IOCFE 2025 Conference
The 1st International Online Conference on Fermentation
12–13 November 2025 | Online
MDPI

Novel strategies for enhancing *Torulaspora delbrueckii* performance in wine fermentation

Alberto Martínez (1), Luis M. Hernández*(1), María L. Franco (2), Manuel Ramírez (1)
(1) Dpto. de Ciencias Biomédicas, Facultad de Ciencias, Universidad de Extremadura, Badajoz, España.
Estación Enológica, Junta de Extremadura, 06200 Almendralejo, España.

INTRODUCTION & AIM

Torulaspora delbrueckii (*Td*) is a valuable non-*Saccharomyces* yeast in winemaking, known for enhancing complexity and imparting attractive dried fruit and pastry notes. However, its biotechnological relevance is hindered by lower fermentation efficiency and smaller cell size compared to *Saccharomyces cerevisiae* (*Sc*).To overcome these limits, four main strategies have been developed:

1. Genetic Improvement: Selecting spontaneous *Td* mutants resistant to stressors like SO_2, ethanol, and CO_2 significantly improved base wine fermentation performance (1).
2. Interspecific Hybridization: Mixing *Td* and *Sc* vegetative cells produced hybrid clones with enhanced intermediate properties, including better stress resistance (2).
3. Increased Ploidy: *Td* naturally increases its ploidy under stress to prolong survival and metabolic activity. Using increased-ploidy inocula boosts fermentation kinetics and may simplify industrial filtration (due to larger cell size) (3).
4. Novel Intra-specific Hybridization: A new method is proposed to cross different *Td* strains by exploiting the yeast's conjugation requirement before sporulation, aiming to generate improved starter strains.

The aim of this presentation is to explore the achievements obtained through the first three strategies mentioned. Intra-specific hybridization is currently under investigation and is the subject of a separate presentation at this conference.

METHOD

STRATEGY 1. Genetic improvement involved isolating spore clones free of growth-retarding alleles from wine *Td* strains.

Mutants resistant to SO_2, ethanol, and high CO_2 pressure were then obtained.

STRATEGY 2. Yeast mixture for eventual hybridization between *Td* and *Sc*.

STRATEGY 3. *Td* is subjected to stressful conditions of culture in which it transitions to diploid or polyploid states.

RESULTS & DISCUSSION

STRATEGY 1. Spore clones of *Td,* free of recessive growth-retarding alleles, were obtained and used for successive selection of mutants resistant to SO_2, then ethanol, and finally high CO_2 pressure.

Figure 1 shows the fermentation kinetics of *T. delbrueckii* SO_2 resistant mutants (*Td* Mut5B-SO_2^R and *Td* Mut6ASO$_2^R$) and SO_2 + ethanol resistant mutants (*Td* Mut6A-SO_2^R-EtOH^R-31 and *Td* Mut6A-SO_2^R-EtOH^R-33) in synthetic must (A) and synthetic must containing 50 mg/L SO_2 (B). The fermentation capability in synthetic must of the SO_2 + ethanol resistant mutants (named *Td* Mut6A-SO_2^R-EtOH^R-31 and *Td* Mut6A-SO_2^R-EtOH^R-33, showed a slight improvement although it became irrelevant after 14 days of fermentation (Figure 3A). However, an evident and relevant improvement was observed in*Td* Mut6A-SO_2^R-EtOH^R-31 and *Td* Mut6A-SO_2^R-EtOH^R-33 that was maintained throughout fermentation (Figure 1B).

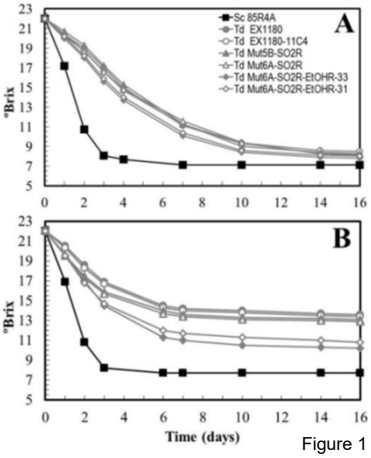

Figure 1

STRATEGY 2. Interspecific hybridization by mixing *Td* and *Sc* was assayed. The resulting *Sc*-mixed *Td* clones exhibited an intermediate phenotype for key biotechnological traits (e.g., resistance to SO_2, ethanol, copper, high CO_2 pressure, and high temperature, and flocculation potential). Crucially, several clones showed improved base wine fermentation, approaching the performance of the *Sc* parent strain.

As shown in Figure 2, Clone *Sc*x*Td*-3 was the best performer, matching the kinetic performance of *Sc* 85R4A in both synthetic must conditions (without or with 100 mg/L SO_2) after seven days of fermentation (A,B). In synthetic base wine (C, D), only *Sc*×*Td*-1 and *Sc*x*Td*-3 completed the fermentation, although they took 7 to 8 days longer than the control *Sc* 85R4A. The fermentations inoculated with the rest of *Sc*-mixed *Td* yeasts and *Td* EX1180 did not start, or they stopped after 2 days.

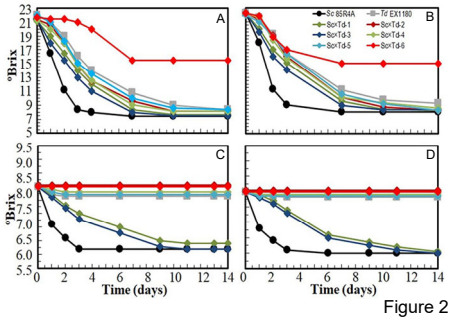

Figure 2

STRATEGY 3. In nutrient-deficient media, *Td* haploid cells became polymorphic, enlarged, and transition to diploid or polyploid states, which seems to be a specific adaptation of *Td* to the stressful fermentation conditions. *Td* inocula with increased ploidy showed enhanced fermentation efficiency compared to haploid inocula of the same strains.

Figure 3. Increased-ploidy *Td* inocula significantly improved fermentation kinetics in synthetic must compared to conventional controls (YEPD stationary cultures) (Fig. 3A). While strains like *Td* EX1180 showed superior capacity to *Td* A58, none initially matched the efficiency of the *Sc* reference yeast. Critically, the best inoculum, *Td* EX1180 prepared with increased ploidy (EX1180-SPO), completed fermentation achieving the performance of *Sc* 229-SPO by the 13th day (Fig. 3C). This demonstrates the superiority of ploidy-enhanced inoculation over conventional methods for boosting *Td* performance and achieving fermentation completion.

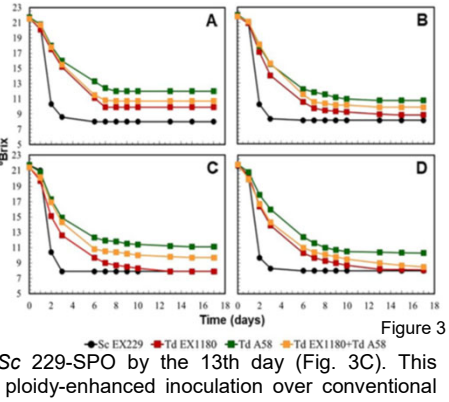

Figure 3

CONCLUSION

This combined strategies (genetic improvement, cell mixture/hybridization, and ploidy manipulation) offer **promising avenues** for optimizing *Td*'s performance in industrial wine production. Its implementation will help maintain the organoleptic benefits of *Td* while overcoming its historical limitations in terms of fermentation efficiency and stress tolerance.

FUTURE WORK / REFERENCES

Currently, our work in the lab is focused on the obtainment and analysis of *Td*x*Td* hybrids with improved wine fermentation properties.

(1) Velázquez et al. (2020) http://doi.org/10.3390/microorganisms8091372.
(2) Martínez et al. (2022) https://doi.org/10.3389/fmicb.2022.1006978
(3) Martínez et al. (2024) https://doi.org/10.1016/j.ijfoodmicro.2024.110894