Stellenbosch University Iyunivesithi Universiteit

SAGWRI South African Grape and Wine Research Institute

Lachancea thermotolerans: Shaping the future of fruit-forward and balanced wines.

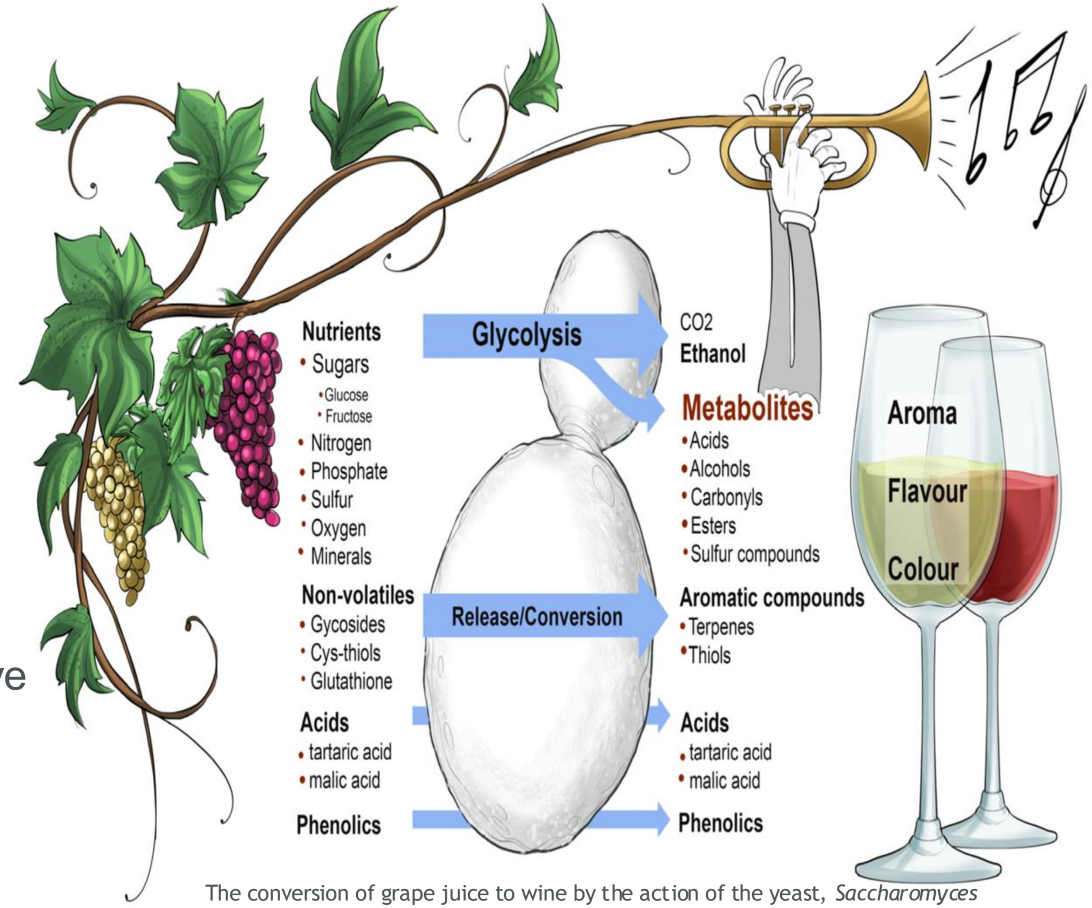
Theo R. Jacobs, Stéphanie Rollero, Arnaud Delaherche, Mathabatha E. Setati and Benoit Divol

SASEV 44th Conference: 24/11/2025

Photo by Stefan Els

Yeasts: Shaping Wine Characteristics:

- Yeasts are essential in wine production.
- Convert grape sugars → ethanol, CO_2 & flavor compounds. Produce/release fruity & floral aromas (esters, terpenes).
- Modern consumers increasingly prefer fresh, well-balanced (acidity and alcohol), fruit-forward wines.
- Enhancing these traits through yeast selection can therefore improve consumer satisfaction & market competitiveness.



The conversion of grape juice to wine by the action of the yeast, *Saccharomyces cerevisiae* (Pretorius, 2016)

(Fleet, 2003; Fleet and Heard, 2003; Jolly et al., 2006; Fleet, 2008; Dashko et al., 2014; Beckner whitener et al., 2015; Hranilovic et al., 2018; Petruzzi et al., 2017; Benito et al., 2016; Jolly et al., 2014).

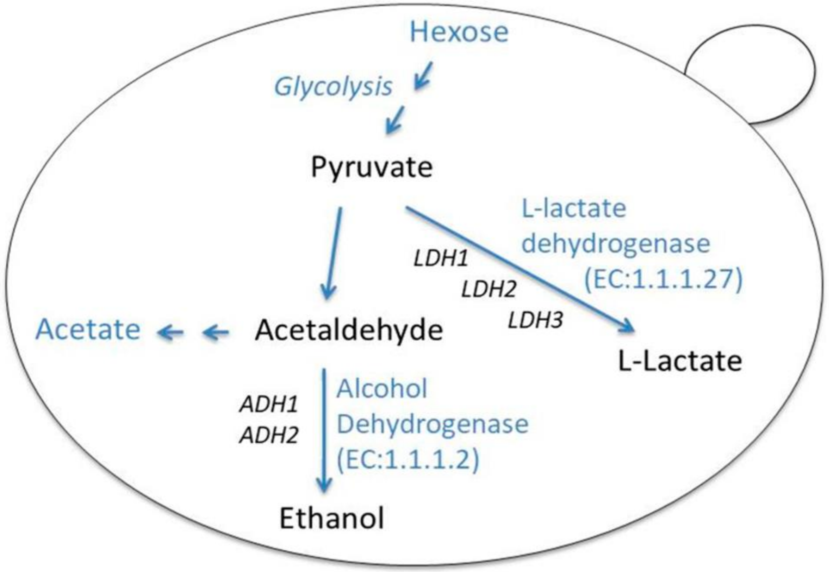
Acidity in Wine: Importance and Climate-Change Challenges

- Acidity strongly influences wine taste (**mouthfeel, freshness and overall balance**), microbial stability.
- **Challenge**: Rising global temperatures accelerate grape ripening and reduce natural acidity at harvest (particularly in South Africa).
- Current solutions: Chemical acid additions to correct acidity loss, but this can be:
  - Expensive
  - Cause stability issues (e.g., tartaric acid crystallization)
  - Chemical inputs increasingly rejected by consumers

➢ Growing need for sustainable, biological solutions to maintain wine acidity under climate change conditions

L. thermotolerans: The Solution

- **Among wine yeasts, *Lachancea thermotolerans* (non-*Saccharomyces* species), has emerged as a leading candidate for biological acidification.**
  - **Why?** Only wine yeast known to produce lactic acid during fermentation.
  - **Key metabolic marker** of the species (widely studied).
  - Produce substantial amounts, naturally increasing wine acidity.

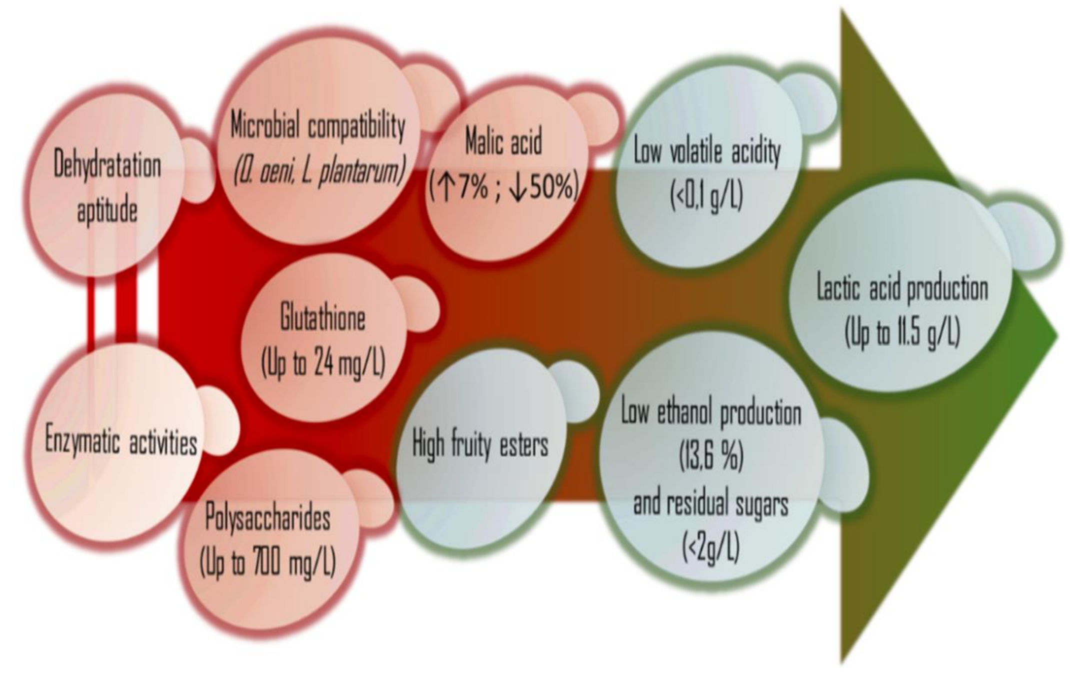


Metabolic pathway in *L. thermotolerans* during fermentation. (Vicente et al.,2021)

(van Leeuwen & Darriet, 2016; Rogiers et al., 2022; Plantevin et al., 2024; Vicente et al., 2025; Benito, 2018; Morata et al., 2018; Vicente et al., 2021, 2023)

More Than Acidity: Additional Benefits of *Lachancea thermotolerans*:

- Research interest has also increased because it enhances wine aromatic diversity:
  - ↑ 2-phenylethanol (rose), ethyl lactate (fruity, creamy), and more.
- Recent studies: *L. thermotolerans* can also improve several other wine traits.
  - Supports current consumer preferences.
- Strain selection criteria have expanded beyond just lactic acid production.



Overview of the proposed criteria for selecting *Lachancea thermotolerans* strains. (Vicente et al.,2021)

Benito 2018; Vilela 2018, 2019; Porter et al. 2019; Vicente et al. 2022; Santillan et al., 2019; DeOrduna, 2010; Volschenk et al., 2006; Porter et al., 2019; Vicente et al., 2022; Vilela, 2019).

High Variability: Provides Opportunities

- These beneficial traits, especially lactic acid production, show **high strain variability**.
- **Even within the same strain**, traits levels can fluctuate under different fermentation conditions.
- Variability arises mainly from **genetic diversity**, but environmental factors also play a role.
- Studying this variability is important: strains differ, but provides more opportunities to find unique, desirable traits.

➢ **Focus of our study**

Parameter	Monoculture	Sequential	Vs S. cerevisiae
Lactic acid production (g/L)	0.2- 20	0.2- 10.4	Significantly higher concentration
pH decrease	0.10- 0.59	0.11- 0.54	Lower
Acetic acid (g/L)	0.03- 0.58	<0.1- 0.54	Lower concentrations
Ethanol (%v/v)	3.98-10.6	11.76- 13.53	Lower concentrations
Malic acid degradation (%)	10-50	27-57	Higher consumption
Polysaccharides (mg/L)	163-260	n.d.a	Higher concentrations
Glutathione (nmol/mg cell)	0.205-0.537	24 mg/L in wine	Higher concentrations
Ethyl esters (mg/L)	57- 163	35.76 ± 1.044	Higher concentrations
Acetate esters (mg/L)	1.80- 2.65	1.446 ± 0.021	Higher concentrations

Snyder et al.. 2021; Hranilovic et al., 2022; Mucalo et al., 2023; Vicente., 2023; Fleming and Threlfall, 2024; Tzamourani et al., 2024; Salopek et al., 2024 and Vicente et al., 2024; 2025

Aim & Objectives:

Aim:

To genetically and phenotypically characterize all *Lachancea* isolates from the SAGWRI's microbial culture collection, with a specific focus on metabolites of oenological interest, to potentially identify strains with desirable traits for better-balanced and fruit-forward wines— ultimately contributing to more sustainable and consumer-preferred wine styles in a warming climate.

Objectives:

1. Twenty *Lachancea* isolates (18 *L. thermotolerans*, 1 *L. lanzarotensis*, 1 *L. fermentati*) were genetically differentiated to confirm their distinctiveness.

2. Phenotypic screening of the *Lachancea* strains was carried out in monoculture fermentations with sterile Chenin blanc grape juice to evaluate their full individual potential on wine characteristics.

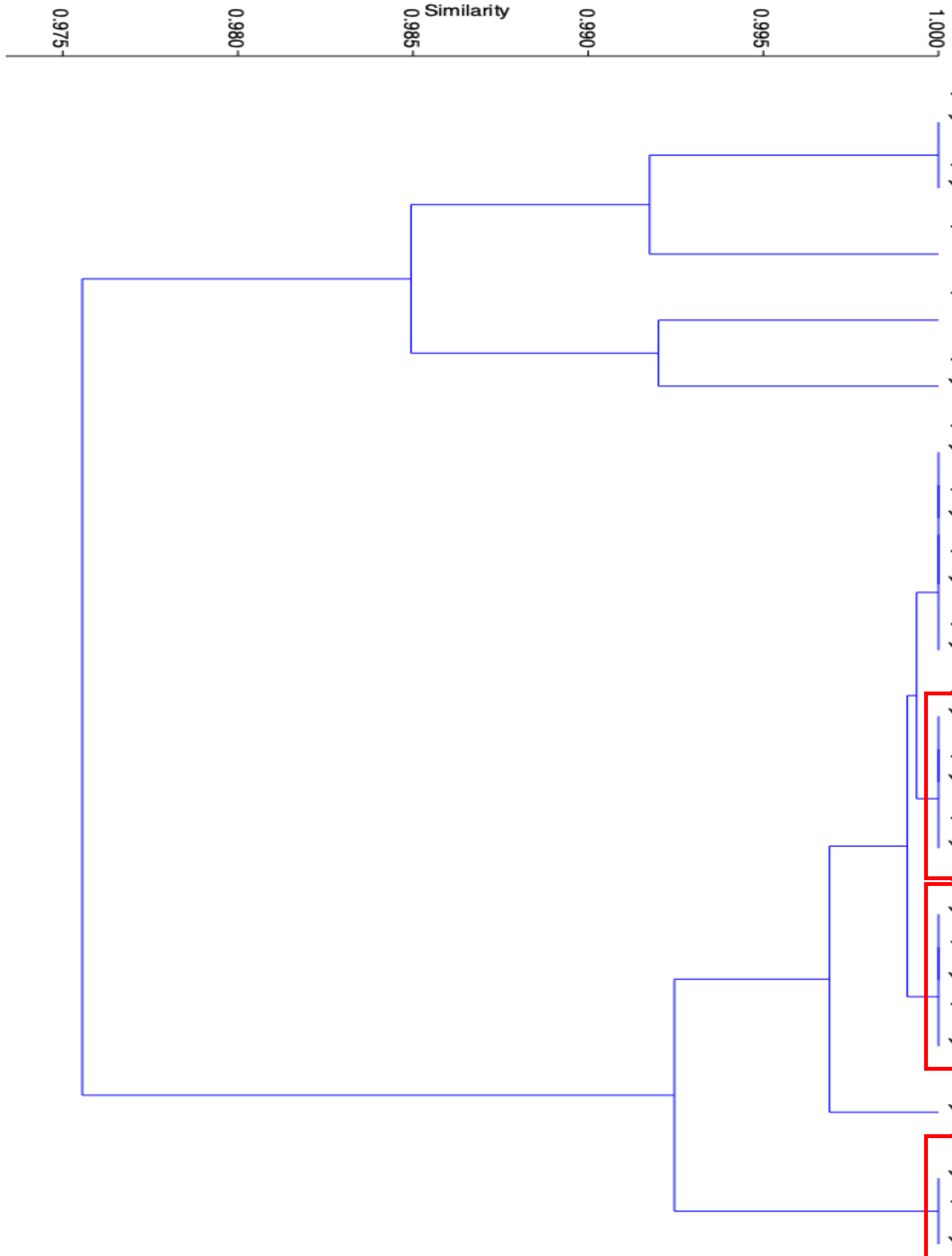
Objective 1: *L. thermotolerans* strain differentiation (Microsatellite typing)

Genetic strain differentiation in *L. thermotolerans* is more challenging than in *S. cerevisiae*, as most methods developed for *S. cerevisiae* are ineffective.

- **Microsatellite typing** has been identified as the most effective molecular method for *L. thermotolerans*.
- **Protocol:** Banilas et al. (2016) – reproducible.

(Vicente et al., 2023; Hranilovic et al., 2017; Banilas et al., 2016)

Objective 1: *L. thermotolerans* strain differentiation (Microsatellite typing) ✅



Strain	Flocculation (YPD)	Origin of isolation	Cluster
Y540	✗	Cabernet Sauvignon, Stellenbosch	1
Y548	✗	Cabernet Sauvignon, Stellenbosch	
Lt1	✗	Chr. Hansen, Hørsholm, Denmark	2
Lt2	✗	Lallemand Inc., Montreal, Canada	3
Y513	✗	Sauvignon blanc, Elgin Valley	4
Y1109	✓	Sauvignon blanc, Somerset West	5
Y1197	✓	Sauvignon blanc, Somerset West	
Y1202	✓	Sauvignon blanc, Somerset West	
Y1295	✓	Sauvignon blanc - Chardonnay blend, Somerset West	
Y1206	✓	Sauvignon blanc, Somerset West	6
Y527	✗	Cabernet Sauvignon, Stellenbosch	
Y940	✗	Shiraz, Stellenbosch	
Y1240	✗	Muscat of Alexandria, Rawsonville	7
Y905	✓	Chenin blanc, Riebeeck Casteel	
Y973	✓	Cabernet Sauvignon, Stellenbosch	
Y1017	✓	Chardonnay, Paarl	8
Y1038	✗	Chardonnay, Sir Lowry's Pass	9
Y519	✗	Cabernet Sauvignon, Stellenbosch	

Discussion:

Dendrogram: High genetic diversity in *L. thermotolerans*

- Microsatellite typing grouped 18 strains into 9 clusters.
- Group validation: flocculation phenotypic trait & origin of isolation; some groups raised concerns.
- Resolution may be insufficient to detect subtle genomic differences → WGS (accurate) may be needed in future.

Objective 2 - Phenotypic screening of the *Lachancea* strains

1

Yeast:

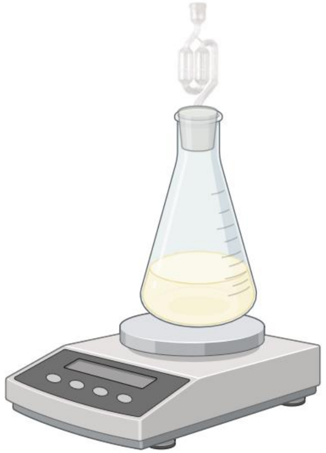
- 20 *Lachancea* strains
- Positive controls: Lt1 & Lt2
- Species control: *S. cerevisiae* (SafOeno™ CKS102)
- Pre-culture: YNB
- Monoculture: 10^6 cells/mL

Fermentation conditions:

- Flash-pasteurised Chenin blanc grape juice
- Small scale (100 mL)
- Triplicates (each strain)
- Shaker: @20°C with 120 rpm agitation

2 Fermentation kinetics

(Daily weight measurements)

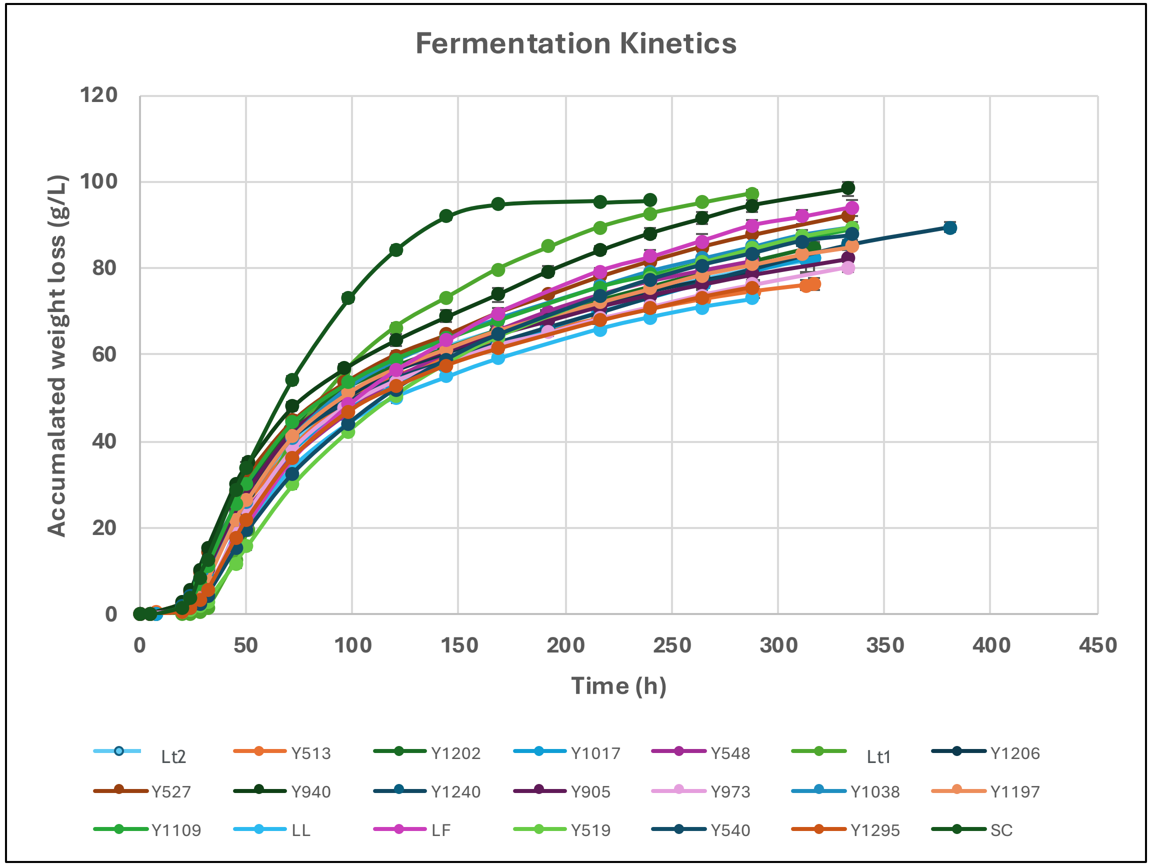


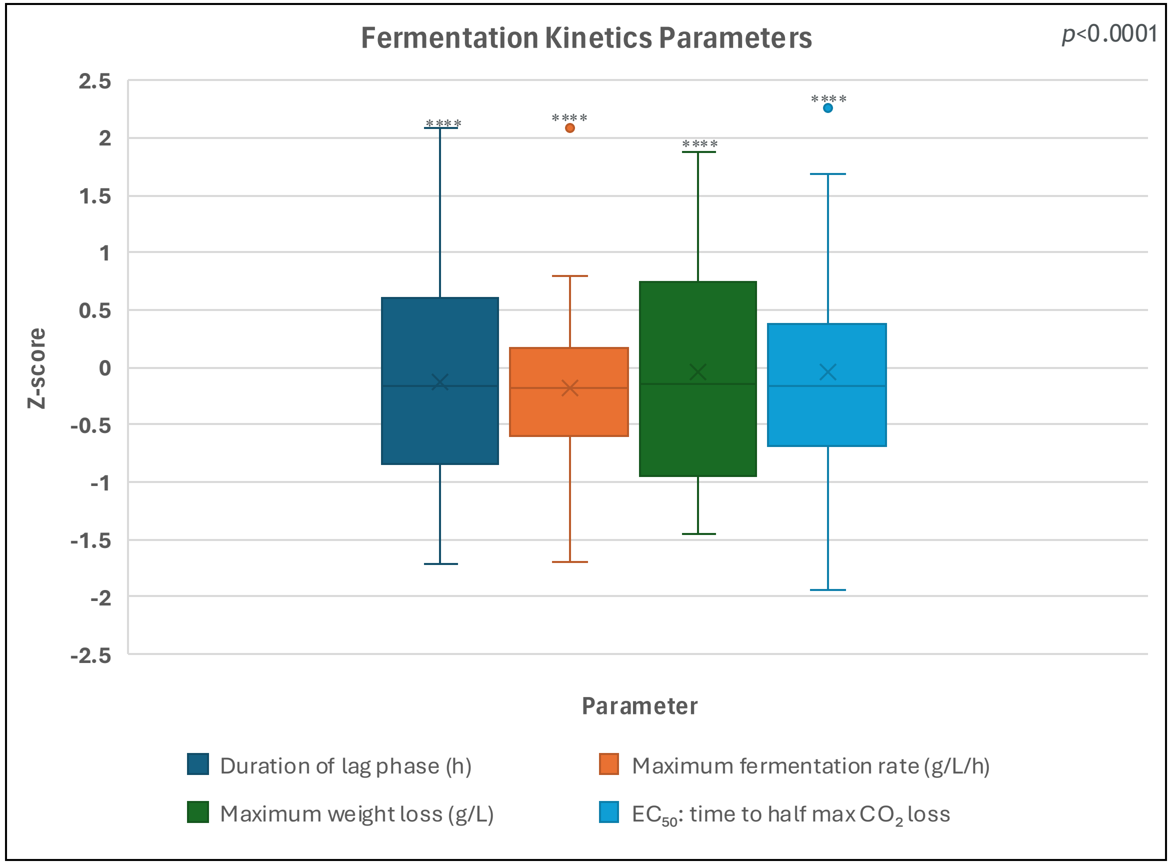
3 Chemical analysis:

Sugars, ethanol, glycerol & organic acids (HPLC): Juice and wine

Volatile organic compounds (Targeted & Untargeted GC-MS): Wine

Fermentation Kinetics





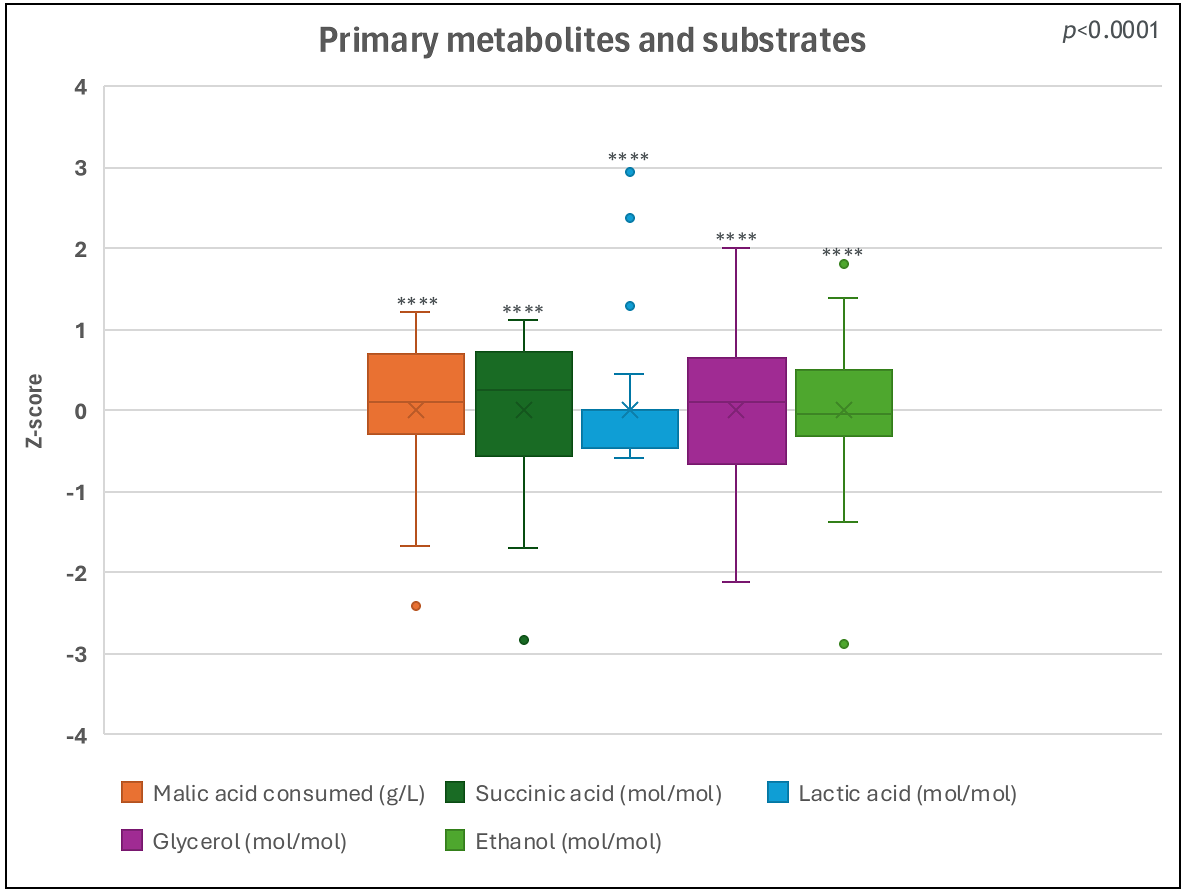
- Only *S. cerevisiae* showed expected sigmoidal curve; all *Lachancea* strains were sluggish.
- Due to the slow/prolonged fermentation of *Lachancea* spp., fermentations were stopped when the rate reached **0.1 g/L/h**.

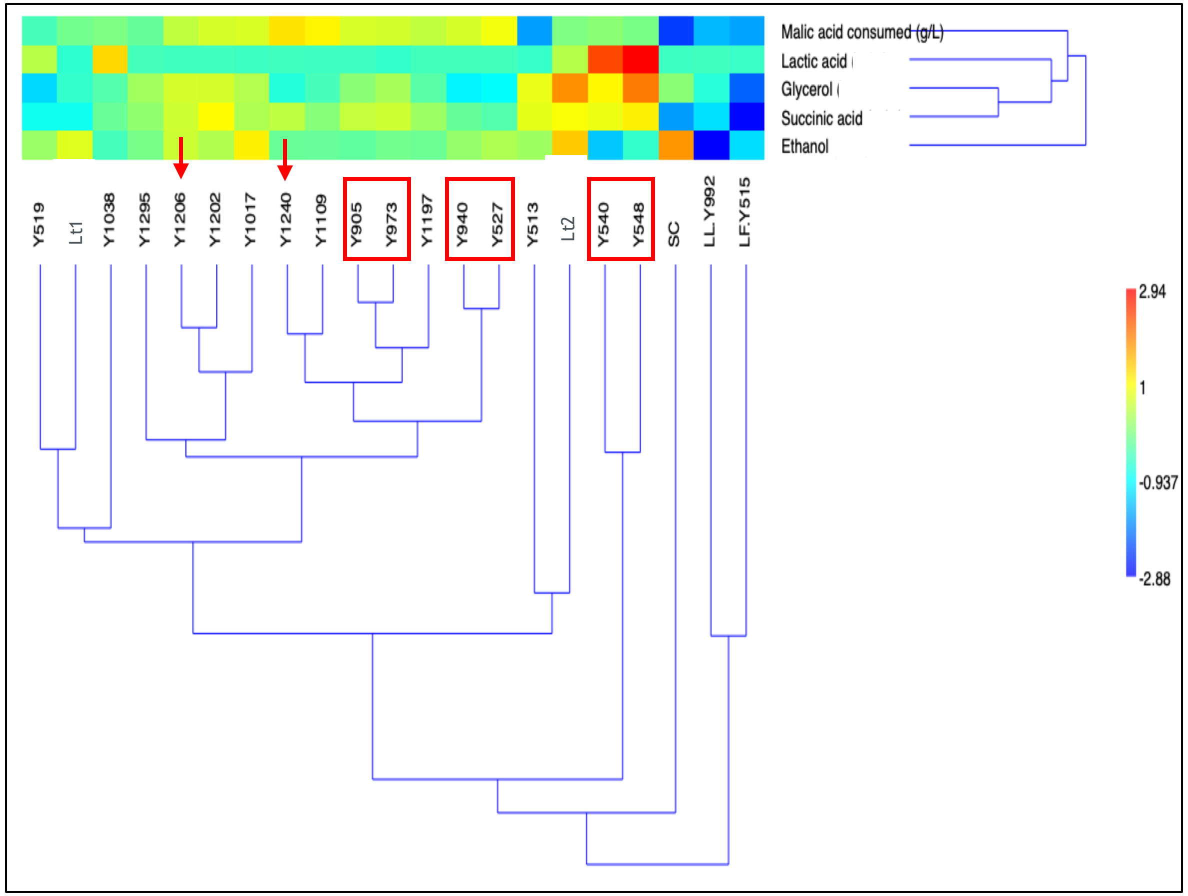
Discussion:

- *Lachancea* strains varied significantly (p<0.0001) in fermentation kinetics, ranging from strong to weak fermenters, but strains within **each genetic cluster mostly behaved similarly**.
- Confirms *Lachancea* **cannot** complete fermentation alone, but some strains came close (future potential for independent AF).
- Max fermentation rate showed **smallest variation**; generally moderate fermenters (consistent with literature)

Vicente et al., 2021; Hranilovic et al., 2022; Mucalo et al., 2023; Fleming and Threlfall, 2024; Tzamourani et al., 2024; Salopek et al., 2024 and Vicente et al., 2024

Primary metabolites (HPLC)





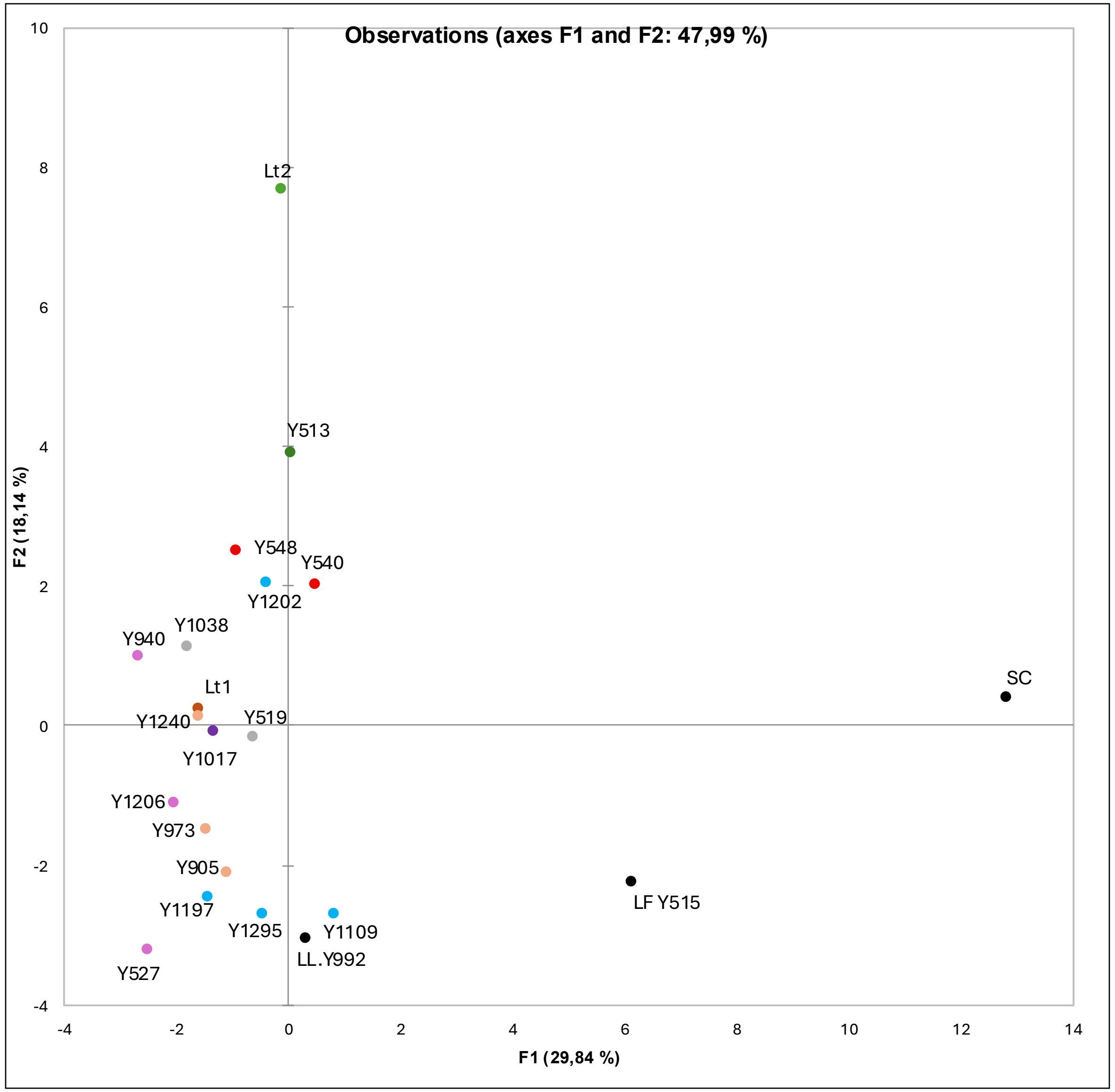
Heat map with UPGMA clustering showing yeast strain grouping based on primary metabolite profiles.

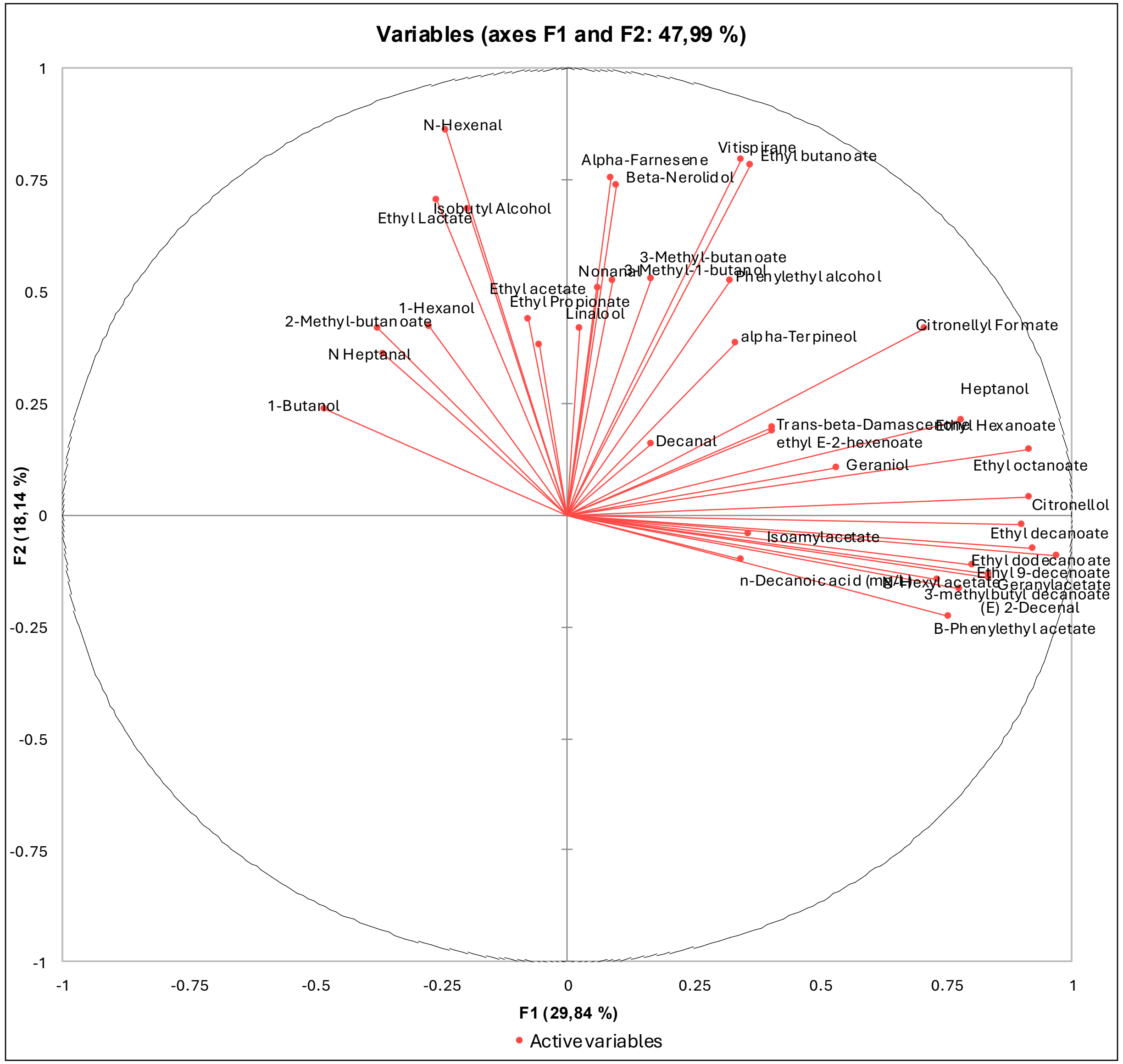
Discussion:

- **Lactic acid** production showed **smallest variation**; surprisingly **<u>only 5 strains produced high levels</u>** (although species marker)
  - Y540 and Y548 produced excessively high levels (~9.9 g/L), significantly reducing pH (~0.15 units). **Strong bio-acidification potential**.
- All strains **consumed** malic acid; some >50%, SC strain the **least** (matches literature): Shorten MLF, lowers spoilage risk, softens harsh acidity **(Improve balance and mouthfeel).**
- Similar trend observed for **succinic acid**. Significant positive correlation with malic acid consumption (r=0.6), directed to succinic acid production and not pyruvate. Enhance wine complexity (slightly salty, bitter).
- **Glycerol** showed largest variation (matches literature). **Strong positive correlation** with succinic acid (r = 0.76), consistent with reported redox balancing. Enhances mouthfeel (adds body and roundness), balances acidity
- All strains yielded **less ethanol** than SC strain, aligning with literature and consumer preference for lower alcohol (better balance, reduced health concerns)
- Primary metabolite clustering **largely aligns** with genetic clustering, with a **few exceptions** (e.g., Y1206 and Y1240).

Vicente et al., 2021; Hranilovic et al., 2021, 2022; Mucalo et al., 2023; Fleming and Threlfall, 2024; Vicente et al., 2024 and Duncan et al., 2024

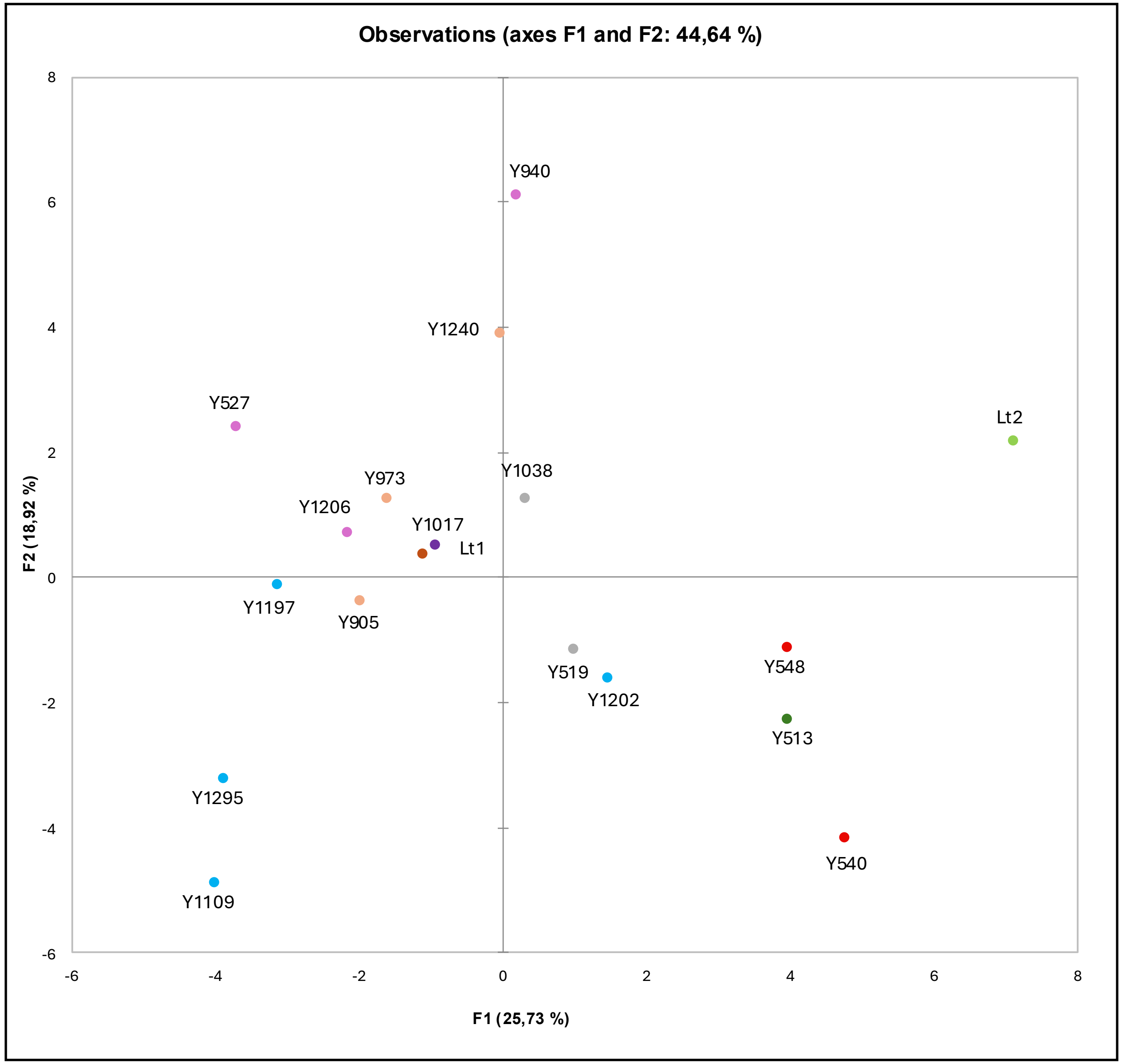
Volatile organic compounds (GC-MS)- PCA of all strains

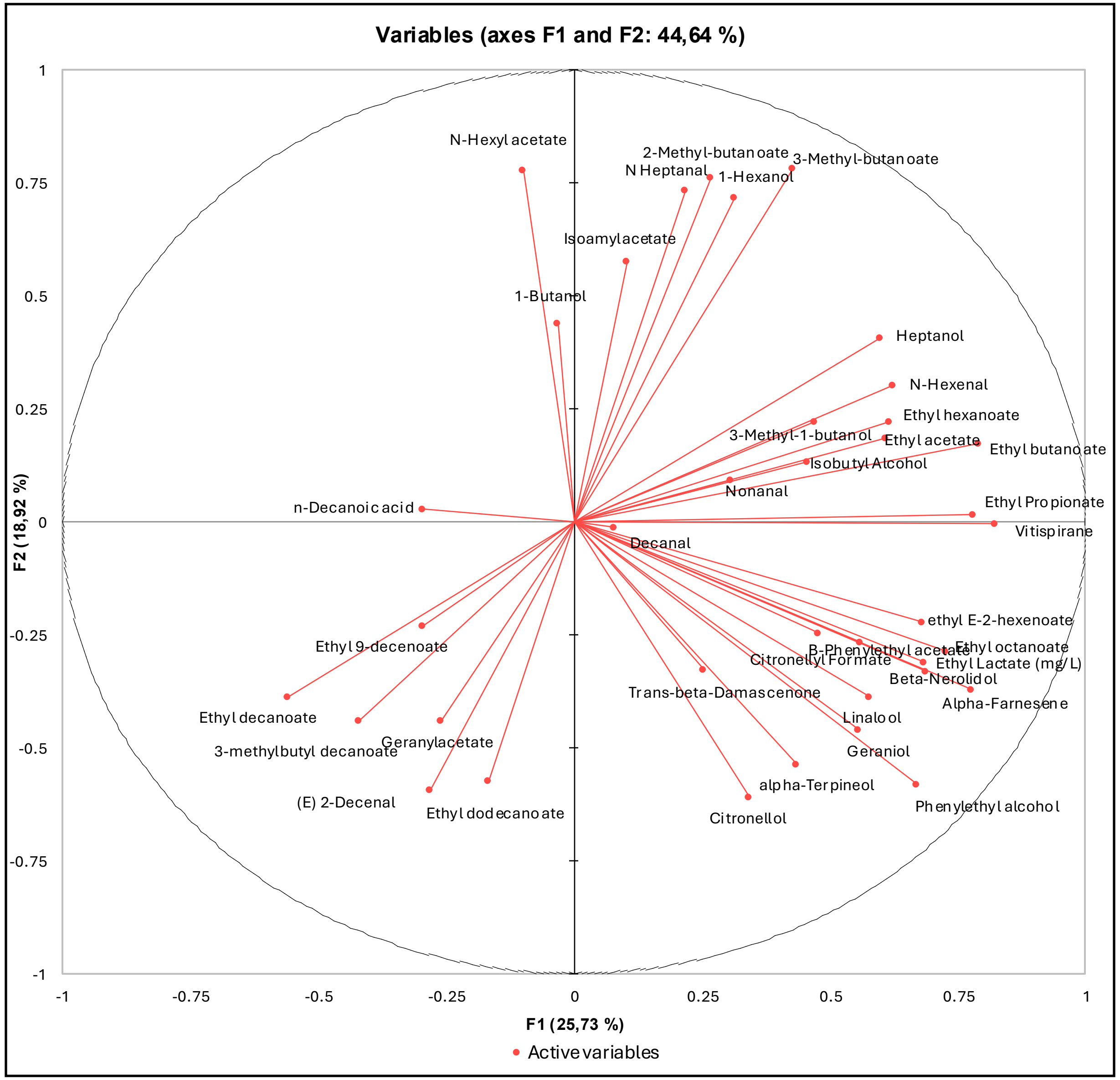




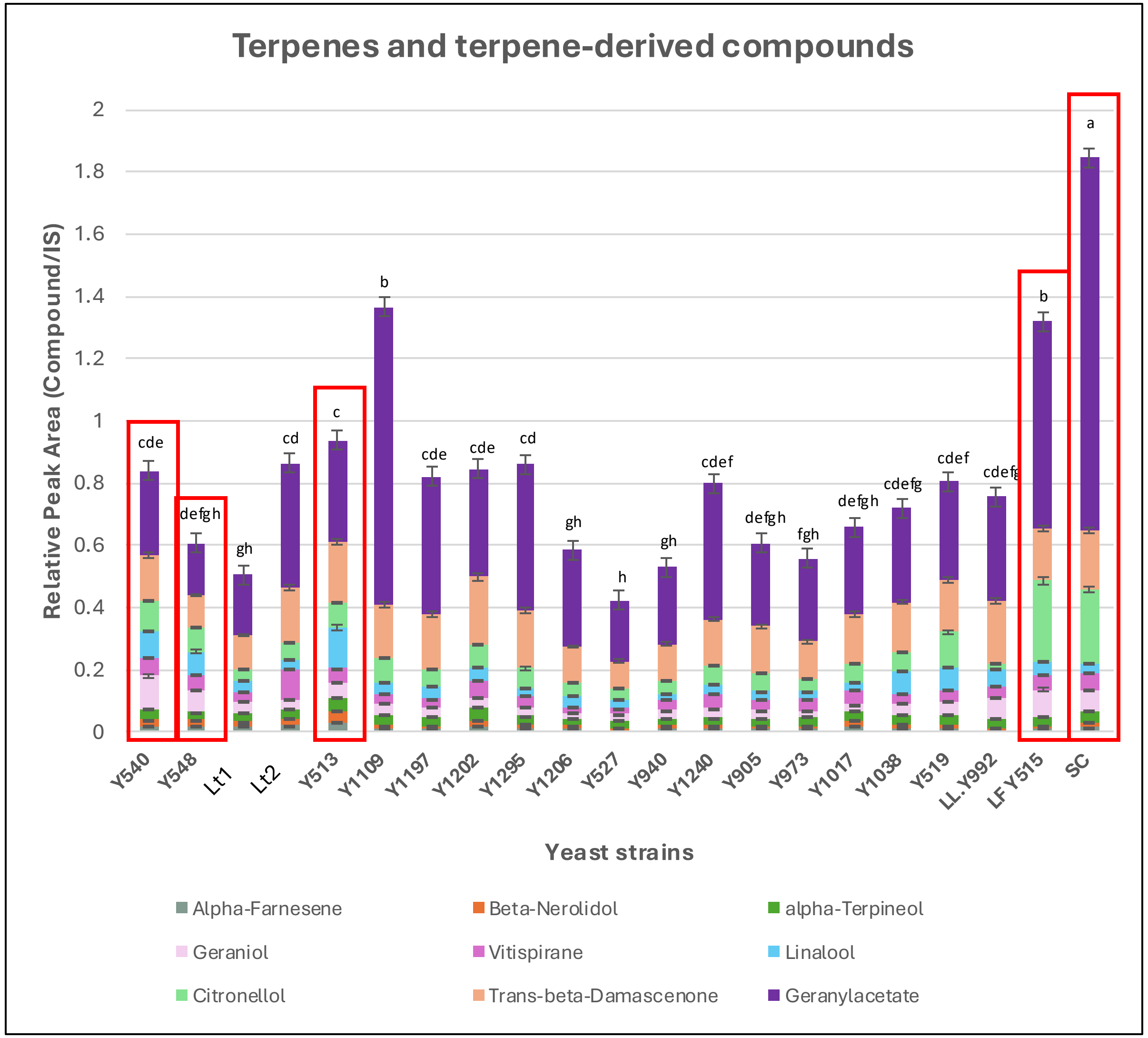
(Vicente et al., 2021 and Hranilovic et al., 2021, 2022)

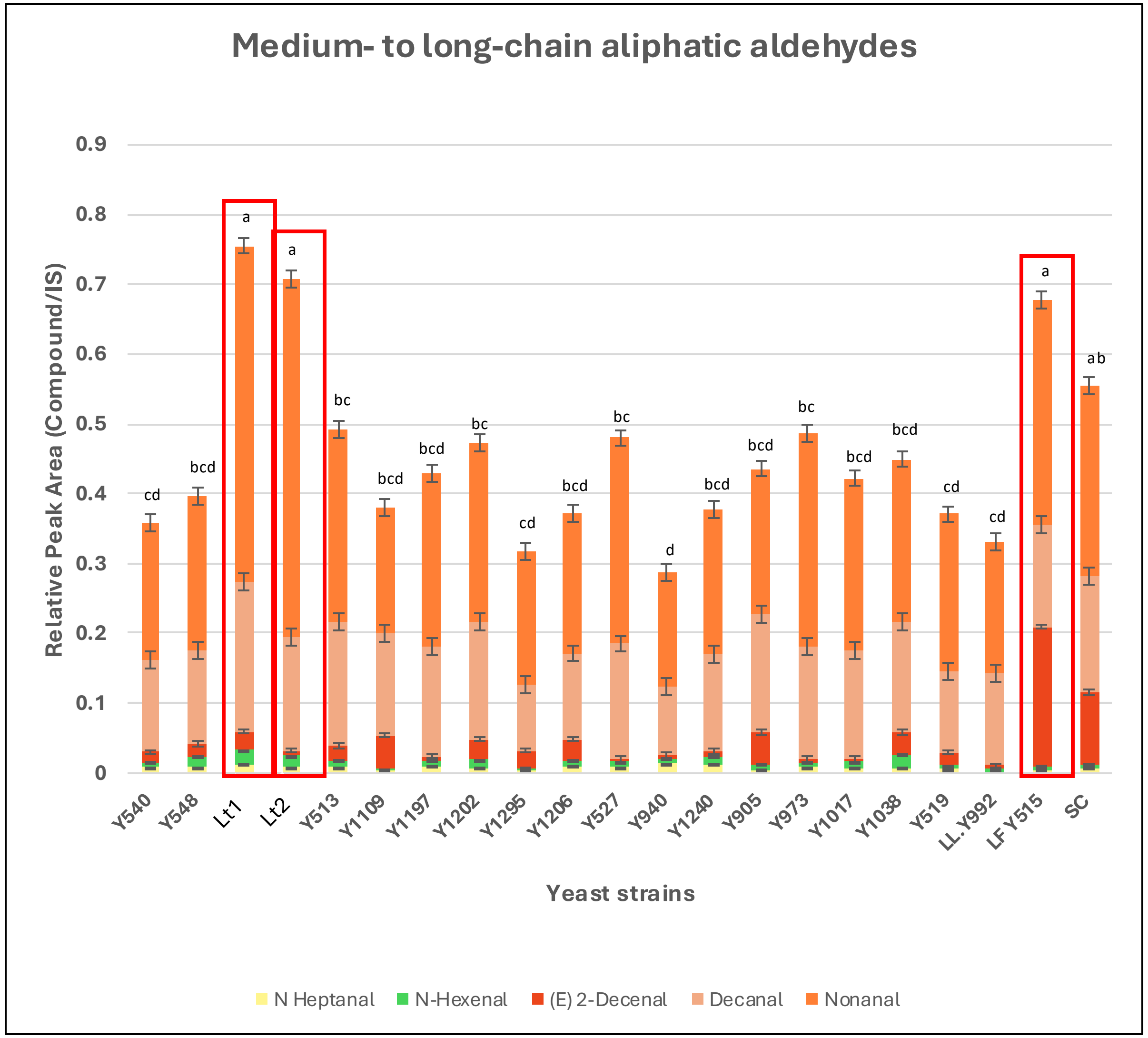
Volatile organic compounds (GC-MS) – PCA of only *L. thermotolerans* strains



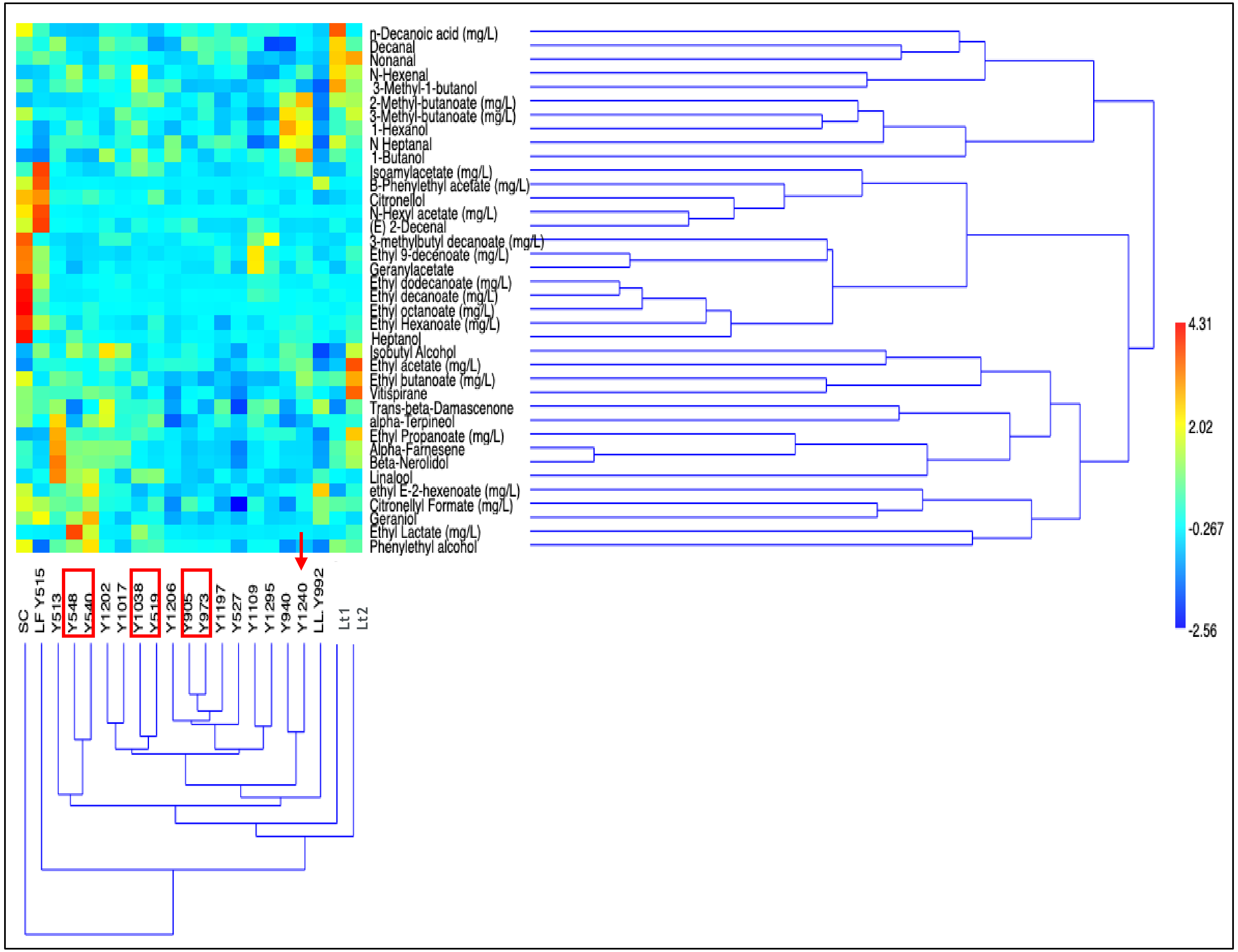


Focus on Terpenes and Aldehydes: Exploring Understudied Volatiles in *Lachancea thermotolerans*





Volatile organic compounds (GC-MS)



- VOC clustering **mostly matches** genetic clusters, with **few exceptions** (e.g., Y1240 not clustering with Y905 and Y973)

Vicente et al., 2021; Hranilovic et al., 2021, 2022; Mucalo et al., 2023; Fleming and Threlfall, 2024; and Vicente et al., 2024

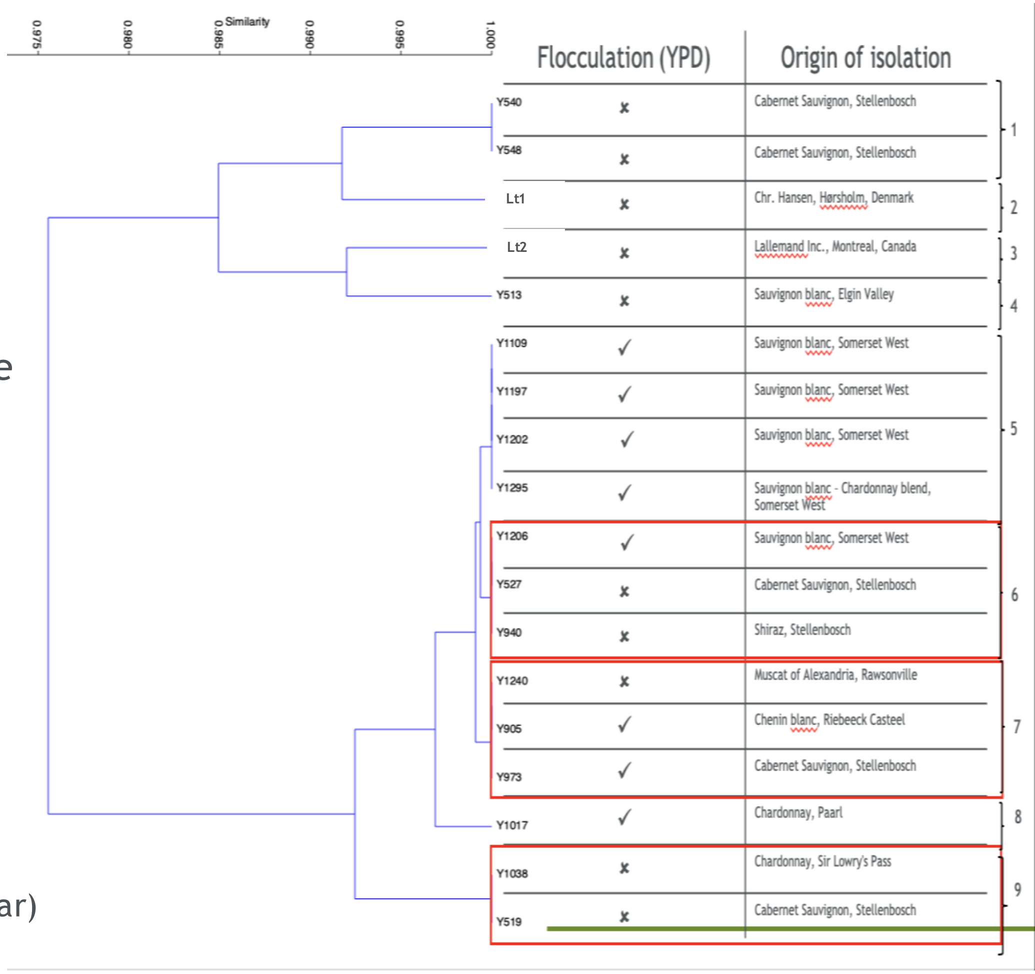
Conclusion (Objective 1 and 2):

Scientific

- Microsatellite typing grouped 18 *L. thermotolerans* strains into **9 genetic clusters** (high genetic diversity).
- **Three groups that showed inconsistencies**:
  - **Group 6**: Y1206 differed from Y527 and Y940.
  - **Group 7**: Y1240 differed from Y905 and Y973.
  - **Group 9:** Y1038 and Y519 behaved the same despite initial uncertainty.
- Flocculation proved useful. Independent of strain origin.
- Strains behaved similarly within respective clusters, each showing distinct metabolite profiles (studying variability is important)
- Shared species-wide trends relevant to consumer preferences.

Applied

- Beneficial traits:
  - High lactic (cluster 1-4) & succinic acid, strong malic acid consumption, and lower ethanol yield → better wine balance; attractive yeast for a warming climate.
  - Volatile compounds: More cluster-dependent
    - ➢ Higher alcohols: phenylethanol (rose), isobutanol (fruity)
    - ➢ Esters: ethyl lactate (creamy), ethyl acetate (fruity), MCFA ethyl esters (tropical/pear)
    - ➢ Terpenes (floral/citrus)
    - ➢ Aldehydes: decanal & nonanal (citrus/green)
    - Enhances fruitiness and aroma complexity.



Take home message: The future of better-balanced, fruit-driven wines is already here — and it starts with choosing the right *Lachancea thermotolerans* strain.

Next step:

- **Limitation:** All work was done in a controlled environment with sterile grape juice and in monoculture – not fully representative of industry conditions.
- **Key questions:**
    - Will beneficial traits of *L. thermotolerans* persist in a more competitive real grape juice environment with *S. cerevisiae* inoculated?
    - How does grape variety or matrix affect these traits?
    - What is the impact of different fermentation conditions?
- **Objective 3: Aim to address these questions, but data analysis is still in progress and therefore not shown.**

Acknowledgements:

- Supervisors: Professor B. Divol and Professor E. Setati
- Fermentis
- NRF
- Central analytical facility (CAF): Rene Veikondis (Senior analyst)
- CA Lab: Dr. Hans Eyeghe-Bickong and Dr. Anke Berry
- Winesteins

Stellenbosch University Iyunivesithi Universiteit

SAGWRI South African Grape and Wine Research Institute

Thank you
Enkosi
Dankie

Photo by Stefan Els