BIOPROTECTION How to use this living technology

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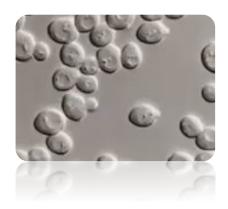






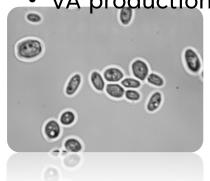
Torulaspora delbrueckii

- Aroma and mouthfeel
- **BIO**Protection



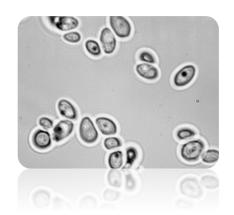
Hanseniaspora uvarum

- Common on grapes
- VA production



Metschnikowia pulcherrima

- BIOProtection
- Lower ethanol production



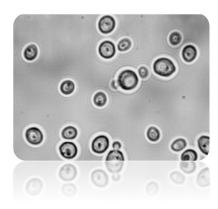
Brettanomyces bruxellensis

Spoilage yeast



Lachancea thermotolerans

- Lactic acid production
- Lower ethanol production



Schizosaccharomyces pombe

Malic acid degradation



1. BIOProtection

2. Aromatic optimization

3. BIO Acidification

LAFFORT® BIOProtection

Use of microorganisms during pre-fermentative phases to:

Alternative to the antiseptic role of SO₂

Alternative for the antioxidative role of SO₂

- Colonize the medium
- Limit the development of the indigenous microflora
- Consume dissolved oxygen

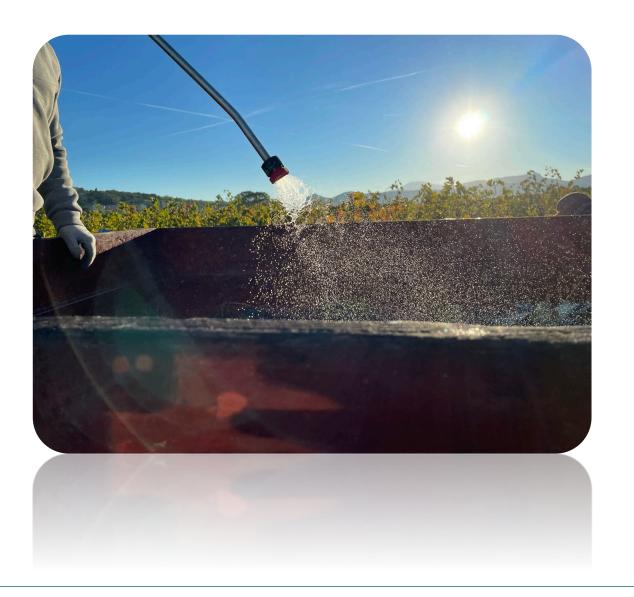
Combine advantages of both species in very different inoculation situations...

ZYMAFLORE® ÉGIDE^{TDMP}

- 50 % M. pulcherrima
- 50 % T. delbrueckii

TD	MP
Less sensitive to SO ₂	Weak fermentative activity
Implantation capacity	Robustness when not rehydrated
Robustness to cold ++	Robustness to cold +++

Spraying **ZYMAFLORE**® **EGIDE**^{TDMP} on materials in contact with grapes.



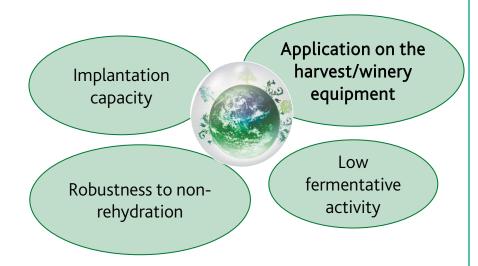
Key points to remember:

- Early contact of grapes with the selected non-Saccharomyces yeasts.
- Minimize the potential development of undesirable flora in hidden zone (juice/organic material)
- Easy to implement.

BIOProtection LAFFORT®

ZYMAFLORE® EGIDE^{TDMP}

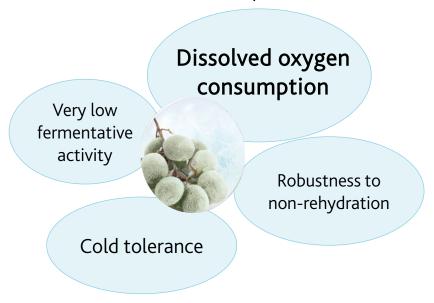
Torulaspora delbruekii & Metschnikowia pulcherrima



- Application on the harvest/winery equipment.
- Vatting of grapes
- Between pressing and racking of grape juice
- Rehydrated or not
- Dosage: 2-5 g/hL

ZYMAFLORE® KHIOMP

Metschnikowia pulcherrima



- Application during pre-fermentatative phases at low temperatures
- Cold maceration grapes
- Stabulation of grape juices
- Rehydrated or not
- Dosage: 2-5 g/hL

BIOProtection - Summary

- Lower VA (acetic acid, ethyl acetate)
- More secure implantation of the inoculated Saccharomyces cerevisiae strain.
- Less SO₂ binding compounds (e.g. acetaldehyde)
- Superior organoleptic profile (purity, fruit character)







ZYMAFLORE® ALPHA^{TD}



1. BIOProtection

2. Aromatic optimization





Modulation of acetic acid by yeasts

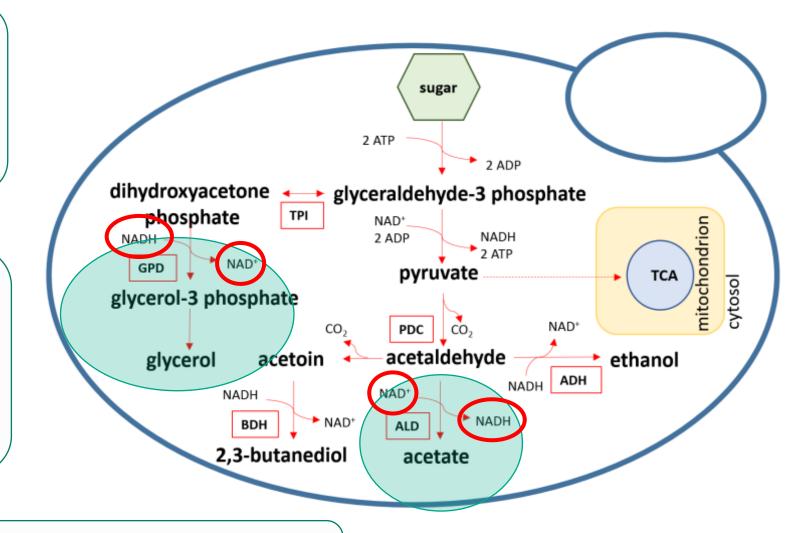
The production of acetic acid by *S. cerevisiae* is favored under hyperosmotic conditions (i.e., matrices rich in sugars).



Link between glycerol production and acetic acid

→ Balance between reduced and oxidized forms of NAD

[NAD+]/[NADH].



Selection of S. cerevisiae strains, but also non-Saccharomyces yeasts.

Torulaspora delbrueckii: Low volatile acidity production

The yeast *Torulaspora delbrueckii* is not very sensitive to high sugar levels → low VA production.

First proposed application:

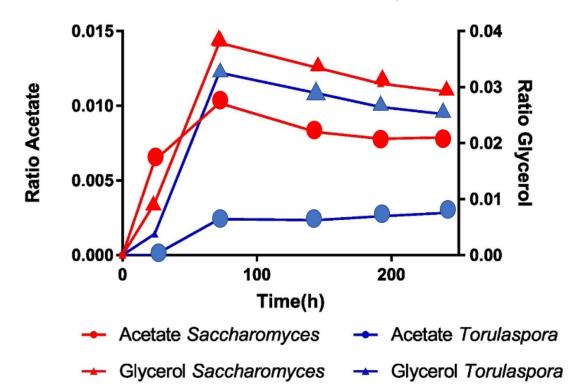
Mixed fermentations of *T. delbrueckii* and *S. cerevisiae* to reduce the VA of sweet wines (\downarrow 50 %).

Bely et al, 2008

High gene expression - routing to glycerol

High expression of the Pyruvate decarboxylase and Alcohol dehydrogenase genes - routing to ethanol

Low expression of the acetaldehyde dehydrogenase gene - Lower acetate production



Evolution of glycerol and acetic acid production during AF.

ZYMAFLORE® OMEGALT



1. BIOProtection

2. Aromatic optimization





Technical characteristics of **ZYMAFLORE® OMEGA**LT

1. Enological use:

- Co-inoculation (simultaneous inoculation) or sequential inoculation with a strain of S.
 cerevisiae.
- SO_2 addition < 40 ppm (or less at low pH).

2. Lactic acid production:

- Variable depending on the conditions of vinification, primarily temperature, type of inoculation and SO₂ addition.
- In optimal conditions > 15 g/L → blending component!

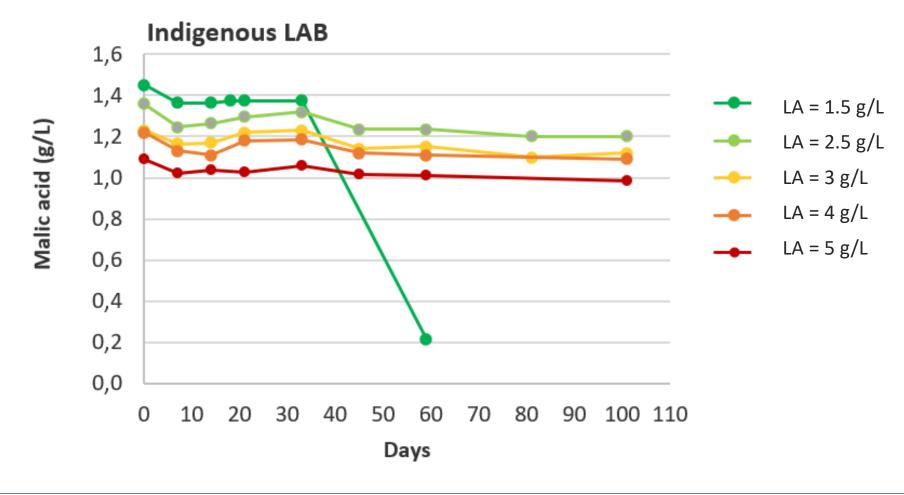
3. Nitrogen demands:

- In co-inoculations, adapted to the requirements of *S. cerevisiae* and enological conditions
- In sequential inoculations, an addition of 100 130 mg/L N coinciding with the inoculation of S. cerevisiae compensates for the nitrogen consumption by ZYMAFLORE® OMEGALT.

Technical characteristics of **ZYMAFLORE**® **OMEGA**^{LT}

Inhibition of MLF

• Indigenous MLF is significantly prolonged or blocked at concentrations > 2 g/L of lactic acid.



LAFFORT®

- High temperature (> 20 °C)
- Sequential inoculation
- Absence of SO₂
- Higher dose
- Low pressure of native flora
- High pH
- Rehydration with SUPERSTART®
- Other parameters (e.g. nitrogen nutrition, trace elements, oxygen...)

- Low temperature (< 18°C)
- Co-inoculation
- Presence of SO₂
- Lower dose
- Strong pressure of native flora
- Low pH
- Rehydration without SUPERSTART®
- Other parameters (e.g. nitrogen nutrition, trace elements, oxygen...)

« Fresh tank » concept : Acidifying tank

- Production of a BIOAcidified tank as a blending component.
- Corrective element for other tanks in terms of acidity and aromatic freshness
 - → current or previous vintages.
- Use conditions favoring strong expression
 of ZYMAFLORE® OMEGA^{LT} e.g.
 - temperature > 18°C (64°F),
 - low SO₂,
 - Inoculate a robust *S. cerevisiae* strain...)



ZYMAFLORE® EGIDE^{TDMP}

Grapes and must:
20 - 50 ppm
With or without rehydration
Direct application on
equipment or grapes









1. BIOProtection

ZYMAFLORE® KHIO^{MP}

Grapes and must:
20 - 50 ppm
With or without rehydration.
Adapted to extreme
temperatures (0 - 6°C).
Strong capacity to consume
dissolved oxygen.

2. Aromatic optimization

ZYMAFLORE® ALPHA^{TD}

Enhanced aroma intensity and volume.

200 – 400 ppm Sequential inoculation with *S. cerevisiae*.









3. **BIO**Acidification

ZYMAFLORE® OMEGALT

Lactic acid production.

Grapes and must: 200 – 400 ppm co-inoculation or sequential inoculation with *S.* cerevisiae.