





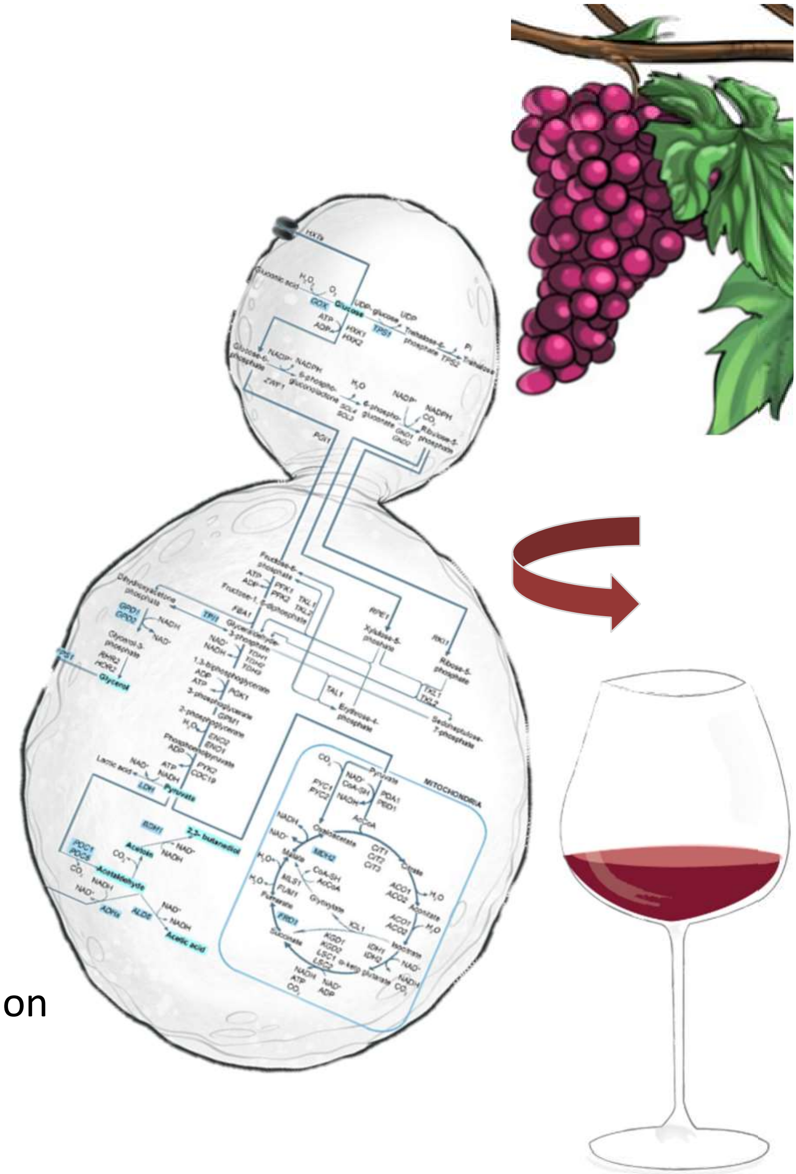

Yeasts from (organic) grapes and wines: Biodiversity and how to manage un-inoculated fermentations

__Ana Hranilovic__, Marina Bely, Vladimir Jiranek, Warren Albertin, Isabelle Masneuf-Pomarede

Yeasts in winemaking

- ***Role:***
  - alcoholic fermentation (AF)
    - conversion of sugars to ethanol and CO_2
  - production/modulation of flavour-active compounds

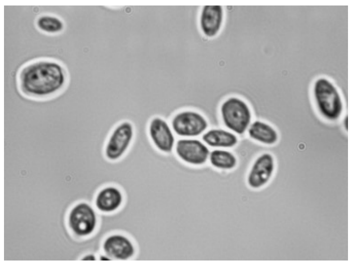
- ***Species:***
  - ***Saccharomyces cerevisiae***
    - 'wine yeast' capable of completing AF
    - rare in the vineyards (grape 1/1000)
  - **Non-*Saccharomyces***
    - different yeasts that occur on grapes/in cellars
    - metabolic contribution to wine profiles
      - e.g.(de)acidification and enhanced aroma production



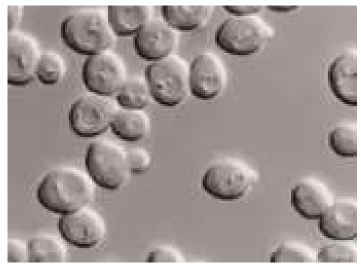
Examples of yeasts from grapes and wines

Wine Server, UC Davis

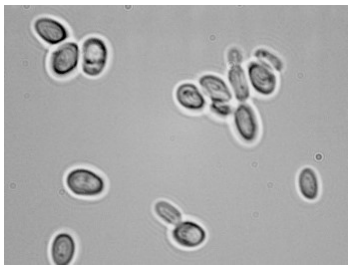
Hanseniaspora uvarum
- common on grapes



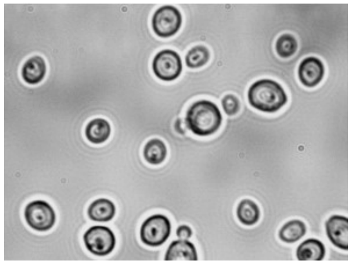
Torulaspora delbrueckii
- aroma/flavour enhancement



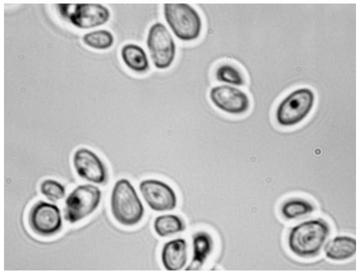
Candida zemplinina
- fructophilic character



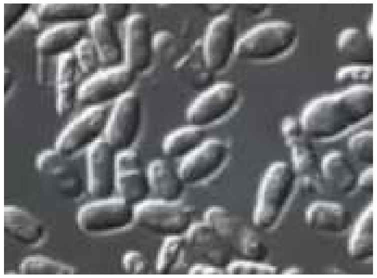
Lachancea thermotolerans
- lactic acid production
- lower ethanol production



Metschnikowia pulcherrima
- lower ethanol production
- bioprotection



Schizosaccharomyces pombe
- malic acid degradation

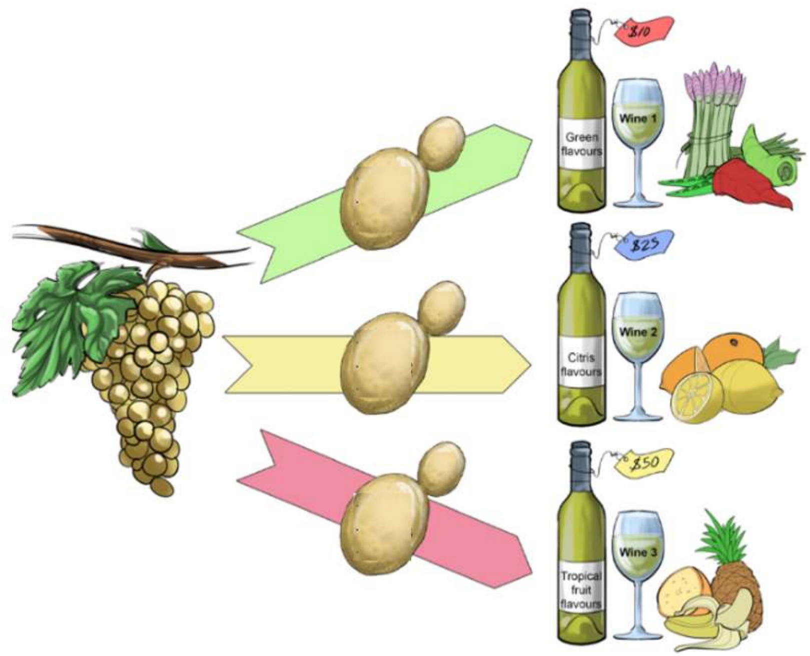


<u>*Different fermentation modalities – pros and cons*</u>

- <u>***Inoculated fermentations with S. cerevisiae***</u>
    - timely and reliabale fermentation
    - costs and wine 'uniformity'

- <u>***Multistarter fermentations with non-Saccharomyces yeasts***</u>
    - modulation of wine chemical and sensory profiles
    - costs and management

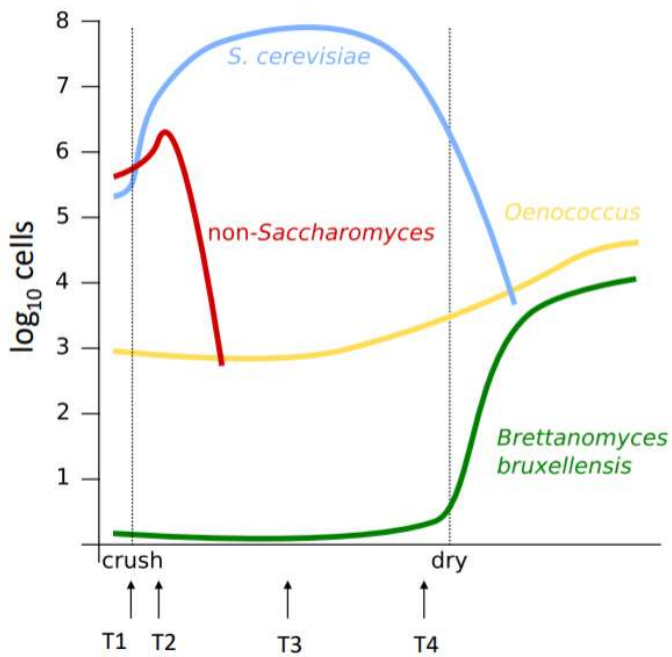
- <u>***Un-inoculated fermentaions***</u>
    - 'microbial *terroir'* and stylistic distinctess
    - lower inputs (ADY cost, refrigeration, nutrients)
    - marketing opportunities (organic/natural/… wines)
    - risks of stuck/sluggish fermentations and erratic results

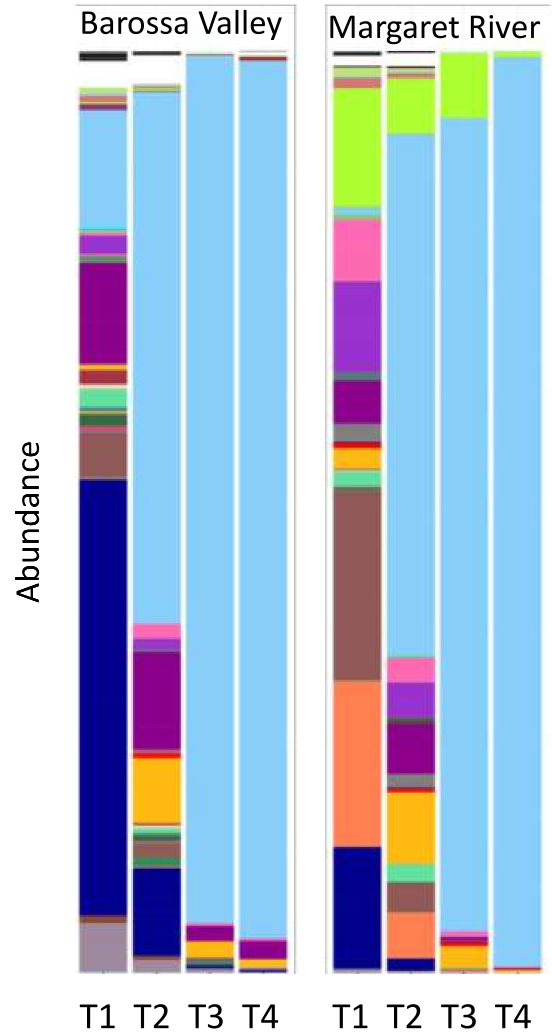


Pretorious, 2016

Population dynamics in un-inoculated fermentations

- **succession of species:**
  - non-*Saccharomyces* at early stages taken over by *S. cerevisaie*
  - variable composition and dynamics

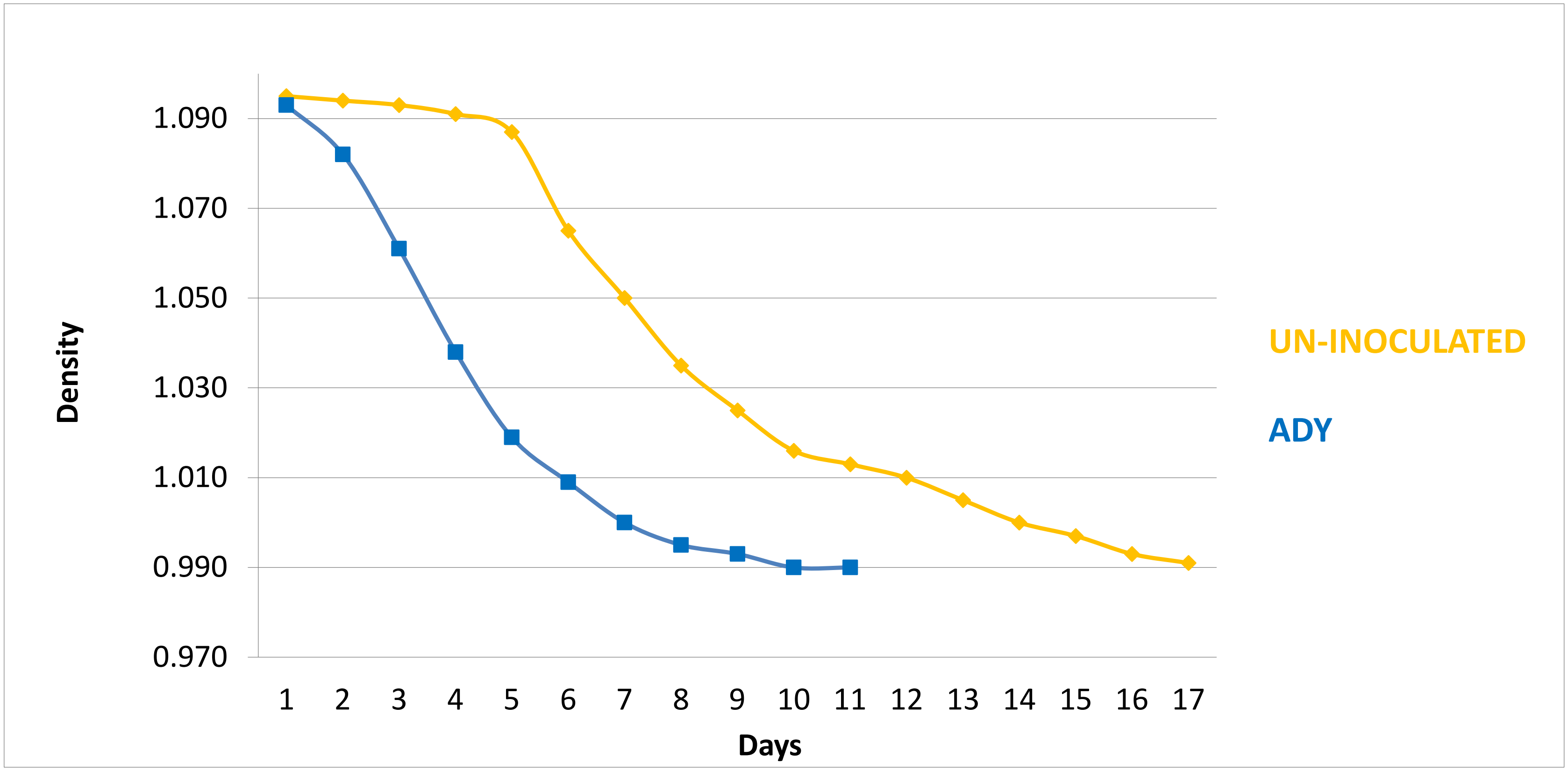




Microbial diversity in Shiraz ferments from two regions (2016 – 2019).

Cuivers et al. 2019 (AWITC)

Managing fermentations with indigenous yeasts



Comparison of fermentation dynamics with indigenous yeasts and ADY.

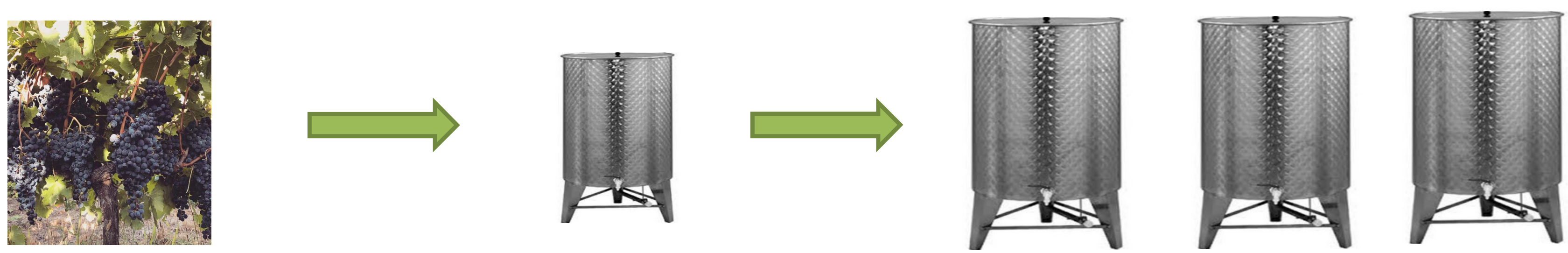
Pied de cuve technology

1) at the beginning of harvest: from grapes

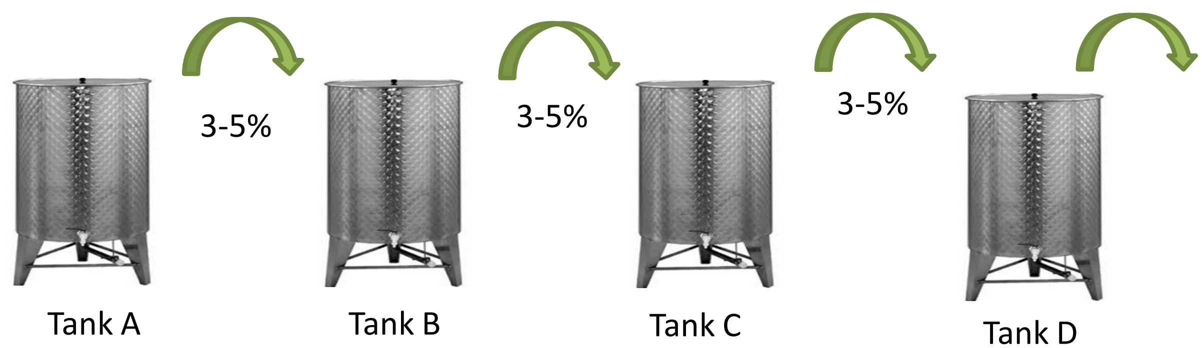
- collecting healthy ripe grapes 8-10 days before harvest (~150 kg for 50 hL)

- pressing the grapes
- 30 ppm SO_2,
- monitoring AF; 20-25 ° C

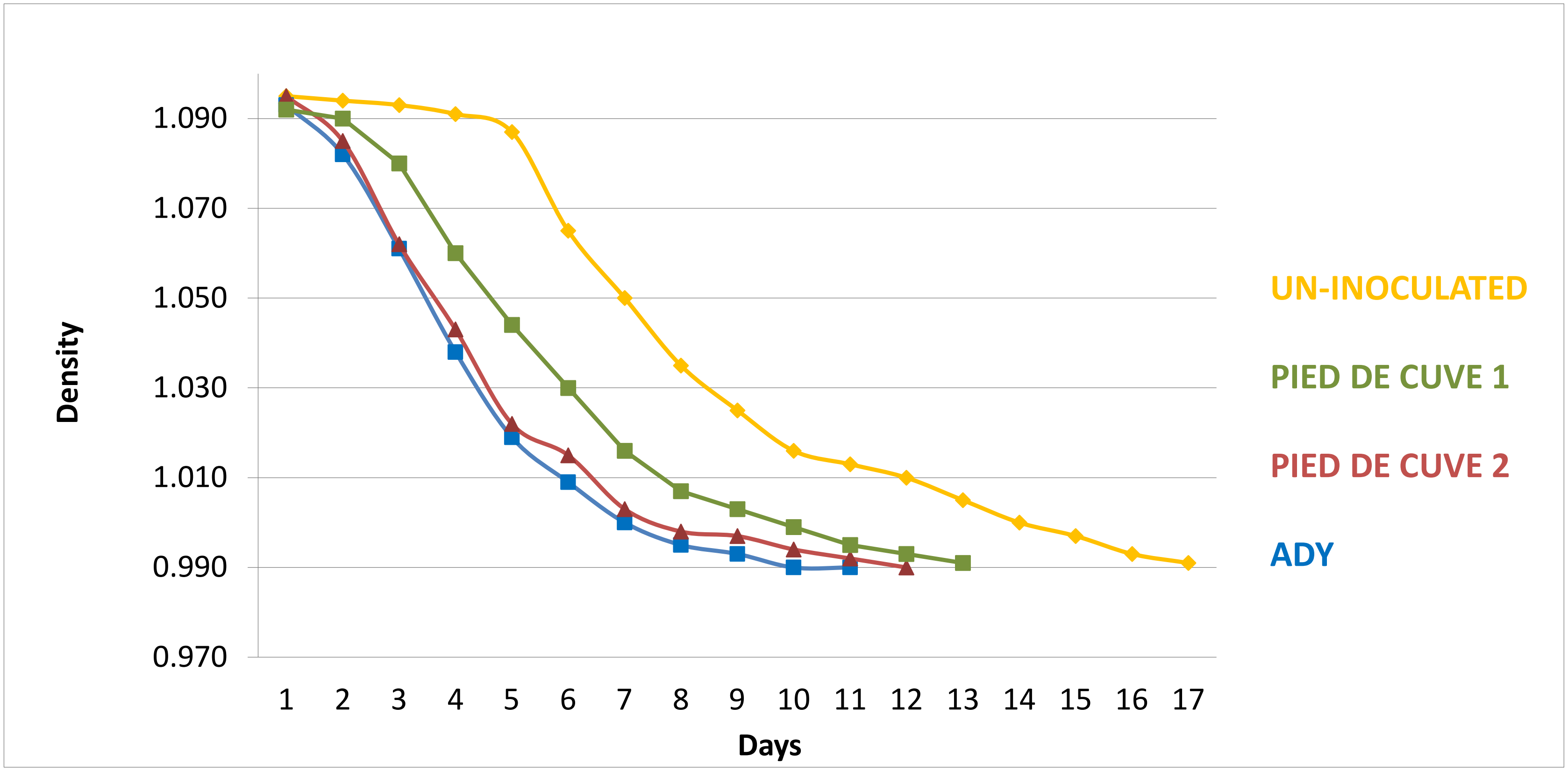
- tasting at mid-fermentation
- use pied de cuve; 3-5% of the tank



2) during harvest time: from fermented juice/must



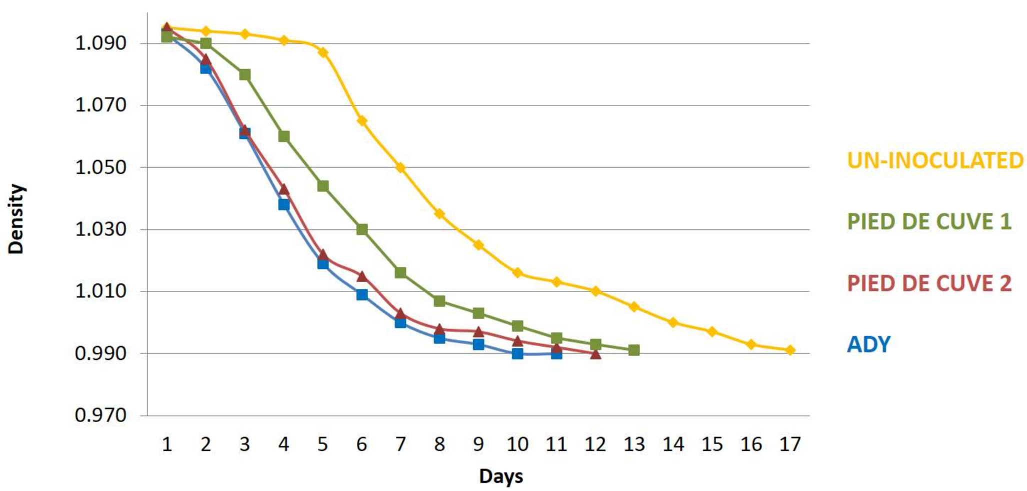
Pied de cuve – ensuring timely and reliable fermentation

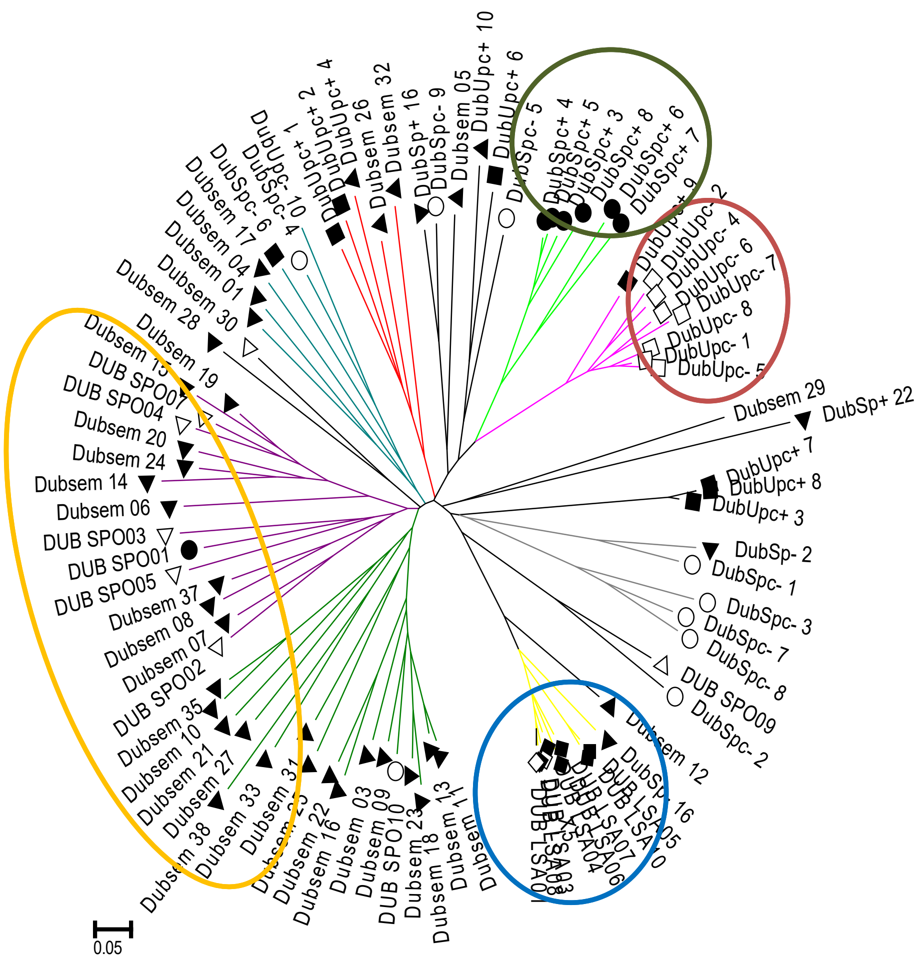


Comparison of fermentation dynamics with indigenous yeasts, ADY and two *pied de cuve* treatments.

Pied de cuve and S. cerevisiae diveristy

- higher initial *S. cerevisiae* rates
- selection of a dominant population

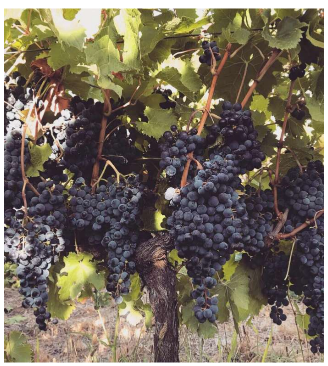




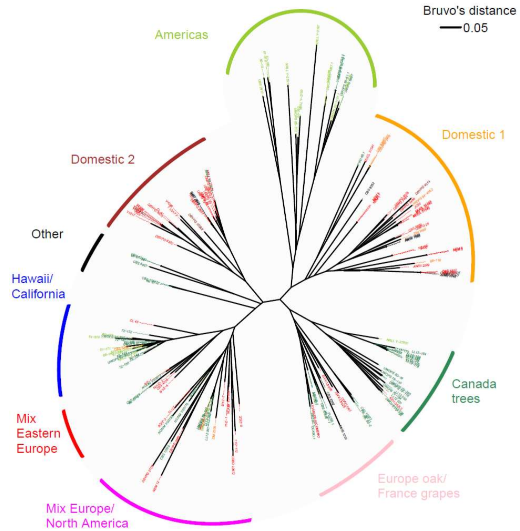
Genetic diversity of *S. cerevisiae* isolates at 75% of AF; dendrogram constructed with genotyping data on 16 microsatellite loci (NJ clustering)

Selection of indigenous yeasts

Isolates from vineyards /ferments → Genetic and phenotypic tests → Lab-scale trials → Cellar trials → ADY / liquid culture



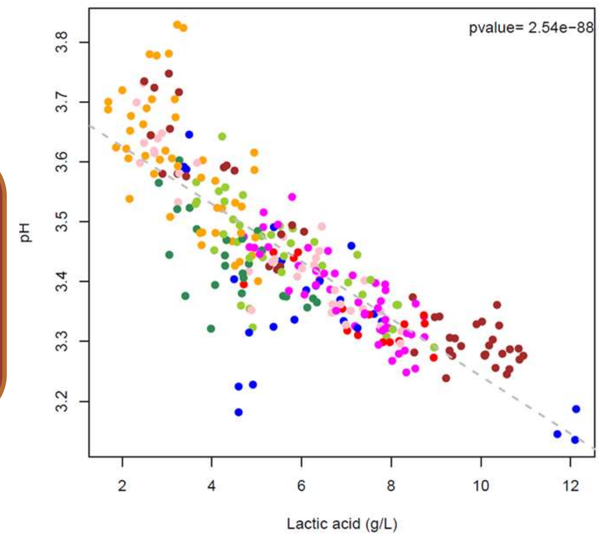

*Population-wide selection of **Lachancea thermotolerans** for wine starters*



Genotyping of ~200 isolates

→

Oenological performance of 94 strains



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RESEARCH ARTICLE

The evolution of *Lachancea thermotolerans* is driven by geographical determination, anthropisation and flux between different ecosystems

Ana Hranilovic[1,2], Marina Bely[3], Isabelle Masneuf-Pomarede[3,4], Vladimir Jiranek[1,2], Warren Albertin[3,5]*

SCIENTIFIC REPORTS

OPEN

Oenological traits of *Lachancea thermotolerans* show signs of domestication and allopatric differentiation

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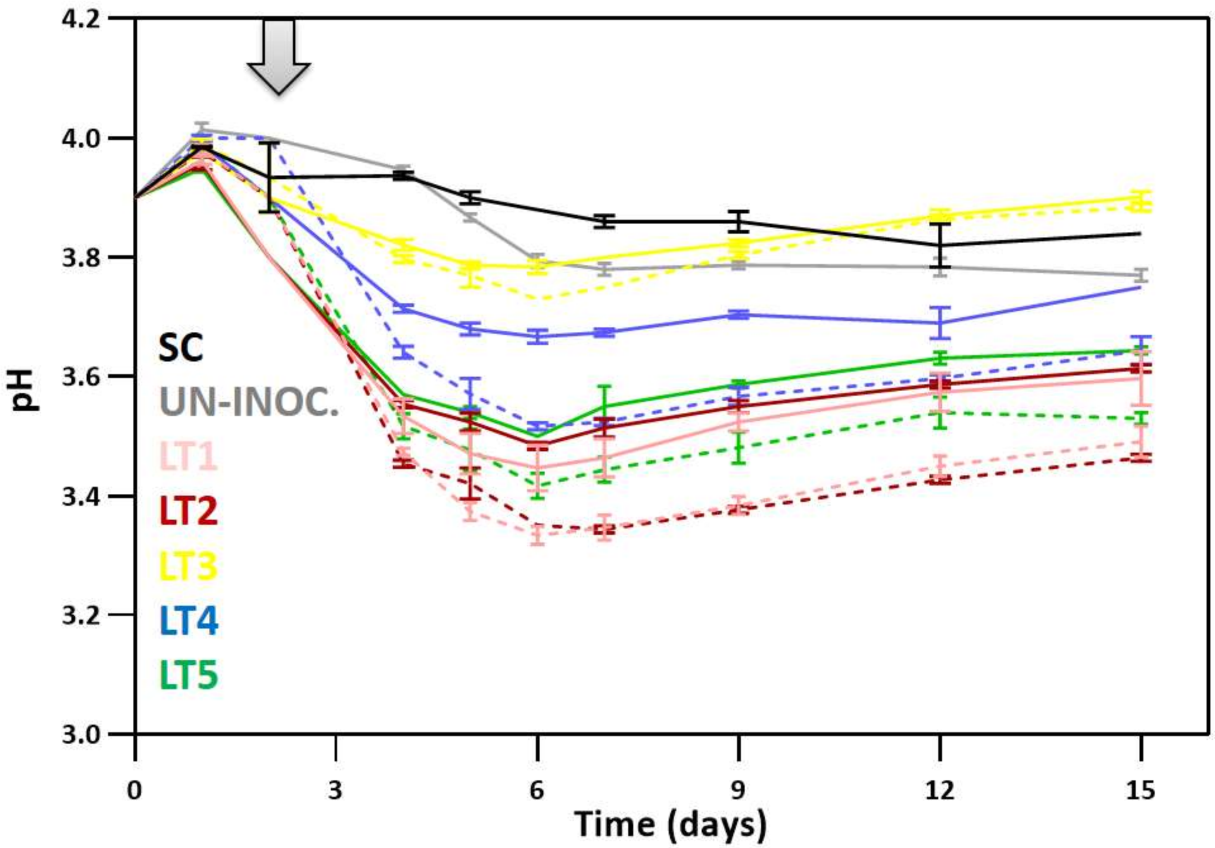
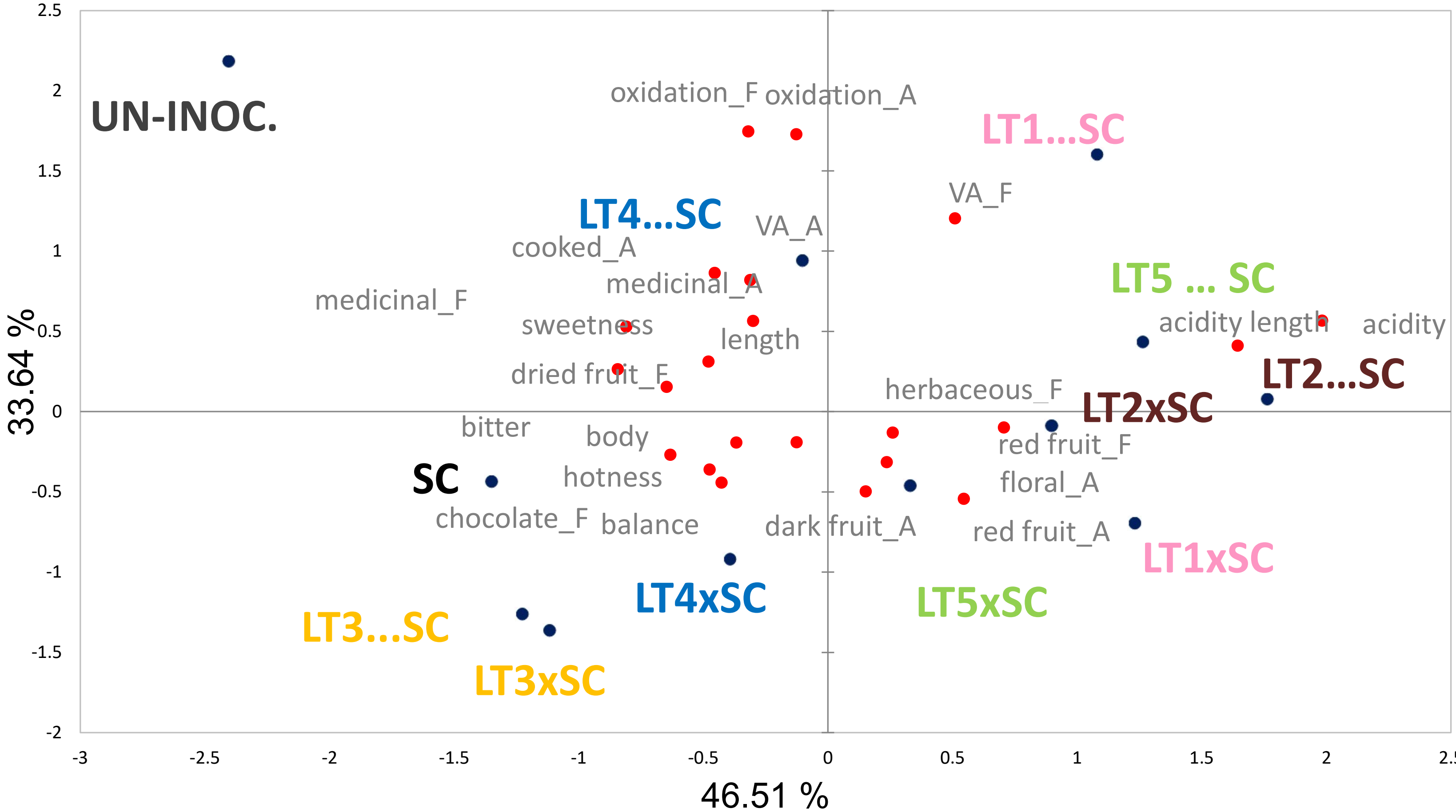
Ana Hranilovic[1,2], Joanna M. Gambetta[3], Leigh Schmidtke[1,3], Paul K. Boss[4], Paul R. Grbin[2], Isabelle Masneuf-Pomarede[5,6], Marina Bely[5], Warren Albertin[5,7] & Vladimir Jiranek[1,2]

Pilot-scale winemaking trials in warm climate Merlot



Fermentation and acidification kinetics in Merlot. Full lines represent co-inoculations and dotted lines sequential inoculations of *L. thermotolerans* and *S. cerevisiae* (arrow).

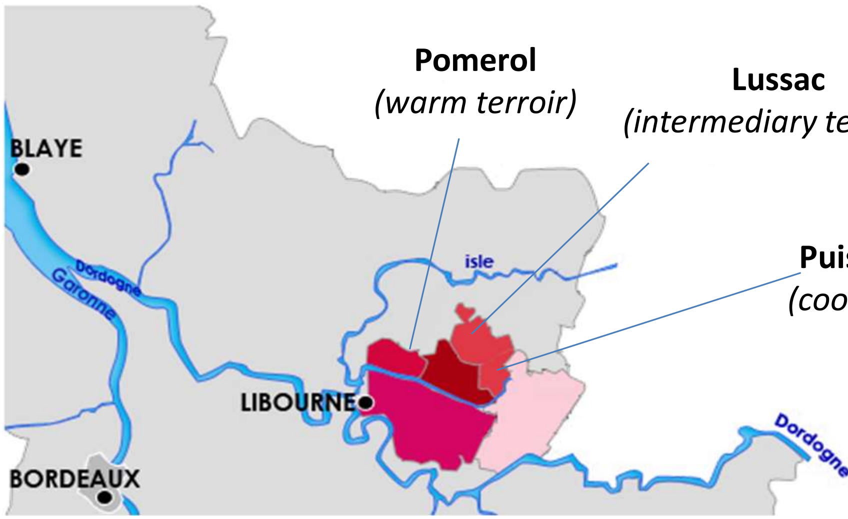
Sensory analysis of Merlot wines



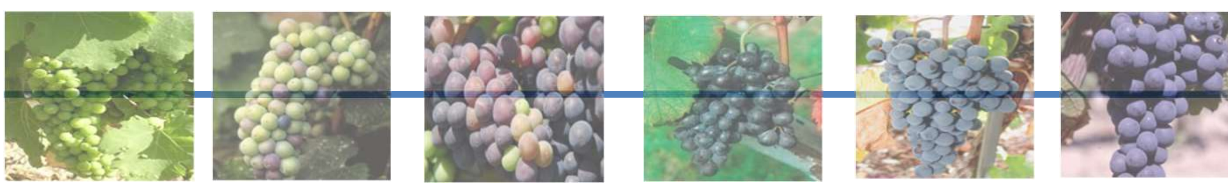
PCA built with attributes significantly ($p < 0.05$) affected by the yeast treatment.
Rate-all-that-apply method; 47 tasters; sequential and co-inoculations are represented with x and … symbols, respectively.

Organic viticulture and grape berry microbiota

PhD thesis Guilherme Martins, 2012



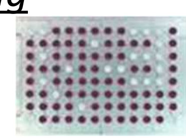
Organic | Conventional



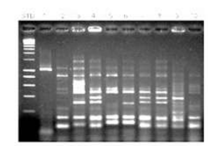
cell suspension

Isolation and enumeration

Metabolic profiling (Biolog™)



Genetic analysis

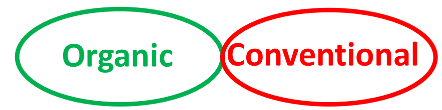


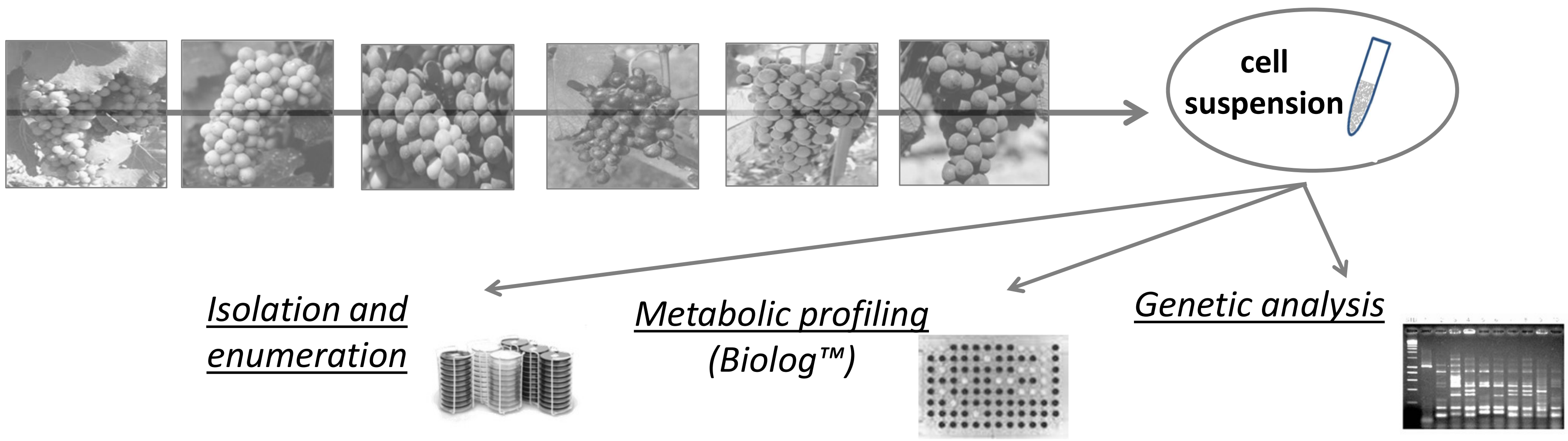
Organic viticulture and grape berry microbiota

PhD thesis Guilherme Martins, 2012

- **Significant effect** of the farming system on population size and diversity

- **Higher microbial** diversity in **organic** compared to **conventional** farming sistem

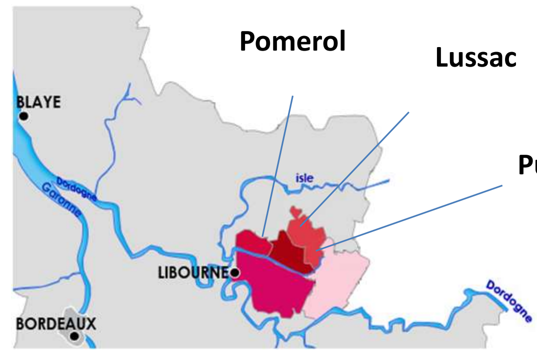




Organic viticulture and grape berry microbiota

PhD thesis Guilherme Martins, 2012

- **Significant effect** of the farming system on microbial population size and diversity
- **Higher microbial** diversity in **organic** compared to **conventional** farming systems
- Preponderant effects of **microclimate** and **phenological stage**



Isolation and enumeration

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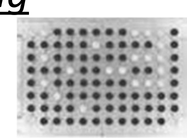

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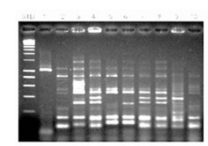
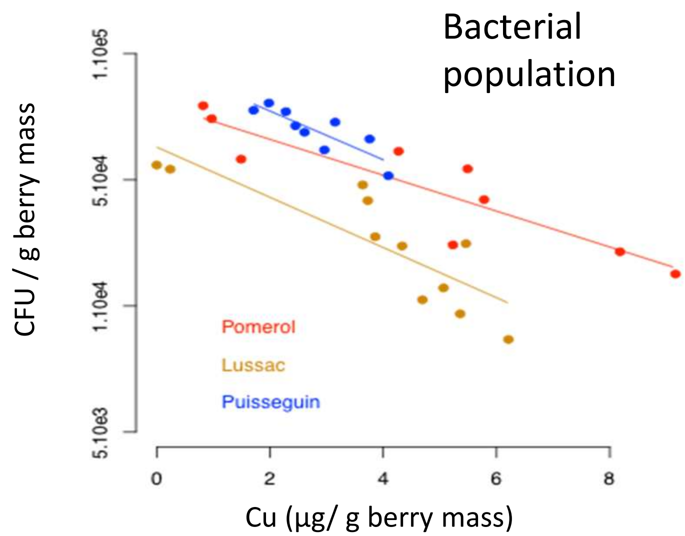
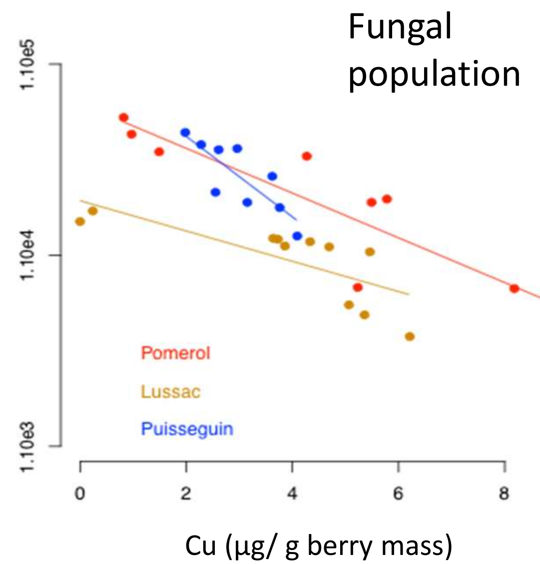


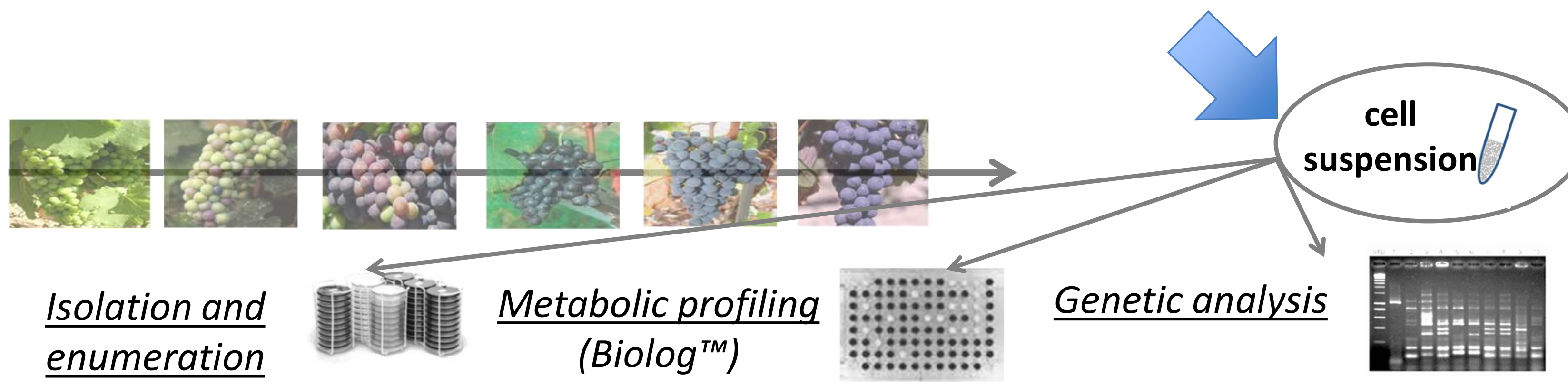
Copper additions negatively affect :

- **Population size**
  (p < 0,01; ρ =0.87 - 0.95)

- **Population diversity:**
  - **metabolic profiles**
    (e.g. Shannon index based on Biolog® metabolic profiles; p < 0,0001; ρ = 0,90)
  - **genetic profiles**
    (e.g. bacterial diversity p < 0,012 ; ρ = 0,58)

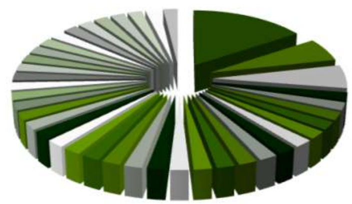






Link between microbial diversity and 'complexity'?

- Not clear answer from the literature
- Microbial diversity ≠ wine 'complexity'
- Chemical complexity ≠ sensory complexity



The complexity of wine: clarifying the role of microorganisms

Sophie Tempère[1] · Axel Marchal[1] · Jean-Christophe Barbe[1,2] · Marina Bely[1] · Isabelle Masneuf-Pomarede[1,2] · Philippe Marullo[1,3] · Warren Albertin[1,4]

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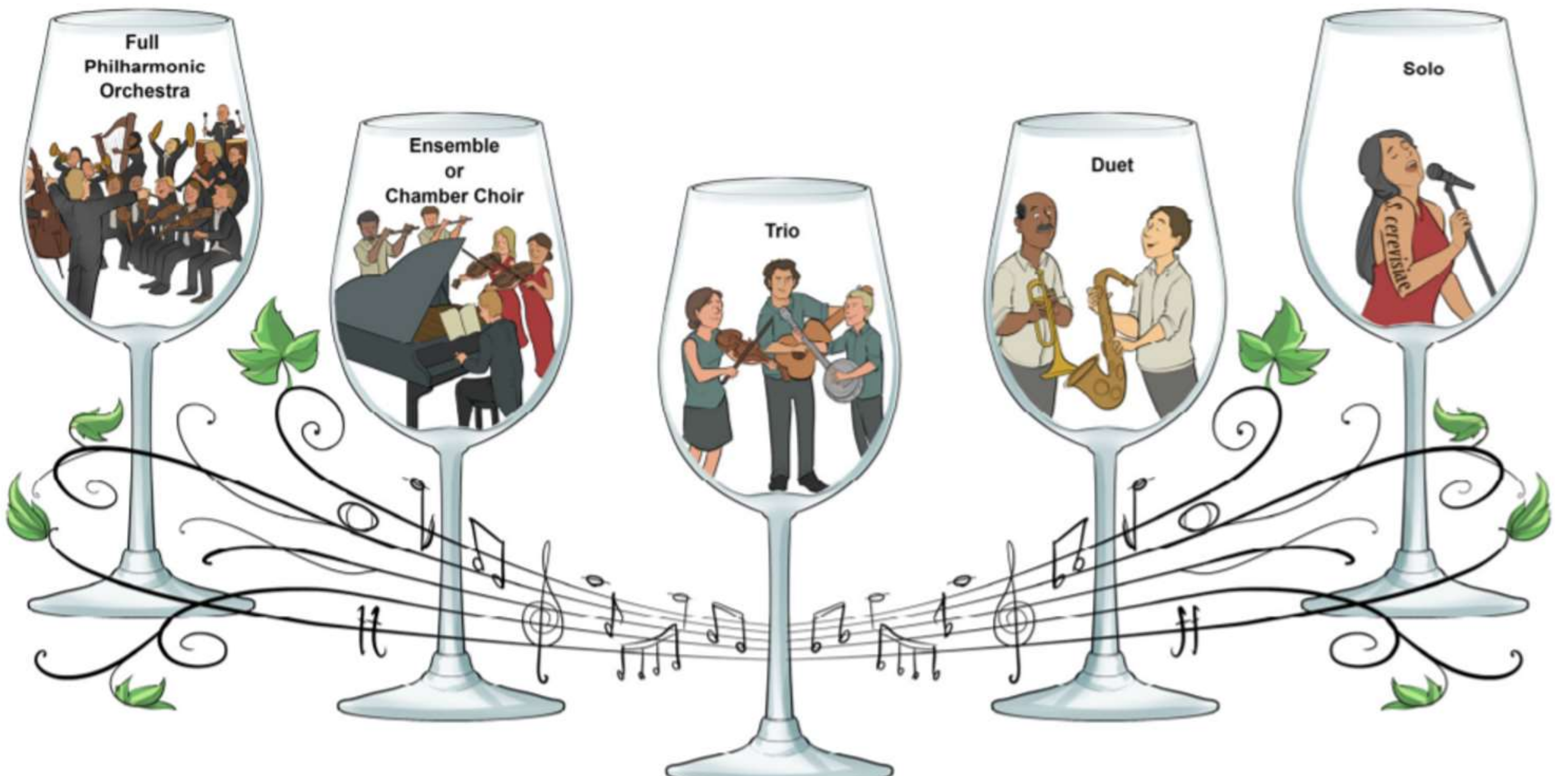
Summary of different fermentation modalities

mix of indigenous *S. cerevisiae* and non-*Saccharomyces* yeasts

pre-selection of multiple species/strains

inoculation of *S. cerevisiae* strain (active dry yeast; ADY)



Pretorious, 2016

Thank you for your attention!

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