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# Adaptive Mechanisms of Wine Yeast Strains Under Ethanol-Induced Stress

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## BACKGROUND AND AIM

During alcoholic fermentation, yeast cells are exposed to multiple stress factors, including the accumulation of ethanol [1]. Ethanol penetrates cell membranes, disrupting membrane fluidity and permeability, which leads to the leakage of intracellular components and compromises cellular homeostasis. It also affects mitochondrial function, reduces ATP production, and induces oxidative stress, potentially causing DNA damage and decreased cell viability [2].

This study aimed to investigate the effects of ethanol stress on the growth, membrane fluidity, and cell surface morphology of enological yeast strains with different fermentative behaviour, including those from both *S. cerevisiae* and *non-Saccharomyces* species (**Table 1**). These strains are commercialized as wine starter by AEB S.p.A. and preserved at the Unimore Microbial Culture Collection (UMCC - www.umcc.unimore.it).

| Commercial Name | Codes | Species |
|---|---|---|
| Fermol Mediterranèe | PB2590 - UMCC 6 | *Saccharomyces cerevisiae* |
| Fermol Blanc | PB2019 - UMCC 19 | *Saccharomyces cerevisiae* |
| Fermol Rouge | PB2023 - UMCC 20 | *Saccharomyces cerevisiae* |
| Fermol Red Fruit | PB2018 - UMCC 2592 | *Saccharomyces cerevisiae* |
| Fermol Lime | PB2101 UMCC 3064 | *Saccharomyces cerevisiae* |
| Fermol Tropical | PB2151 - UMCC 3065 | *Saccharomyces cerevisiae* |
| Fermol Fleur | PB2171 - UMCC 3066 | *Saccharomyces cerevisiae* |
| Fermol Arome Plus | PB2010 - UMCC 24 | *Saccharomyces cerevisiae* |
| Fermol Sauvignon | PB2530 - UMCC 263 | *Saccharomyces cerevisiae* |
| Levulia Pulcherrima | MCR 24 - UMCC 15 | *Metschnikowia pulcherrima* |
| Levulia Torula | BBMV3FA5 - UMCC 5 | *Torulaspora delbrueckii* |

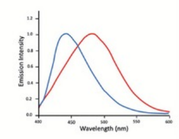
***Table 1.*** *Yeast strains used in the current study.*

## EXPERIMENTAL DESIGN AND MAIN GOALS

1 MICROVINIFICATION ASSAY

2 CELL GROWTH ASSAY

3 LAURDAN GENERALIZED POLARIZATION (GP) ASSAY




$GP = (I_{440} - I_{490})/(I_{440} + I_{490})$

4 ATOMIC FORCE MICROSCOPY (AFM) MEASUREMENTS

### 1 EVALUATION OF FERMENTATIVE PERFORMANCE

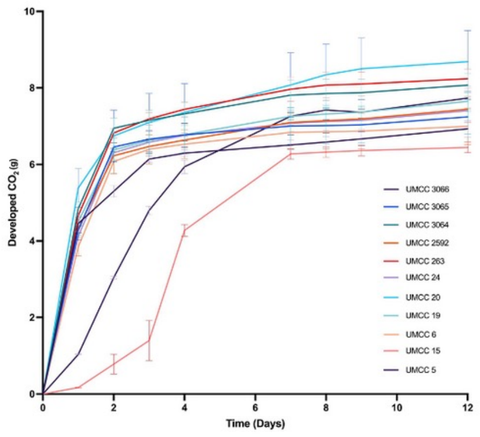


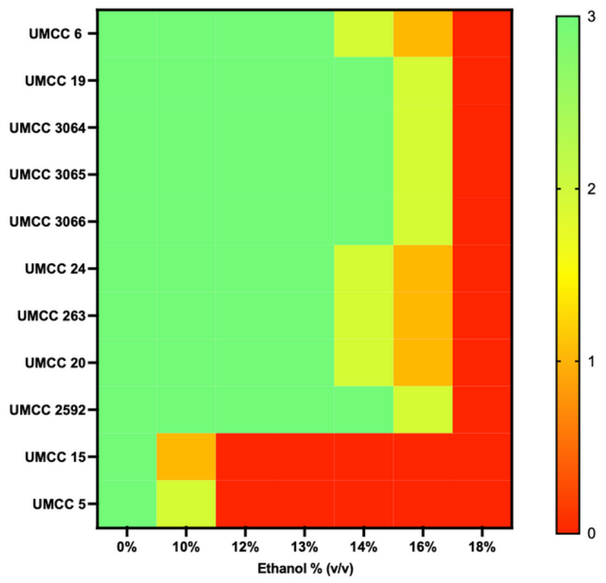

***Figure 1.*** **Developed $CO_2$** over the course of 12 days microfermentative trials in grape juice.

| Sample | FR | FV (g) |
|---|---|---|
| **UMCC 20** | 1 | 7.11 |
| **UMCC 6** | 0.84 | 6.08 |
| **UMCC 24** | 0.85 | 6.31 |
| **UMCC 19** | 0.91 | 6.38 |
| **UMCC 5** | 0.29 | 3.04 |
| **UMCC 263** | 0.67 | 6.82 |
| **UMCC 2592** | 0.89 | 6.23 |
| **UMCC 15** | 0.19 | 0.78 |
| **UMCC 3066** | 0.90 | 5.30 |
| **UMCC 3065** | 0.92 | 6.46 |
| **UMCC 3064** | 0.95 | 6.95 |

***Table 2.*** **Fermentative ratio (FR) and fermentative vigor (FV)** for the eleven yeast strains.

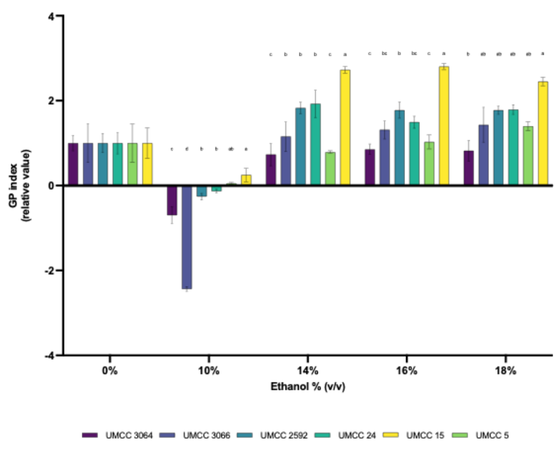
### 2 EVALUATION OF GROWTH AT DIFFERENT ETHANOL CONCENTRATIONS

***Figure 2.*** **Growth of yeast strains in YPDA medium supplemented with 0%, 10%, 12%, 13%, 14%, 16%, and 18% (v/v) ethanol.** The legend indicates growth levels: red represents no growth, while green represents maximum growth.




### 3 EVALUATION OF MEMBRANE FLUIDITY

***Figure 3.*** **Membrane fluidity** expressed as relative GP index values, which are inversely correlated with fluidity, for *S. cerevisiae* strains UMCC 3064, UMCC 3066, UMCC 2592, UMCC 24, and *non-Saccharomyces* strains UMCC 15 and UMCC 5 after exposure to 0%, 10%, 14%, 16%, and 18% (v/v) ethanol for 24 hours.




### 4 EVALUATION OF CELL SURFACE MORPHOLOGY

***Figure 4.*** **AFM images** of *S. cerevisiae* UMCC 3066, UMCC 24, and *non-Saccharomyces* strains UMCC 5 and UMCC 15 after exposure to 0%, 10%, and 18% (v/v) ethanol for 24 hours.

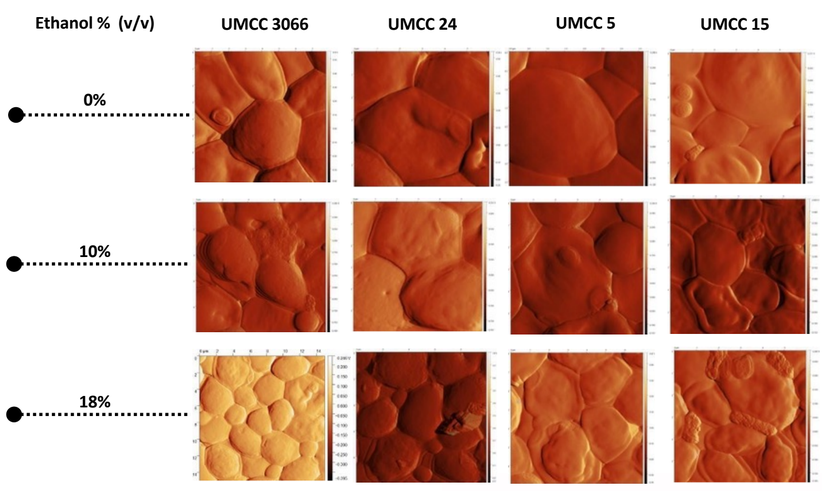
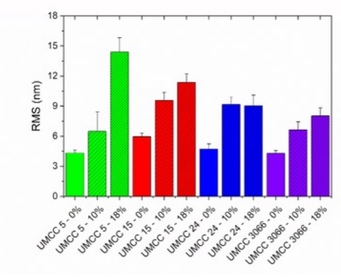



***Figure 5.*** **Root Mean Square (RMS) values** for strains *S. cerevisiae* UMCC 3066, UMCC 24, and *non-Saccharomyces* strains UMCC 5, UMCC 15 after exposure to 0%, 10%, and 18% (v/v) ethanol.




## TAKE-HOME MESSAGES

- At low ethanol stress (10% v/v), membrane fluidity increased [3], especially in strains capable of growing at ethanol concentrations up to 16% (v/v) and exhibiting superior fermentative performance. In contrast, less tolerant strains, such as *T. delbrueckii* UMCC 5 and *M. pulcherrima* UMCC 15, showed smaller increases.
- *Non-Saccharomyces* strains exhibited higher surface roughness (RMS values) at 18% ethanol, suggesting greater instability under high ethanol stress. In comparison, more tolerant strains showed lower RMS values, indicating better adaptability.

## REFERENCES

*[1] Learmonth, Robert P. "Membrane fluidity in yeast adaptation: insights from fluorescence spectroscopy and microscopy." Reviews in fluorescence 2010. New York, NY: Springer New York, 2011. 67-93.*
*[2] Lairón-Peris, María, et al. "Lipid composition analysis reveals mechanisms of ethanol tolerance in the model yeast Saccharomyces cerevisiae." Applied and environmental microbiology 87.12 (2021): e00440-21.*
*[3] Yang, Yijin, et al. "Membrane fluidity of Saccharomyces cerevisiae from Huangjiu (Chinese rice wine) is variably regulated by OLE1 to offset the disruptive effect of ethanol." Applied and environmental microbiology 85.23 (2019): e01620-19.*