Comparative Ethanol Stress Response and Membrane Fluidity in *Saccharomyces* and Non-*Saccharomyces* Yeast Strains

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UNIMORE UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA — Unimore Microbial Culture Collection

BACKGROUND

Yeast cells are subjected to various stresses during alcoholic fermentation, including ethanol-induced stress, which can alter membrane fluidity, compromise its integrity and increase its permeability (Fig.1). Furthermore, ethanol can deactivate key enzymes, including ATPases and glycolytic enzymes, resulting in reduced cell growth [1].

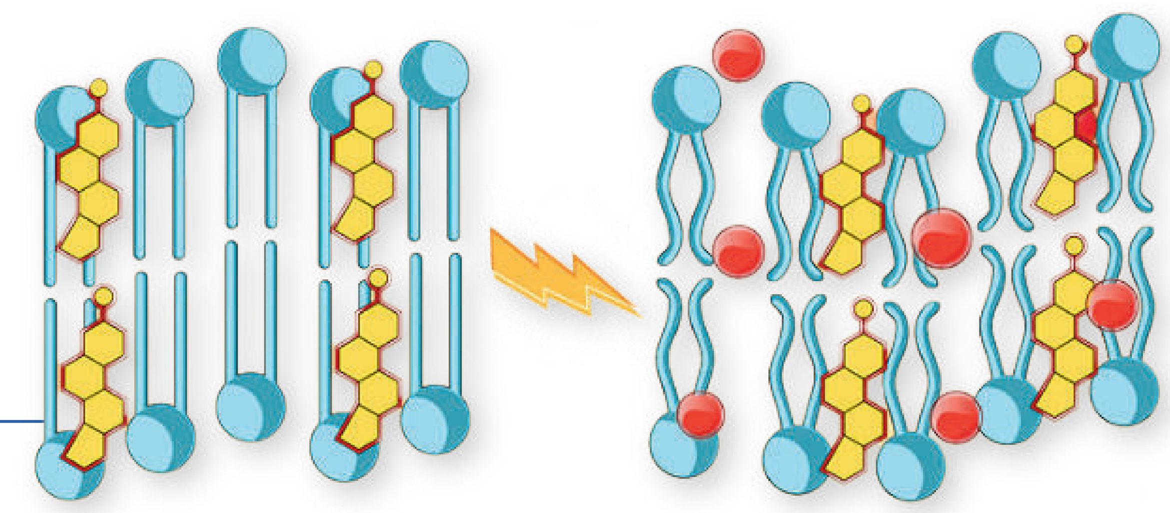


Figure 1. Plasma membrane modification after ethanol stress.

Commercial Name	Code	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	*Toluraspora delbrueckii*

Table 1. Yeast strains used in the current study.

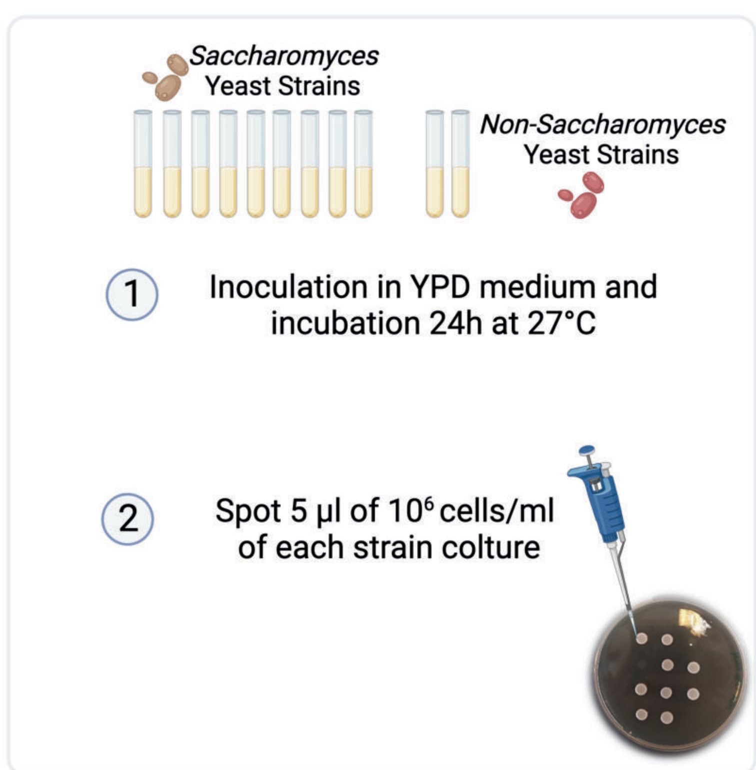
AIM OF THE STUDY

This study examined ethanol's effects on growth and membrane fluidity of eleven yeast strains, including nine *Saccharomyces* and two non-*Saccharomyces* strains (Tab.1). These strains are commercialized by AEB S.p.A. and preserved at the Unimore Microbial Culture Collection (UMCC); www.umcc.unimore.it.

METHODS AND RESULTS

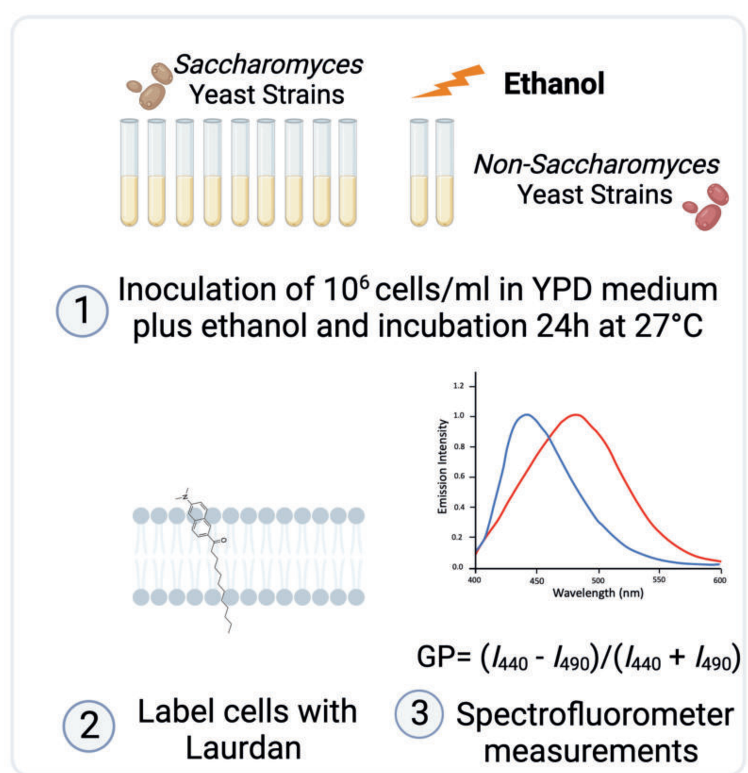
The impact of ethanol on yeast growth was assessed using selective media, while membrane fluidity was evaluated by measuring the generalized polarization of Laurdan (GP) with a spectrofluorometer, following the protocol outlined by Learmonth [2] with slight modification.

EVALUATION OF ETHANOL TOLERANCE



Spot Growth assay on solid media containing YPD or YPD plus ethanol 10-14%, 16% and 18%

EVALUATION OF MEMBRANE FLUIDITY



Laurdan GP assay after 24h of treatment with different concentration of ethanol (10%, 14%, 16% and 18%)

ETHANOL TOLERANCE

All ***Saccharomyces*** **strains** tolerated ethanol well up to 14% (v/v). Only UMCC 3066, UMCC 3065, UMCC 3064, UMCC 2592, and UMCC 19 maintained good tolerance even at 16% (v/v).

Non-*Saccharomyces* strains exhibited impaired growth when subjected to concentrations exceeding 10% (v/v) ethanol.

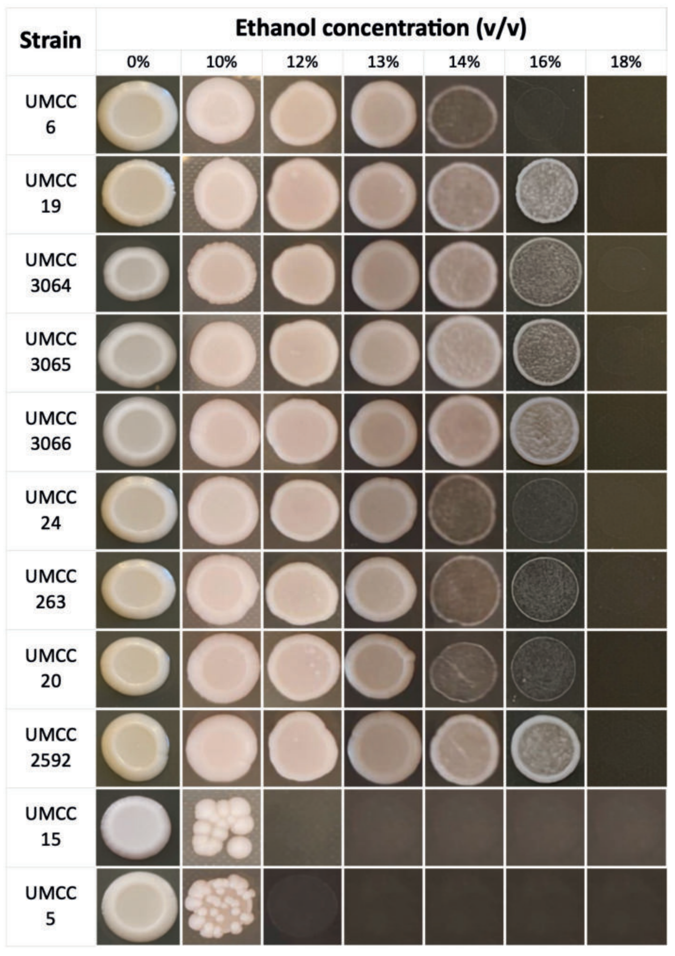


Table 2. Growth on selective solid media.

MEMBRANE FLUIDITY

Saccharomyces strains showed increased membrane fluidity and lower GP index at 10% ethanol. At 14%, fluidity decreased but stabilized at 16% and 18% in more tolerant strains (Fig.2), while less tolerant strains showed greater fluctuations (Fig.3).

Among **non-*Saccharomyces***, T. *delbrueckii* (UMCC 5) increased fluidity at 10% ethanol and decreased at 14%, similar to *Saccharomyces* strains. In contrast, M. *pulcherrima* (UMCC 15) had no significant fluidity increase at 10% but showed a decrease at 14% (Fig.4).

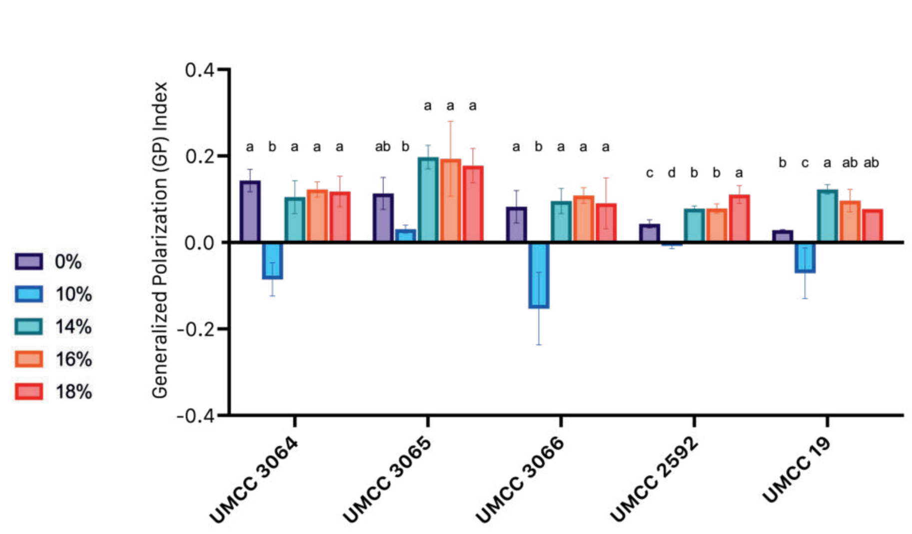


Figure 2. GP index in Saccharomyces strains with Enhanced Ethanol Tolerance

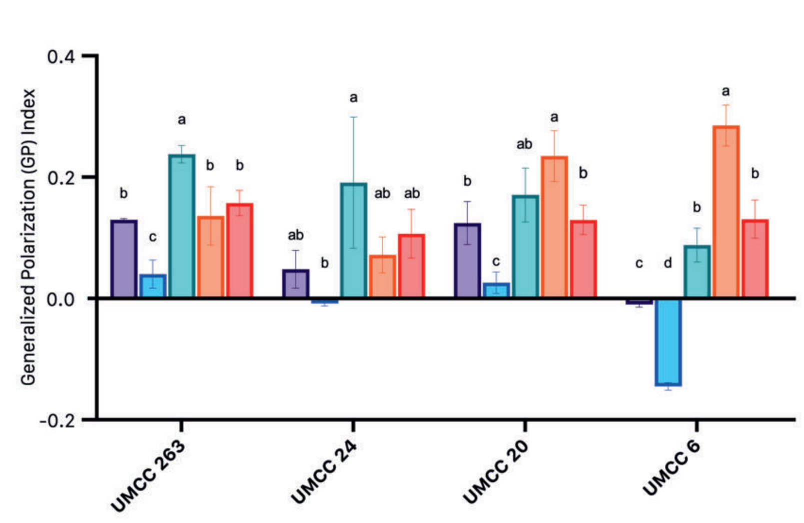


Figure 3. GP index in Saccharomyces strains with Lower Ethanol Tolerance

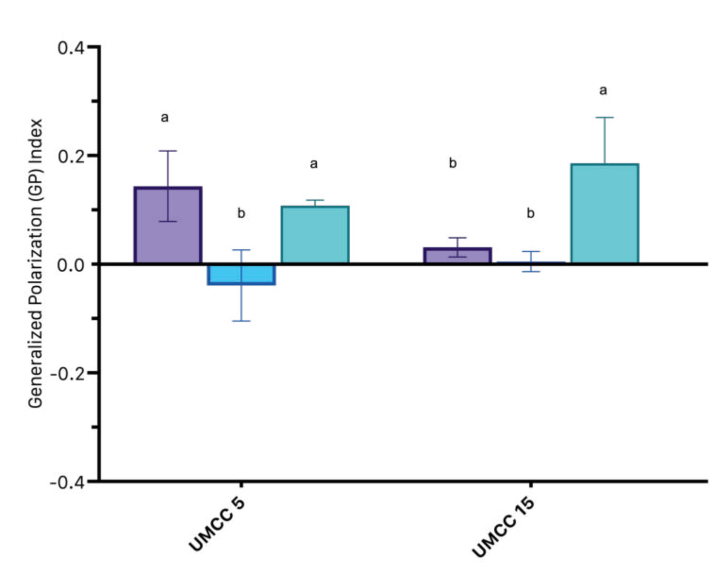


Figure 4. GP index in Non-Saccharomyces strains.

OUTPUTS

- *Saccharomyces* strains displayed robust growth even at high ethanol levels, with fluidity increasing at lower concentrations and decreasing at higher ones [3]. Strains tolerant up to 16% ethanol exhibited stable stiffening at higher concentrations, indicating better adaptability.
- Growth of non-*Saccharomyces* strains was inhibited above 10% ethanol. UMCC 5 increased fluidity at 10%, unlike UMCC 15. Both had reduced fluidity at higher levels, likely due to decreased growth [2].

This study highlights the distinct abilities of these strains to cope with ethanol stress in terms of growth and membrane fluidity. Future research will test grape must substrates to develop more resistant yeasts.

References:

[1] 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.

[2] 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.

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