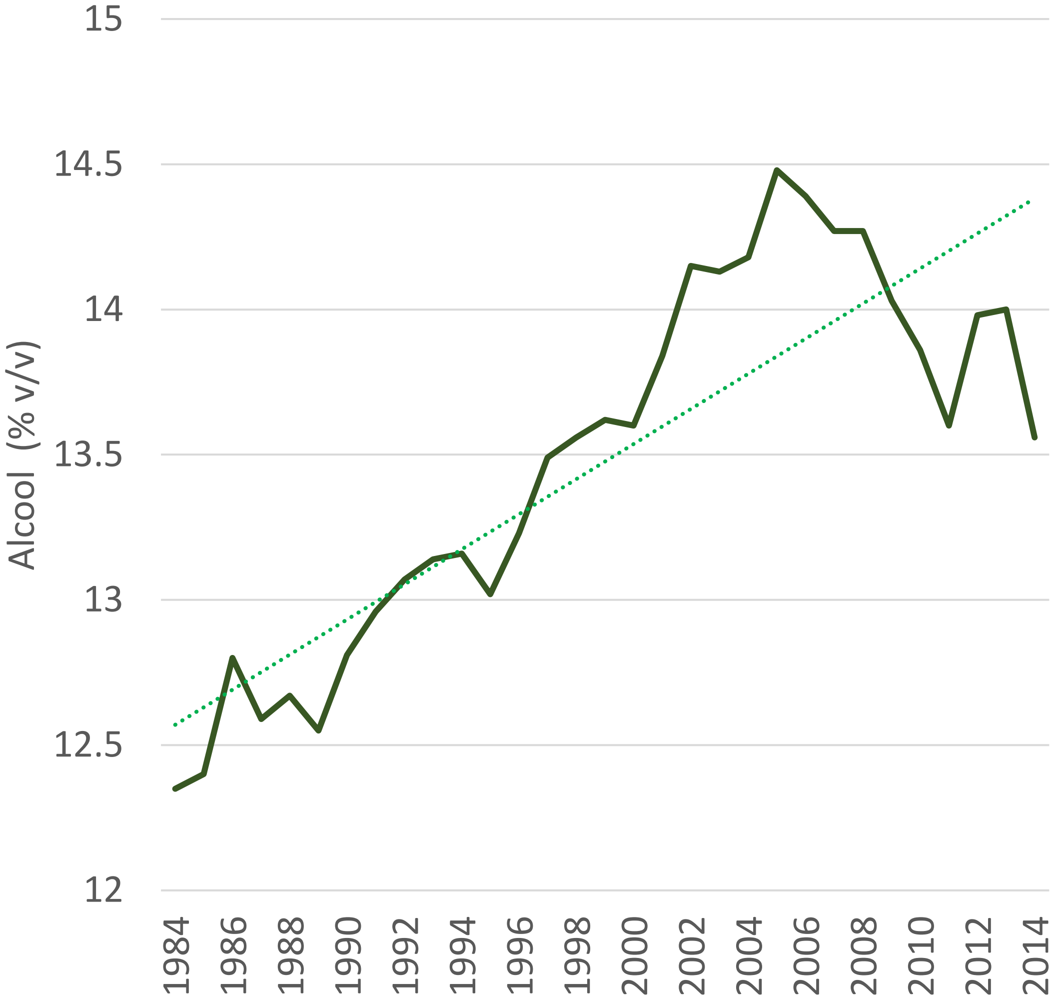
LAFFORT® BIOACIDIFICATION

Jolly et al., 2014

Global tendances of increasing pH and alcohol levels in wines

Example of evolution of pH and alcohol in Australian wines (1984-2014)





Adapted from Godden et al. 2015

Problematic of wines with high pH and alcohol levels

NEGATIVE IMPACT ON:

- Alcoholic and malolactic fermentation
- Physicochemical and sensory profiles
- Microbiological stability
- Ageing potential
- Production costs
- Consumer demand

- **Various solutions** are implemented at different stages of grapes and wines production cycle
- An **acidifying yeast** with low **ethanol yield** is the **"Holy grail"** of oenology

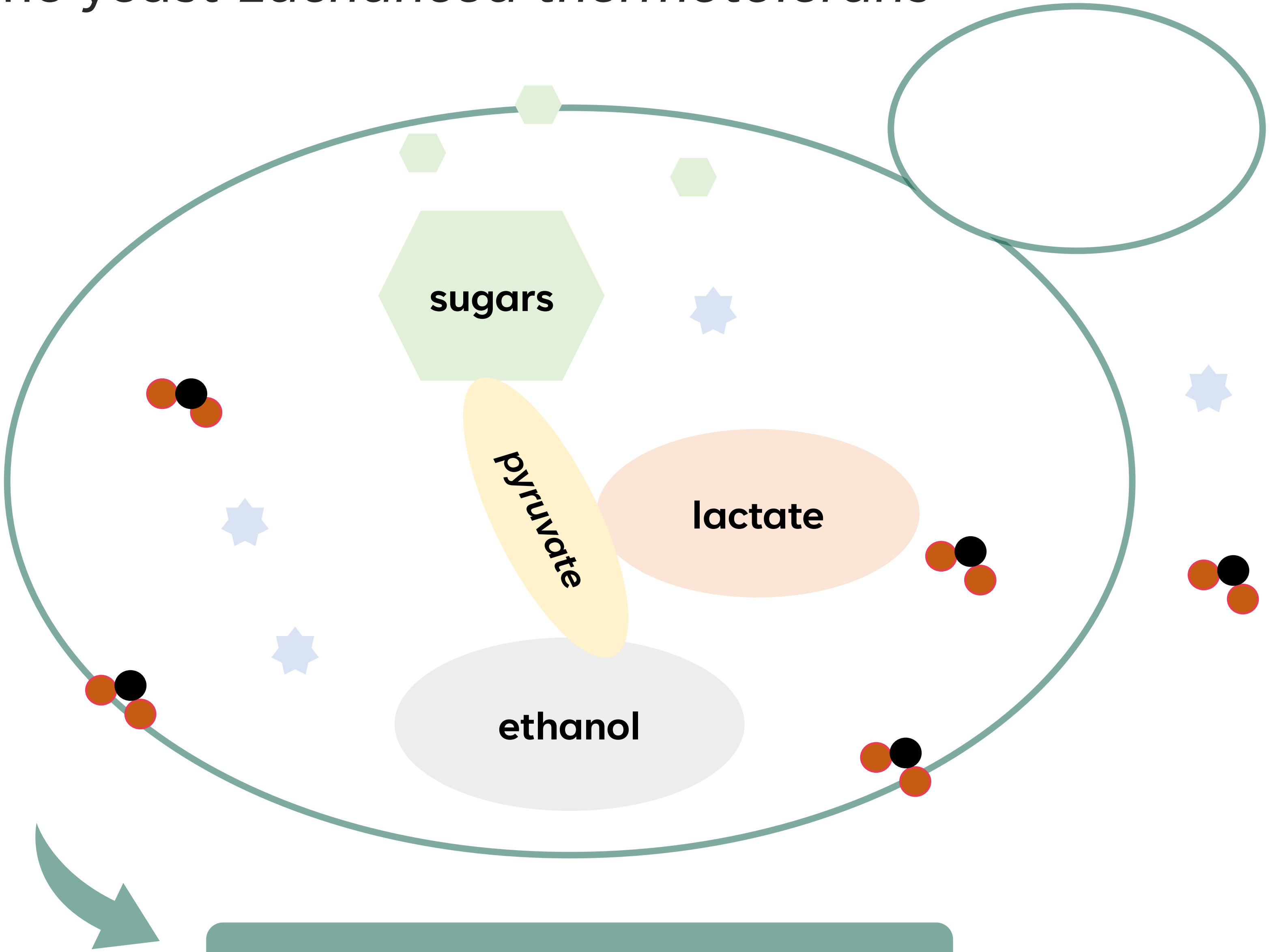
The unique metabolism of the yeast *Lachancea thermotolerans*

Yeast species indigenous to grapes and wine

Fermentative species (< 10 % vol.)

Production of lactic acid

BIOAcidification accompanied by ethanol reduction

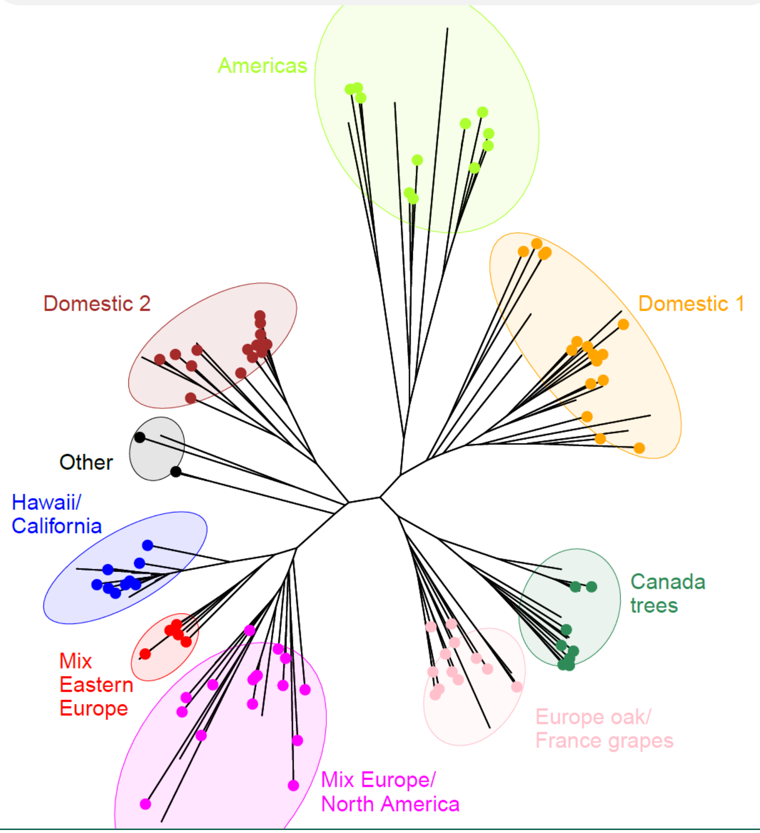


Variation between the strains

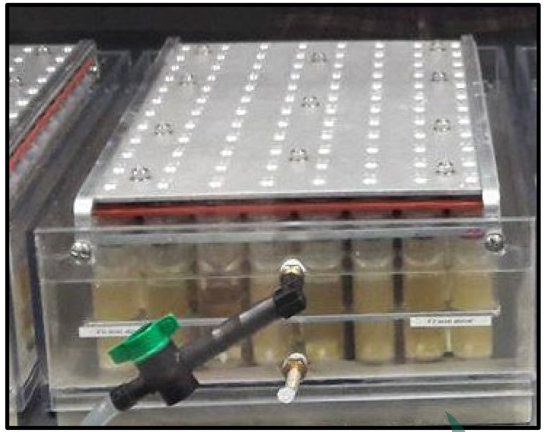
Diversity of *Lachancea thermotolerans*

Genetic characterisation of **~200 strains** from all over the world

Oenological characterisation of **~100 strains** (in triplicates)

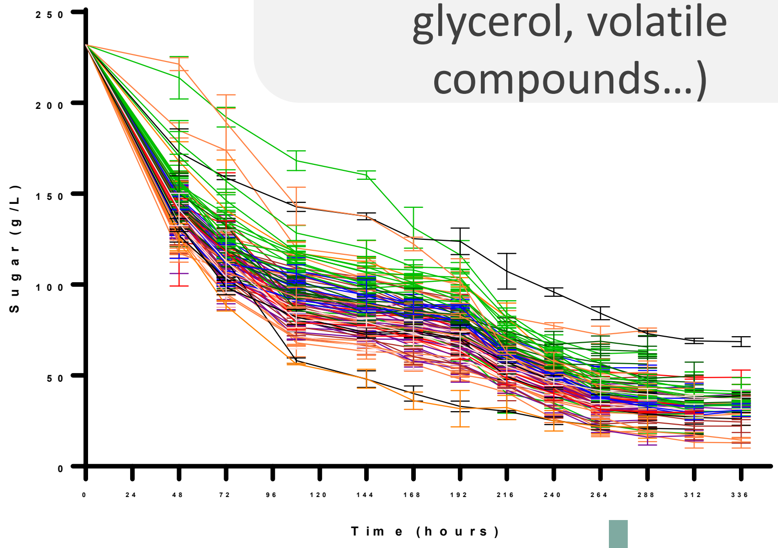


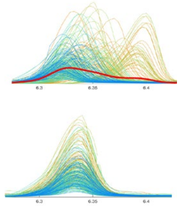
Hranilovic et al. 2017

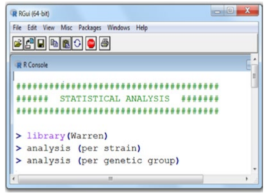


Fermentation trials with individual strains; Chardonnay (sterile) 14 % vol.; pH 3.6

Analysis of **>110 parameters** of each wine (acidity parameters, ethanol, glycerol, volatile compounds…)







Hranilovic et al. 2018

LAFFORT®

Selection of **ZYMAFLORE® OMEGA*LT***

Journal of *Fungi*

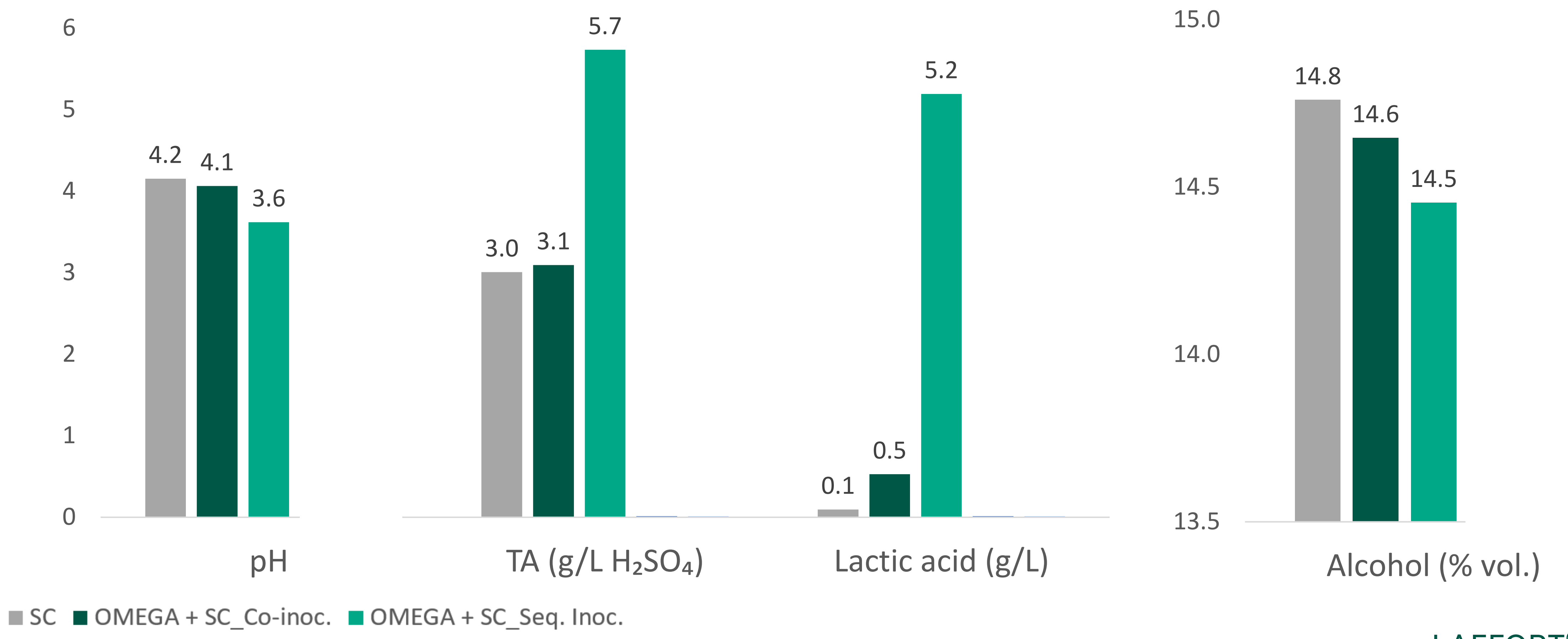
Impact of *Lachancea thermotolerans* on Chemical Composition and Sensory Profiles of Viognier Wines

by Ana Hranilovic [1,2,*], Warren Albertin [1,3], Dimitra L. Capone [2,4], Adelaide Gallo [2,†], Paul R. Grbin [2,4], Lukas Danner [2,‡], Susan E. P. Bastian [2,4], Isabelle Masneuf-Pomarede [1,5], Joana Coulon [6], Marina Bely [1] and Vladimir Jiranek [2,4,*]

Modulation of acidity and ethanol content of wines:

→ **Higher lactic acid** production in **sequential inoculations** than in co-inoculations

- The presence of *S. cerevisiae* inhibits *L. thermotolerans*



LAFFORT®

Viognier. 2019. pH 3.9 ; AF at 18°C/65 °F; averages of triplicates (Hranilovic et al. 2022 ; https://www.mdpi.com/2309-608X/8/5/474)

Parameters that affect the development of **ZYMAFLORE® OMEGA**^{LT}

Favorable parameters

- **Sequential inoculation**
- **High temperature (> 20 °C)**
- **Absence of SO_2**
- **Higher dose**
- Low pressure of native flora
- High pH
- Rehydration with SUPERSTART®
- Other parameters (e.g. nitrogen nutrition, trace elements, oxygen...)

Limiting parameters

- **Co-inoculation**
- **Low temperature (< 18°C)**
- **Presence of SO_2**
- **Lower dose**
- Strong pressure of native flora
- Low pH
- Rehydration without SUPERSTART®
- Other parameters (e.g. nitrogen nutrition, trace elements, oxygen...)

The advantages of **BIOAcidification** are numerous:

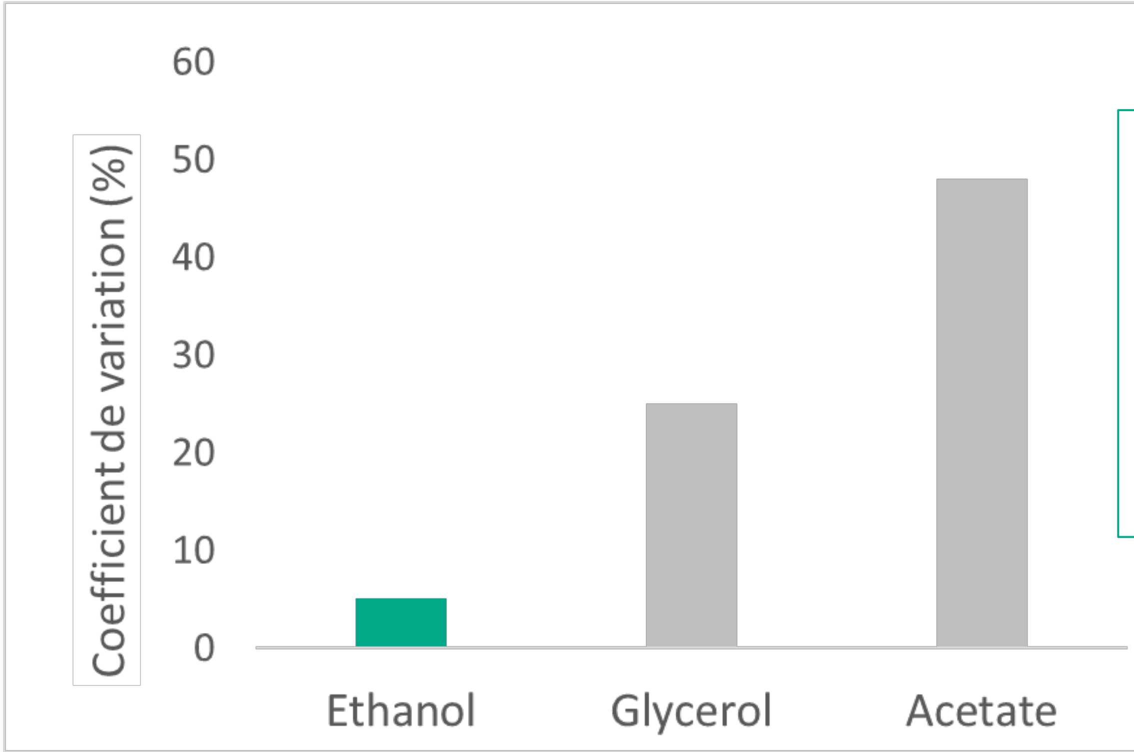
BIOAcidification allows:

- Decrease of pH / increase of TA
- Slight ethanol reduction
- Accentuation of aromatic freshness
- Microbial stabilization
- Increased aging potential
- Correction of acidity without labeling

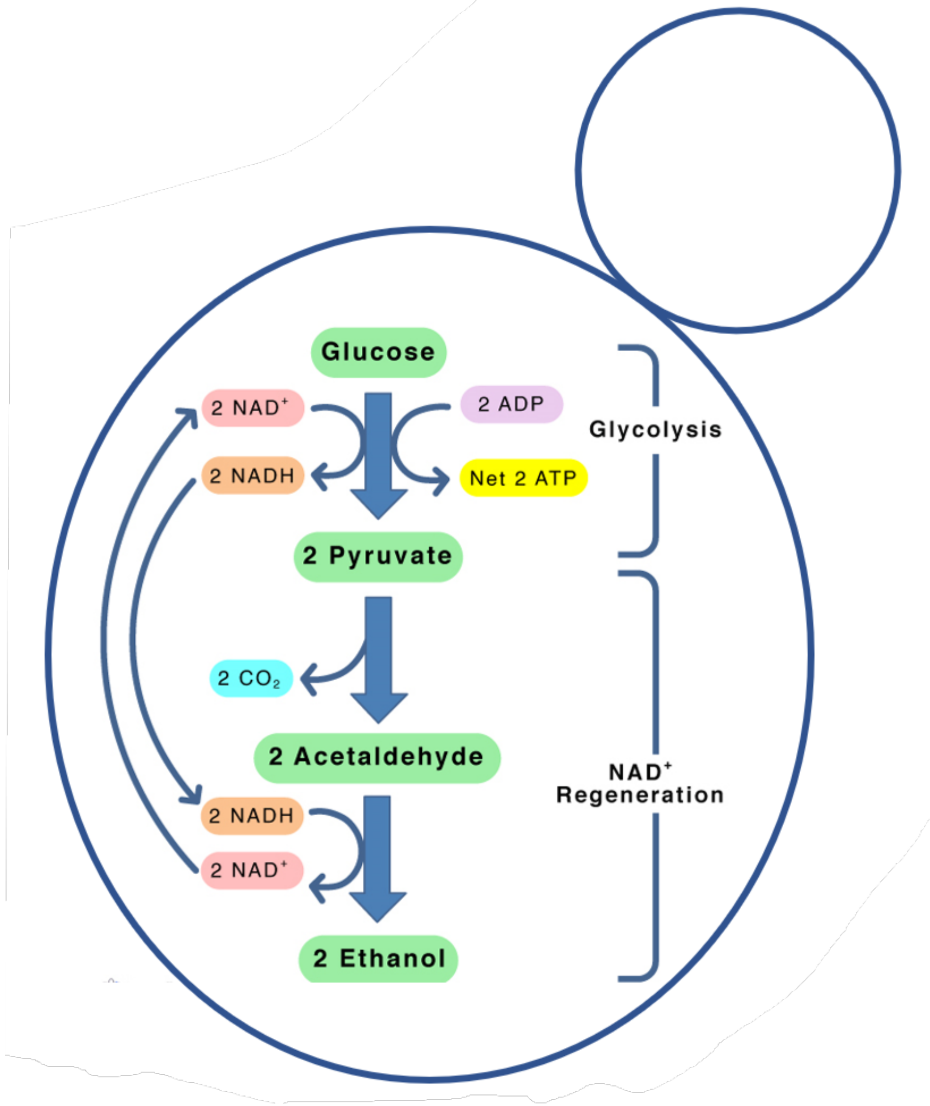
Can ***Saccharomyces cerevisiae*** strains affect the acidity and ethanol levels in wines?

Impact of *Saccharomyces cerevisiae* strains on **alcohol content**

- **Efficient transformation of sugars to ethanol:**
  → 1 g of sugars generally yields 0.47 g of ethanol (>90 % of the theoretical yield of AF; the rest goes to glycerol, biomass etc.)
- **Little variation between the strains**



Variation of 51 strains of S. cerevisiae (AF in the synthetic must) in the production ethanol, glycerol and acetate.



Impact of *Saccharomyces cerevisiae* strains on **wine acidity**

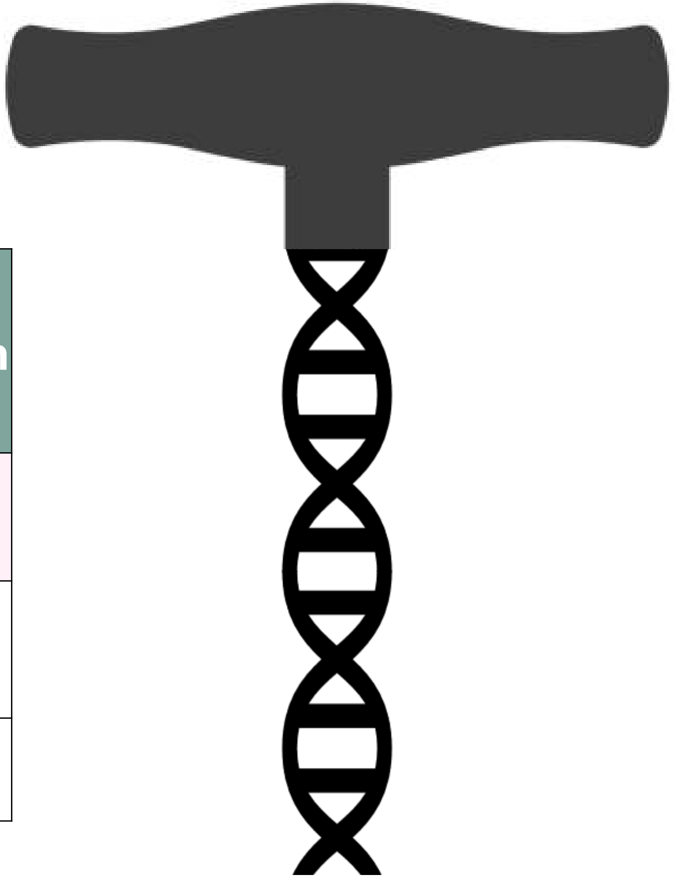
- **Lactic acid** is **not produced** by *S. cerevisiae*
- **Malic acid** is generally **partially consumed** during AF

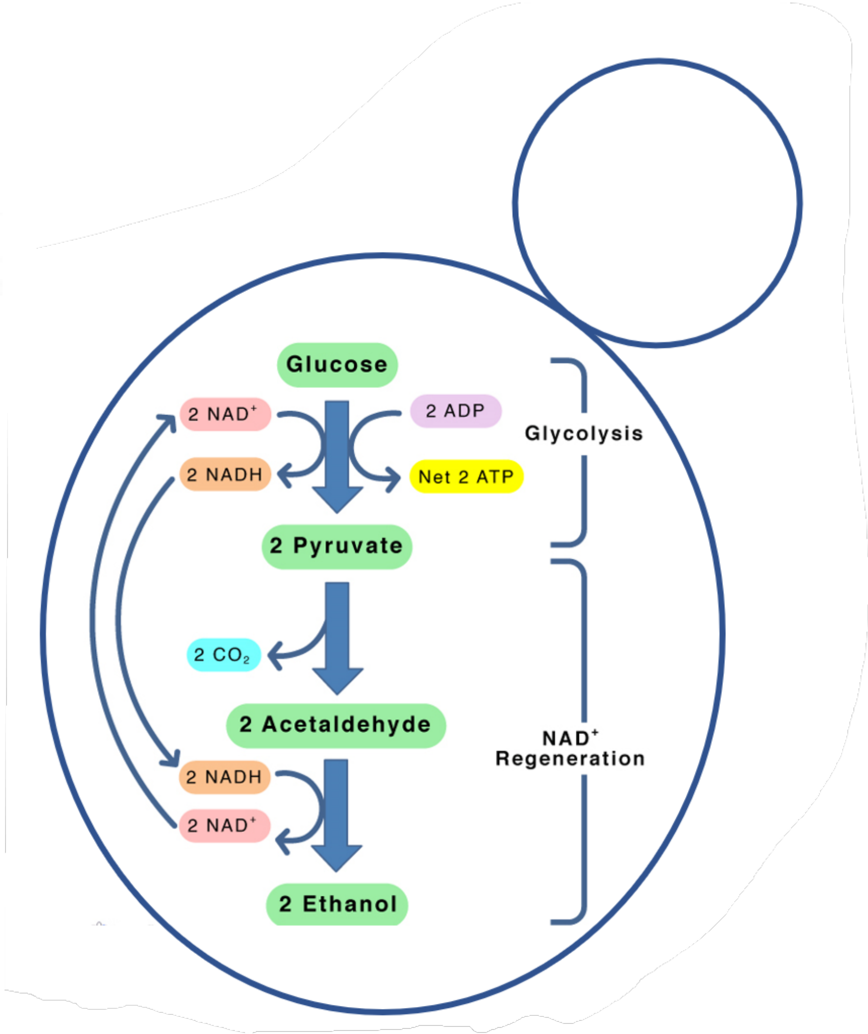
	Alcohol (% vol.)	Acetic acid (g/L)	TA (g/L H_2T)	pH	Lactic acid (g/L)	Malic acid (g/L)	Malic acid consumption (MAC %)
Rosé juice	/	/	4.8	3.58	/	3.57	
BO213	12.79	0.27	5.5	3.63	0	**2.52**	+30 %
X16	12.74	0.20	6.0	3.57	0	**3.03**	+15 %

Most *Saccharomyces cerevisiae* strains ferment efficiently and partially degrade malic acid

→ Expertise LAFFORT®

	Malic acid (g/L)	Malic acid consumption (MAC %)
Rosé juice	3.57	
BO213	**2.52**	+30 %
X16	**3.03**	+15 %





ZYMAFLORE® KLIMA: Experimental results in whites

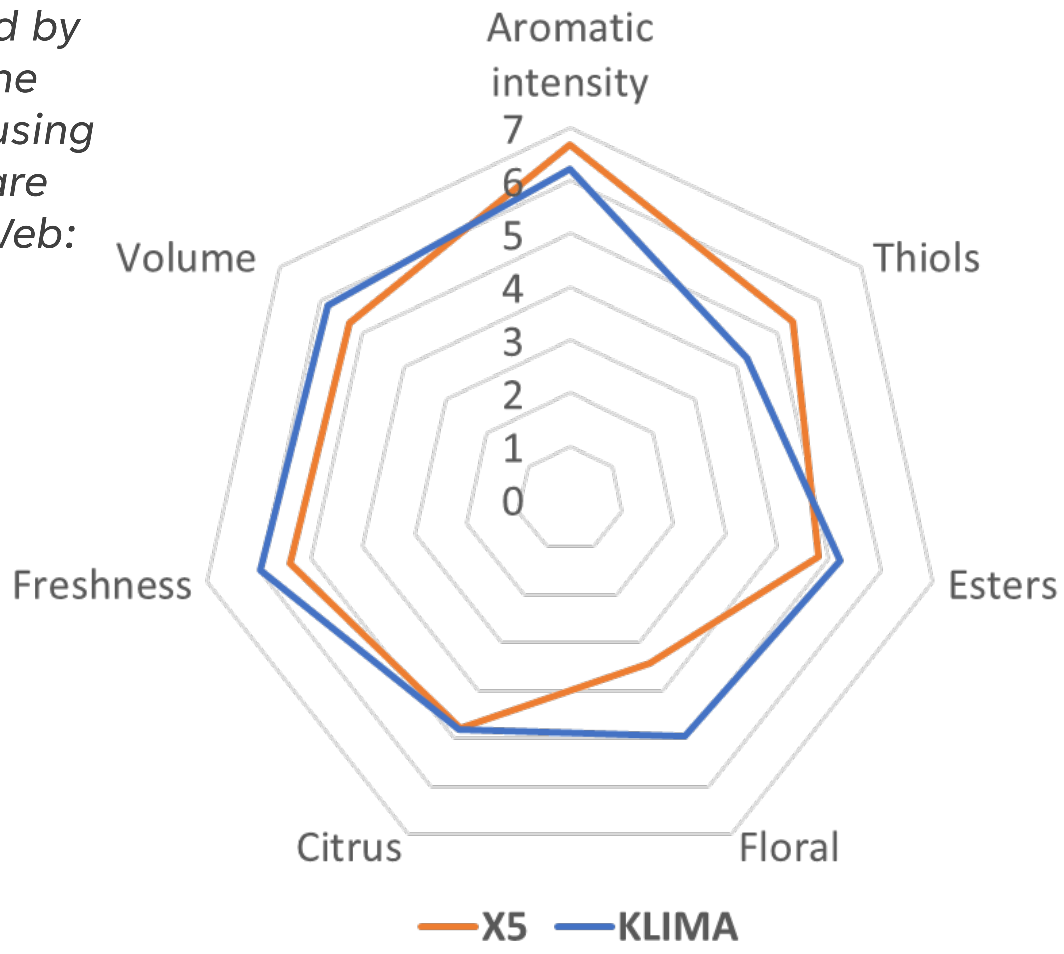
Sauvignon blanc, Bordeaux, 2022

- pH 3.4; malic acid 1.7 g/L
- 150 NTU; YAN 158 mg/L;**THIAZOTE® PH**
- Inoculation at 14°C, AF 16 - 18°C
- **X5** & **KLIMA** 20 g/hL + **SUPERSTART® BLANC**

Analytical profiles of wines:

Parameters	**X5**	**KLIMA**
Alcohol (% vol)	12.74	**12.44**
pH	3.31	3.26
TA (g/L H_2T)	5.94	**6.64**
Acetic acid (g/L)	0.36	**0.17**
Malic acid (g/L)	1.46	**1.86**
Glycerol (g/L)	5.84	**7.69**
SO_2 T (mg/L)	17	**<LQ**
TL35 (mg/L)	102	92

Sensory profile obtained by 12 wine experts using software Tastel Web:



ZYMAFLORE® KLIMA: Experimental results in reds

Merlot, WEC, 2022

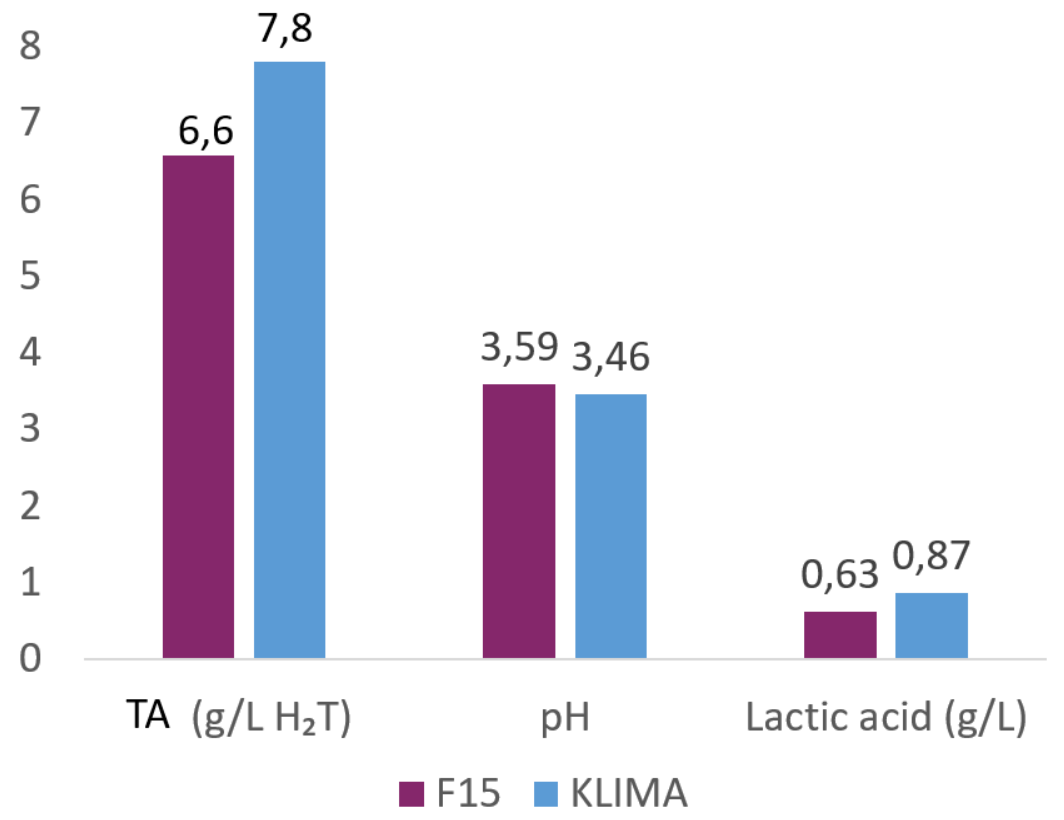
- pH 3.5; malic acid 1.1 g/L
- YAN 122 mg/L, **THIAZOTE® PH**
- Inoculation at 20 °C; AF 24-26 °C
- F15 & KLIMA 20 g/hL + **SUPERSTART® ROUGE**

Analytical profiles post-FA:

Paramètres	F15	KLIMA
Alcohol (% vol)	13.62	**13.14**
pH	3.48	**3.41**
TA (g/L H_2SO_4)	7.2	**8.9**
Acetic acid (g/L)	0.27	0.16
Malic acid (g/L)	1.19	**1.59**
Glycerol (g/L)	9.7	9.7
SO_2 T (mg/L)	<LQ	<LQ
TL35 (mg/L)	79	**61**

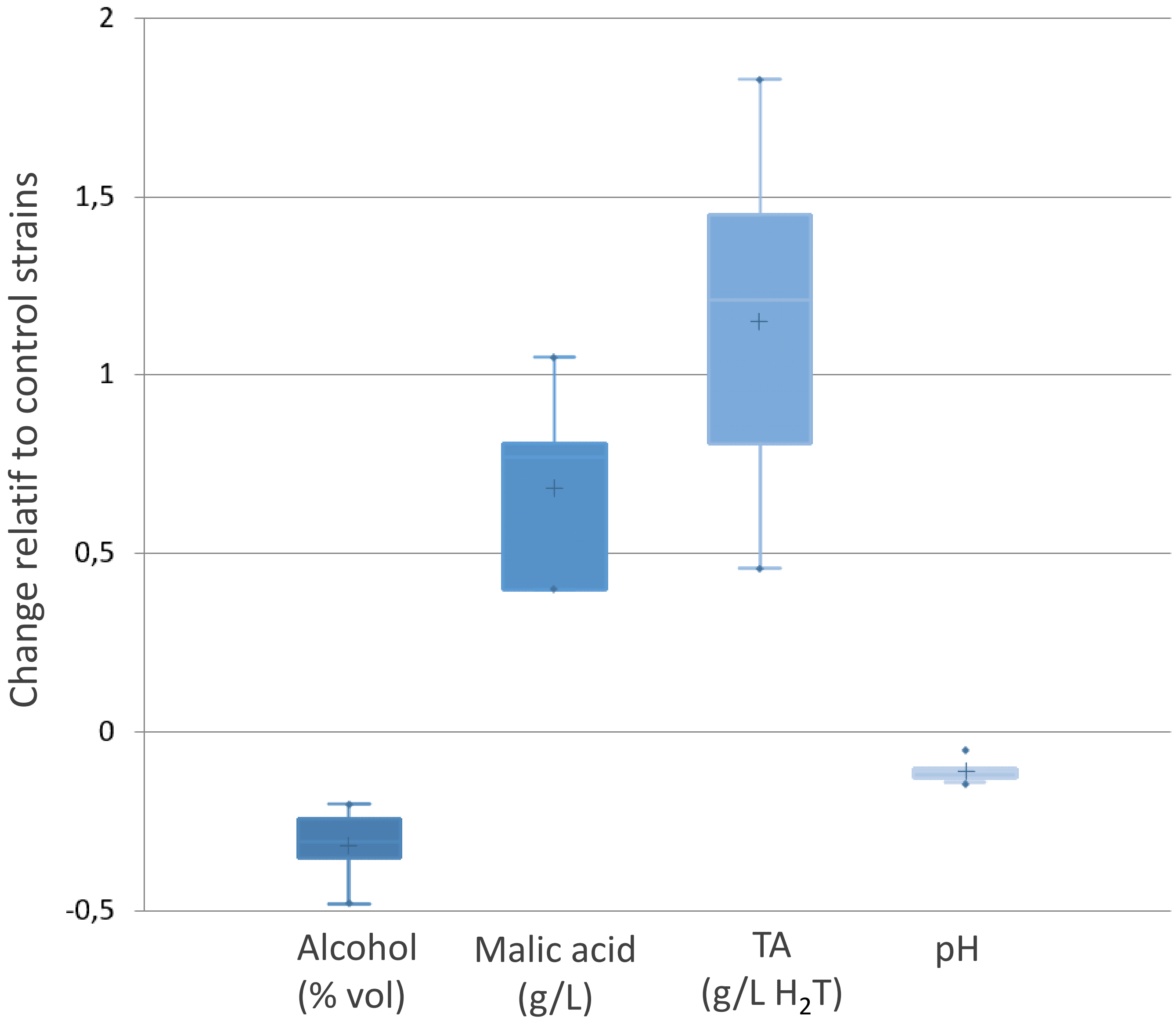
Acidity parameters post-MLF

→ higher lactic acid content using KLIMA



ZYMAFLORE® KLIMA: Cummulative results of winemaking trials

- Relative to the control *S. cerevisiae* strains, **ZYMAFLORE® KLIMA** allows:
  - **Decreases of alcohol** content ranging between **0.3–0.5 % vol.**
    → **maximum of the species**
  - **Increases of malic acid** post AF and/or lactic acid post-MLF
  - Increases of TA (~1 g/L H_2T) and decreases of pH (~ 0.1 units)



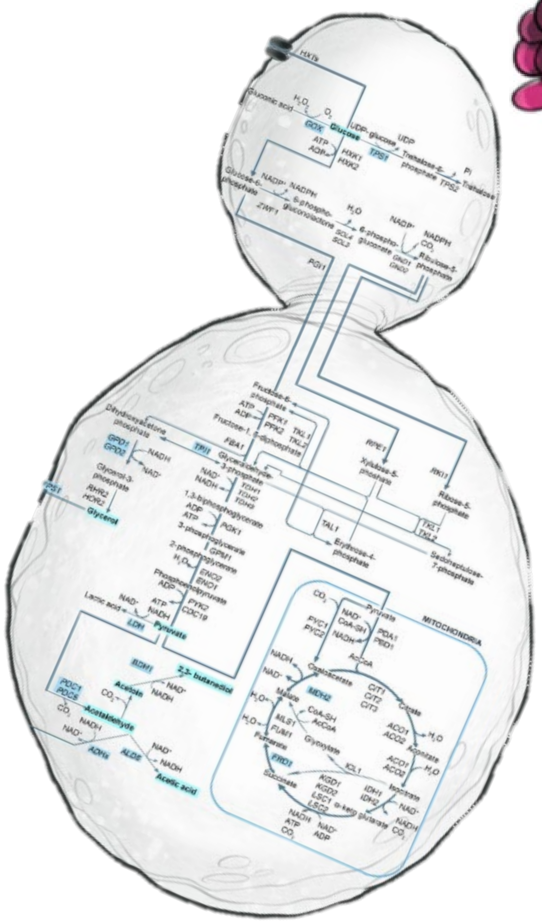
Oenococcus oeni

Saccharomyces cerevisiae

- **Main oenological yeast** responsible for alcoholic fermentation
- Rarely isolated from vineyards (1/1000 grapes)

Non-*Saccharomyces* yeasts

- **Heterogenous group** of yeasts predominant on the grapes
- Their presence and **metabolic activity** can affect the **physicochemical and sensory profile** of wines



Oenococcus oeni
Heterogenous species of lactic acid bacteria most frequently associated with malolactic fermentation (MLF) in wine.

Goold et al., 2017

Oenococcus oeni: main species responsible for MLF

- **The role of MLF :**

  1. **Diminution of acidity**

     → conversion of a strong diacide to a weak monoacide

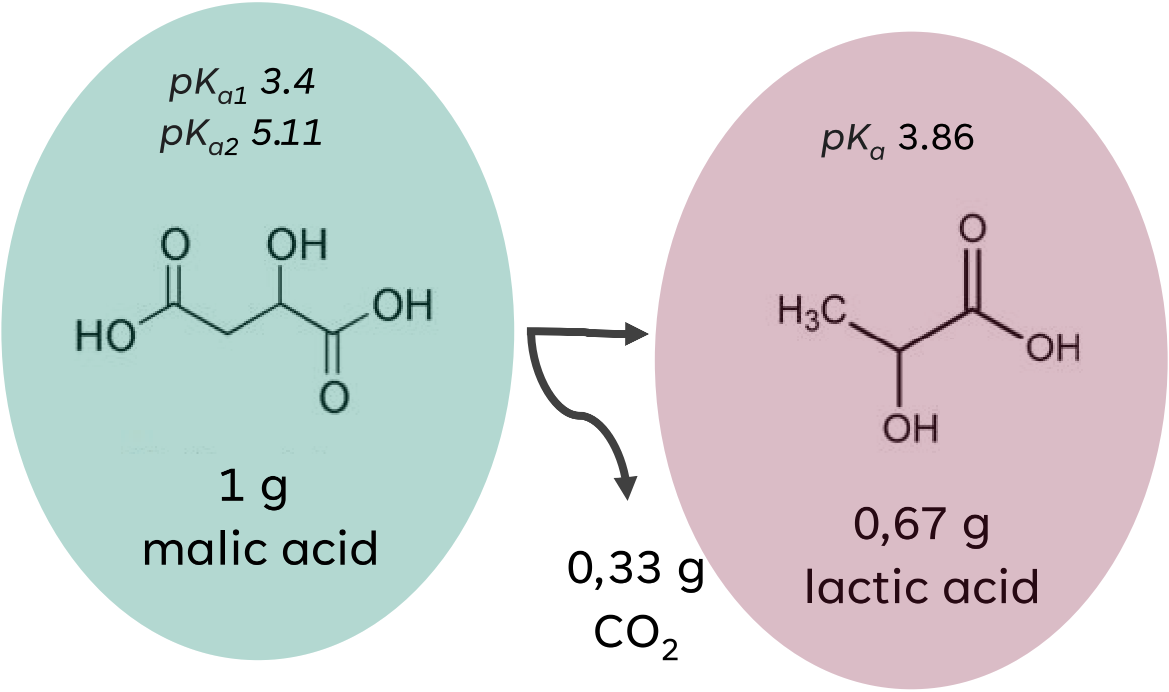
  2. **Microbiological stabilization**

     → malic acid is microbially unstable; lactic acid is stable

     → depletion of nutrients

  3. **Sensory modulation**

     → besides acidity modulation, impact on the aromatic profile of wines (e.g., production of diacetyl, esterase and glycosidase activities etc.)

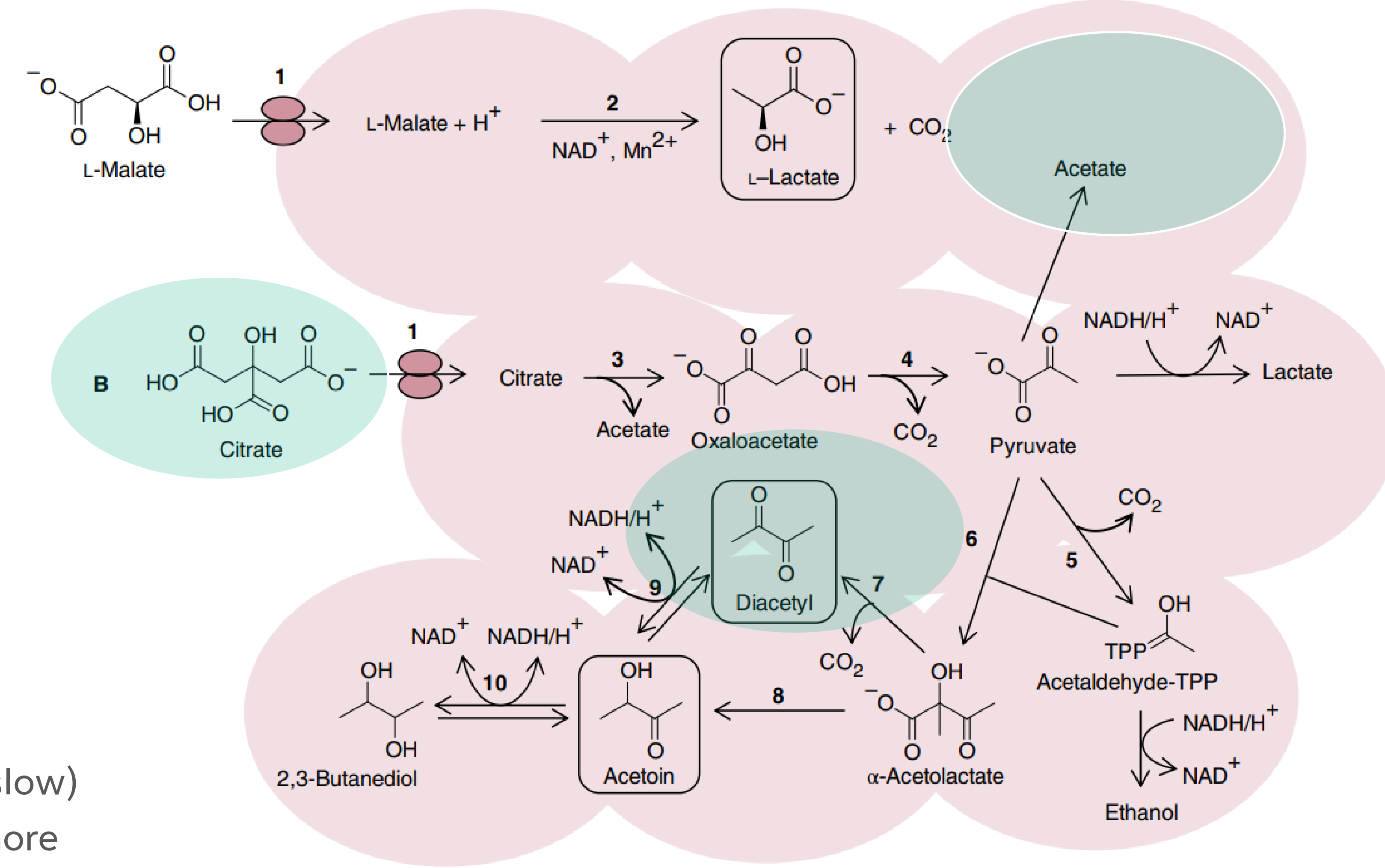


Lower pK_a values indicate stronger acids

Citric acid metabolism: variability between the strains of *O. oeni*

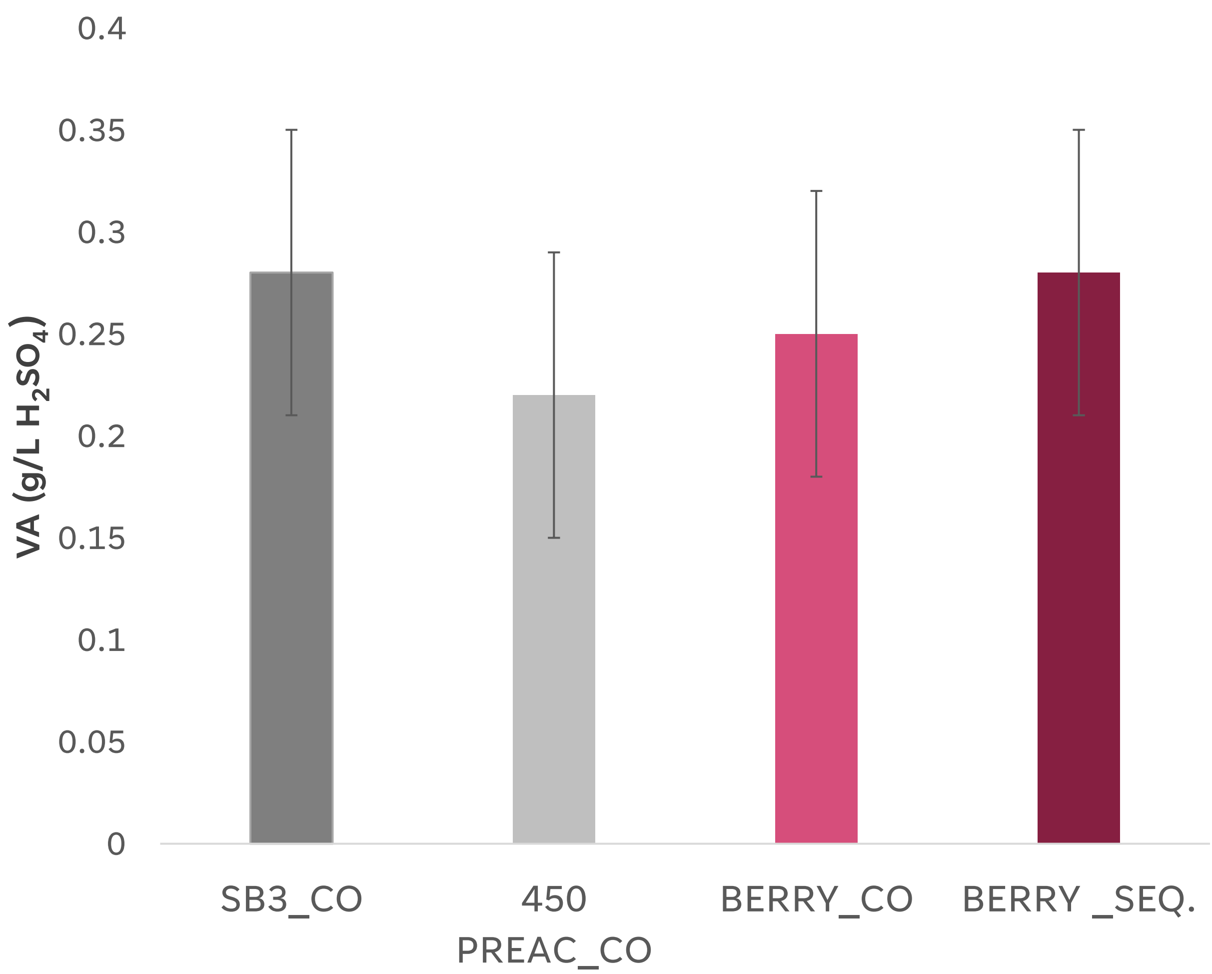
- **Malic acid metabolism**

- **Citric acid metabolism**
  - **Citrate negative strains (CIT-)**
    →Absence of genes involved in consumption of citric acid
  - **Citrate positive strains (CIT+)**
    →Differences in citric acid **consumption kinetics** (fast vs slow)
    →Slow citric acid metabolism: more citric acid, higher TA and lower diacetyl post-MLF



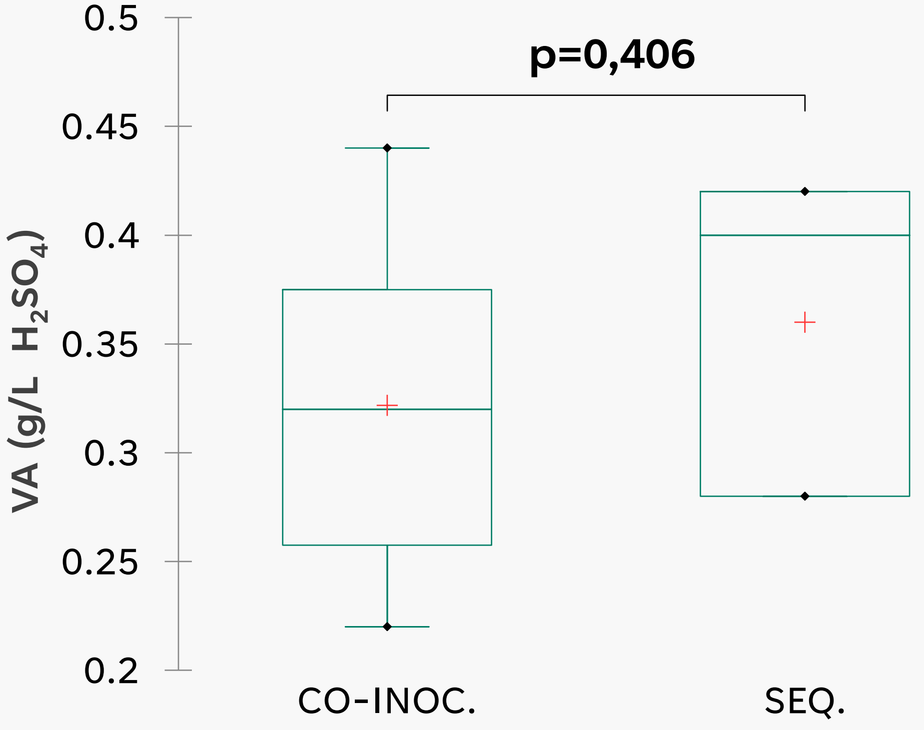
Waterhouse et al, 2016

Co-inoculation of *O. oeni* does not induce increases in VA



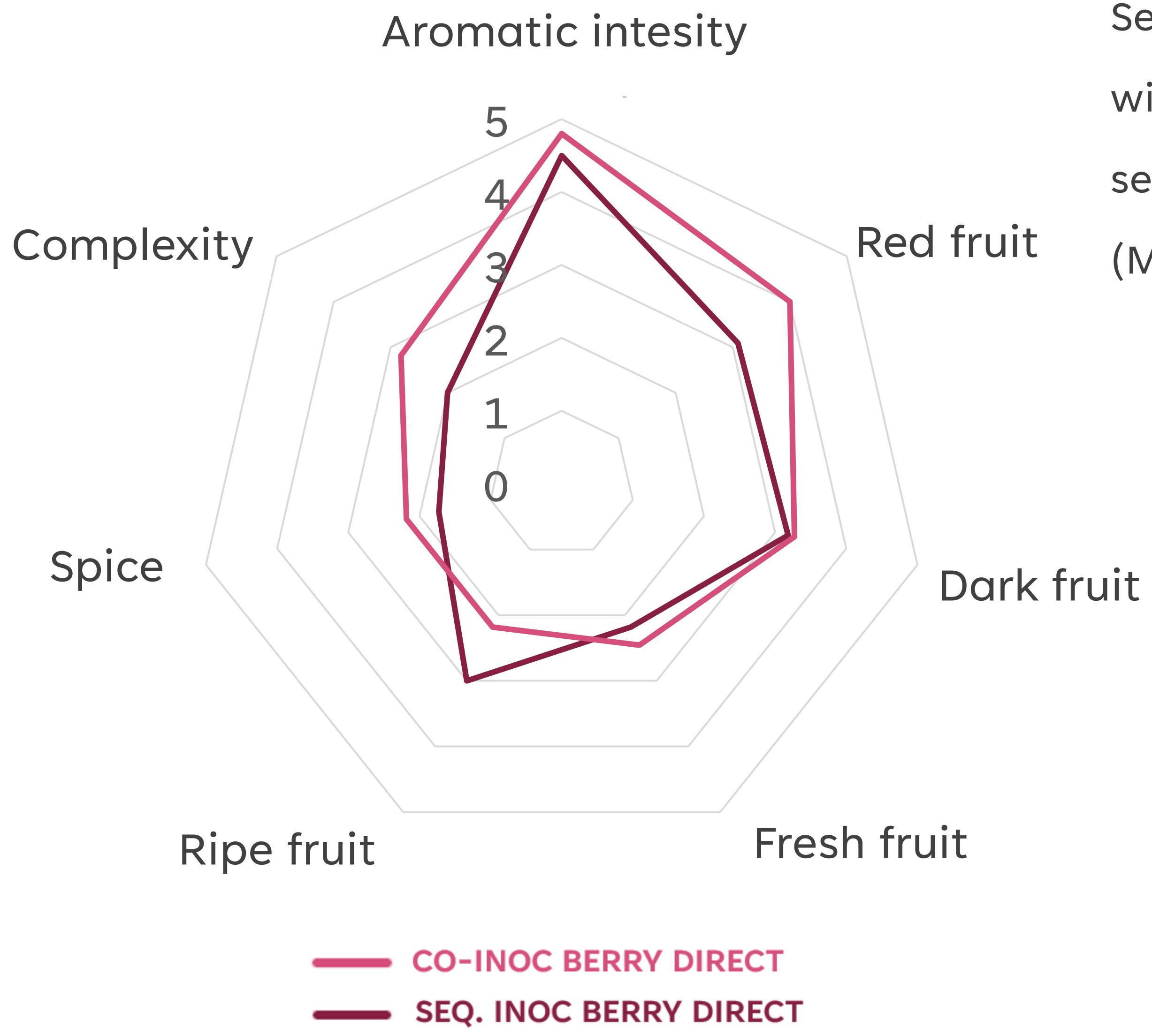
VA of finished wines (Merlot, Bordeaux, 2022)

Regardless of the strain, co-inoculation with bacteria from the **LACTOENOS®** range does not result in an increase in VA :

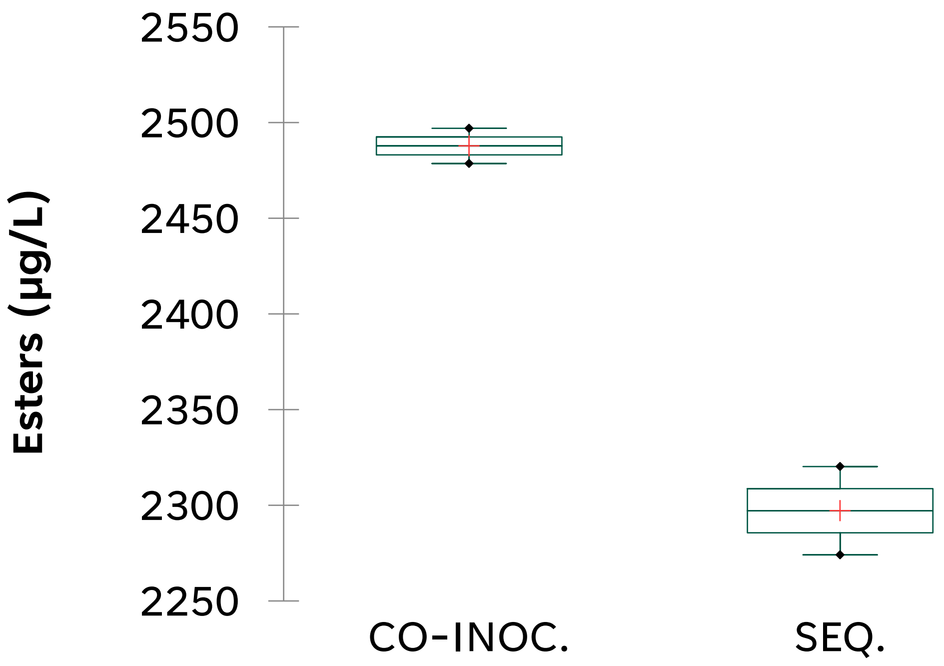


Cumulative results from multiple trials

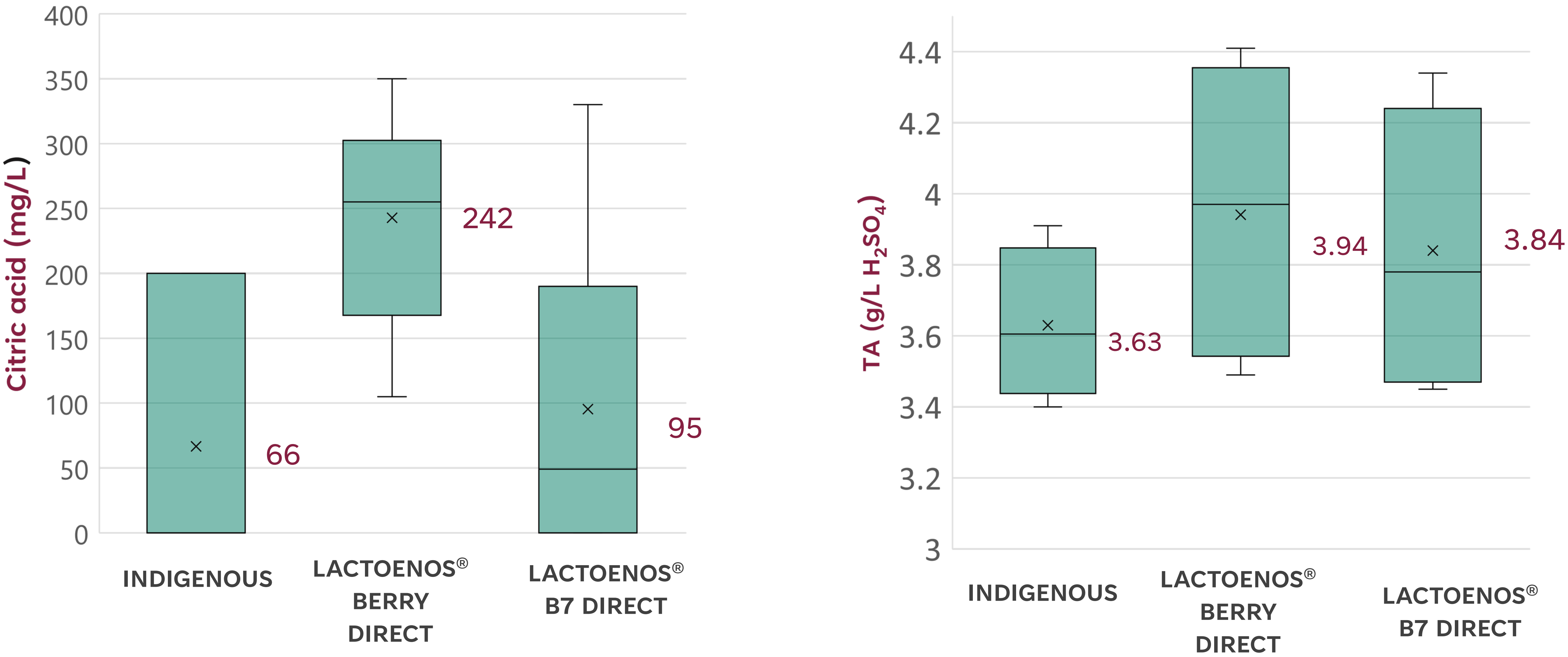
Co-inoculation is suitable for elaboration of fresh and flavorful wines



Sensory profiles and concentrations of esters in wines mede with **ZYMAFLORE FX10®** and either co-inoculated or sequentially inoculated **LACTOENOS® BERRY DIRECT** (Merlot, Bordeaux, 2022).



Regardless of the inoculate type, **LACTOENOS® BERRY DIRECT** preserves >80% of citric acid

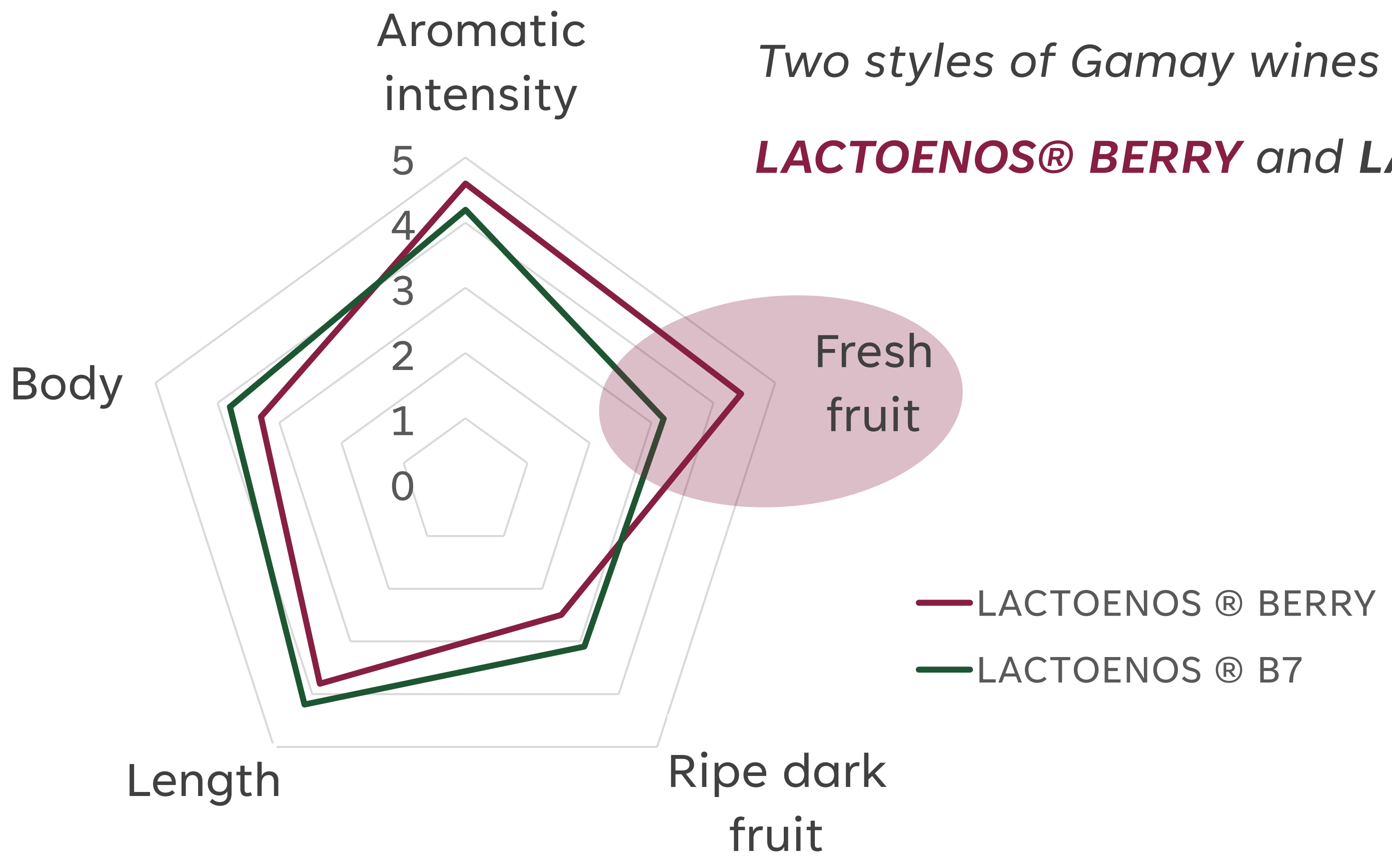


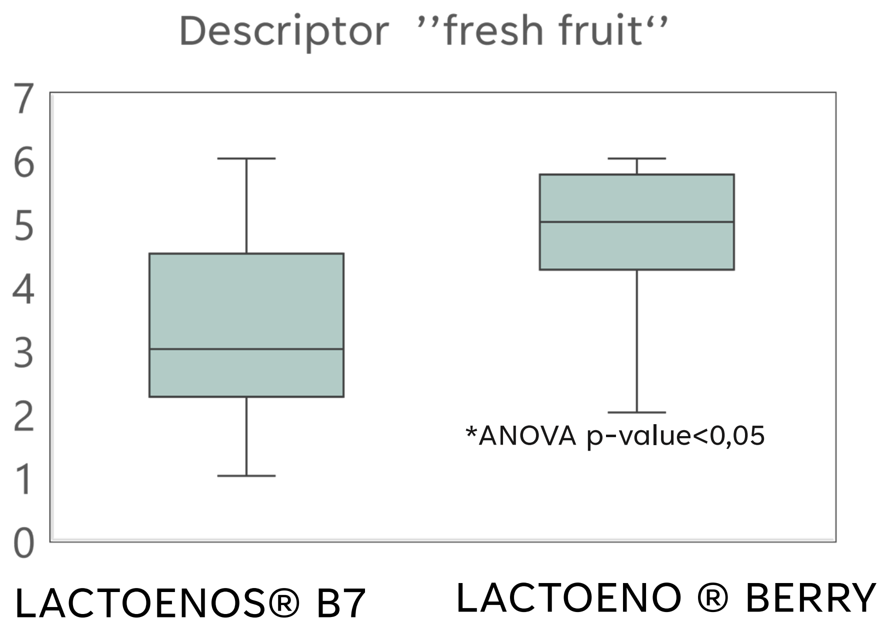
Citric acid and TA post-MLF: Cumulative results of 8 field trials

LACTOENOS® BERRY DIRECT Accentuates freshness and aromatic intensity

Two styles of Gamay wines inoculated with

LACTOENOS® BERRY** and **LACTOENOS® B7



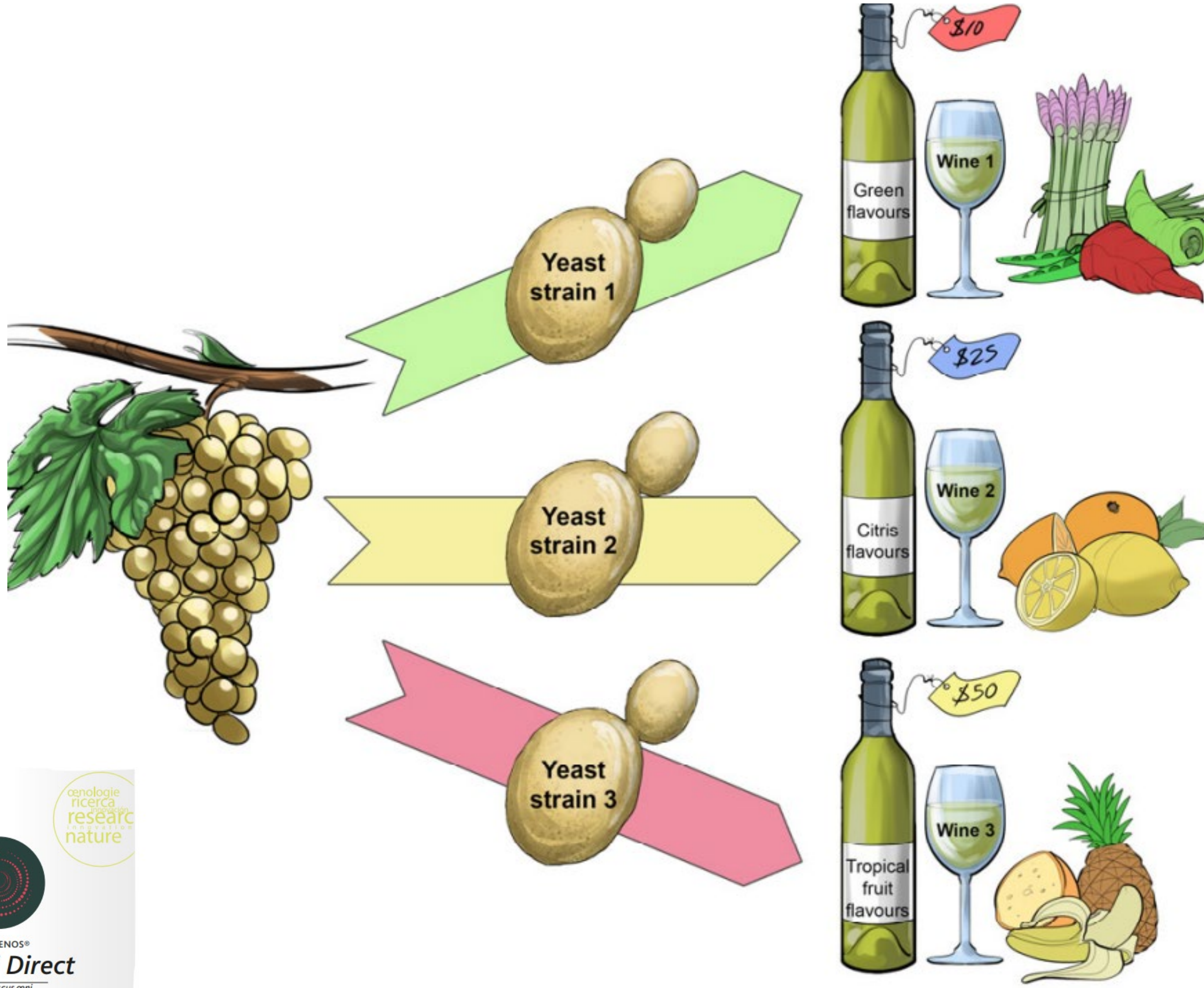


LACTOENOS® BERRY enhances ''fresh fruit'' character: FRESHNESS AND AROMATIC INTESITY

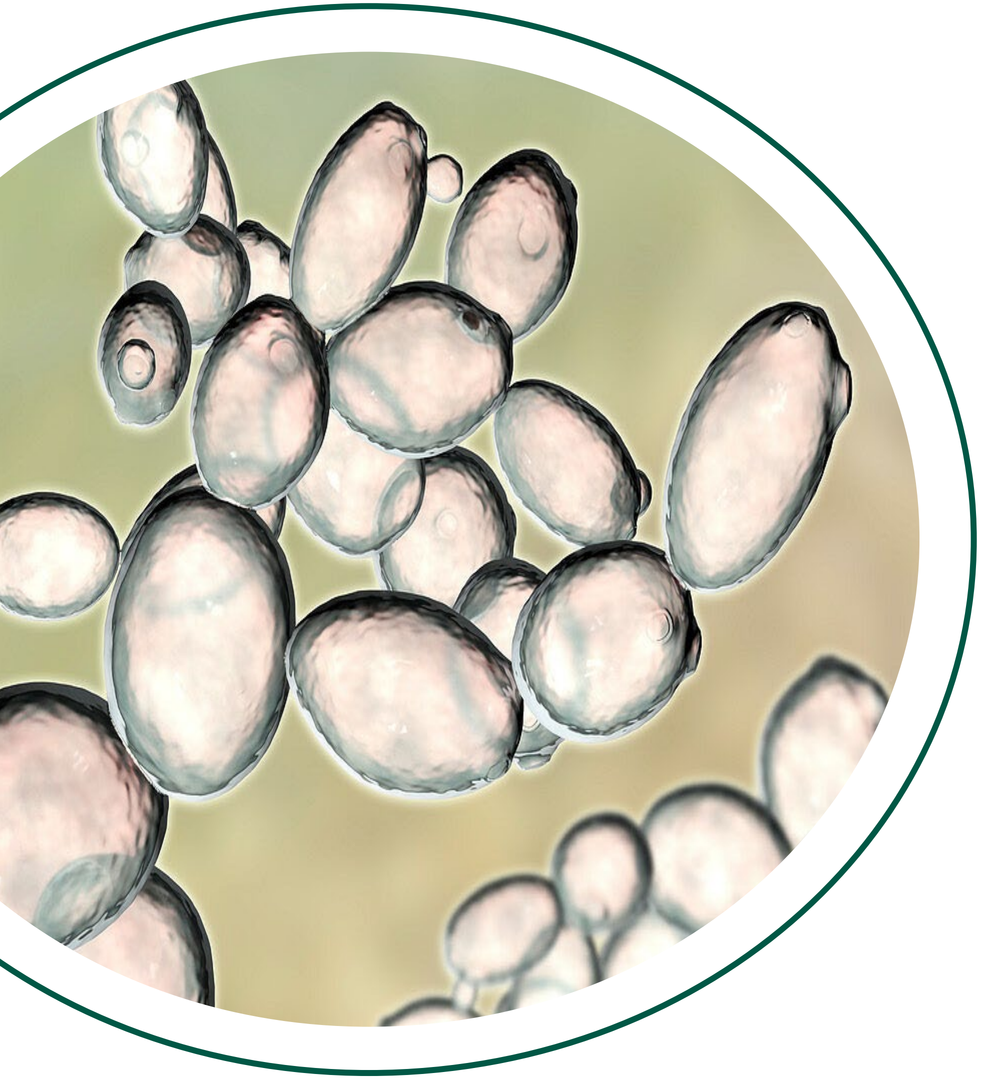
LACTOENOS® B7 favorizes ''ripe dark fruit'' : COMPLEXITY

Gamay; analytical profile post-AF: TAP 12.2 % alc, TA 6.3 g/L H_2T, pH 3,23, Malic acid 1.32 g/L

Innovative solutions for wine microbiology



Pretorius 2016



Thank you for your attention!

LAFFORT

l'œnologie par nature