CONSIDERATIONS ON THE USE OF *Torulaspora delbrueckii* FOR WINEMAKING

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INTRODUCTION

Of the non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* is probably the most suitable for use in winemaking. This is because it has a good fermentation performance compared to other non-*Saccharomyces* yeasts that might be considered for winemaking, such as *Hanseniaspora uvarum*, *H. vineae*, *Candida zemplinina*, *C. pulcherrima*, *C. stellata*, *Schizosaccharomyces pombe*, *Hansenula anomala*, *Metschnikowia pulcherrima*, *Lachancea thermotolerans*, *Pichia fermentants*, *P. kluyveri*, and *Kazachstania aerobia*.

Additionally, it has been claimed that *T. delbrueckii* can be used to optimise some wine parameters with respect to usual *S. cerevisiae* wines such as a low amount of acetic acid, lower ethanol concentration, increased amount of glycerol, greater mannoprotein and polysaccharide release, promoted malolactic fermentation, increased amounts of interesting aromatic compounds (fruity esters, lactones, thiols, and terpenes), and decreased amounts of unwanted aromatic compounds (such as higher alcohols); these may improve wine quality or complexity (reviewed by Benito in (1)). These additional features constitute the main reason why modern oenologists are conducting trials with this yeast, as well as with other non-*Saccharomyces* yeasts, for industrial winemaking.

However, this option entails additional complications and economic costs in controlling must fermentation with respect to the use of conventional selected strains of *S. cerevisiae*, which is undoubtedly the most reliable yeast species for this purpose. Some of the complications of using *T. delbrueckii* in commercial wineries come from its physiological properties under the stressing conditions that are usual in the winemaking process.

In this work, we review these specific properties, with a focus on their relevance for some applied aspects of winemaking.

RESULTS

Torulaspora cells mostly have a spherical shape (torulu), and are smaller than *Saccharomyces* (Fig. 1A, E). Its life cycle has yet to be elucidated. It was long believed to be haploid yeast, mainly because of its small cell size, and because tetrads are absent or rarely observed in sporulation media. However, it has recently been suggested that may actually be mostly diploid and homothallic. This small size results in lower biomass yields from batch cultures, and may be a handicap for large-scale production.

A strong dominance of *T. delbrueckii* is usually achieved in wine fermentation by using clarified must, and a killer strain for inoculation. In this way, the relative initial growth of the *T. delbrueckii* population is high, and favours its becoming the clear protagonist during fermentation. However, since is less resistant than *S. cerevisiae* to high ethanol concentrations (Fig. 2), the fermentation

rate slows down, and cell death increases after the tumultuous fermentation of sugar-rich musts (Fig. 3).

As a consequence, fermentation may stop, slow down and become sluggish, or continue mainly because of the contaminating wild *Saccharomyces* yeasts. This situation reduces the domination ratio of *Torulaspora*, that ocurrs even more rapidly in co-inoculation/sequential inoculation.

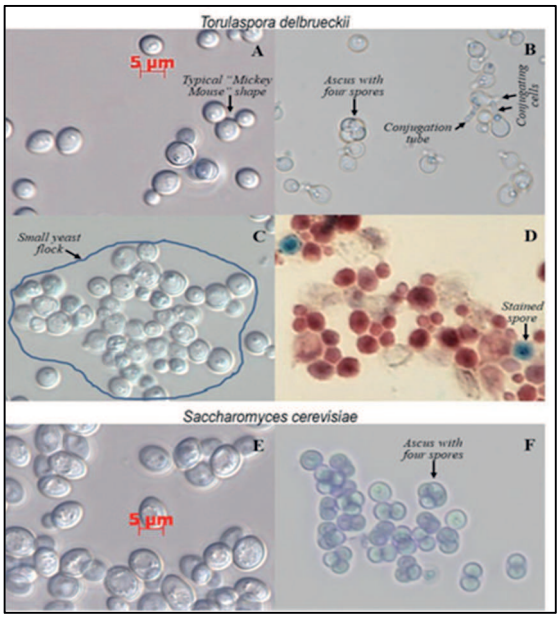

Figure 1. Vegetative cells (A, C, E) and spores (B, D, F) of T. delbrueckii and S. cerevisiae. Photomicrographs using Nomarski (vegetative cells) or bright-field illumination (spores). B, F: spores from minimal sporulation medium. D: spores from YEPD-agar stained with malachite green.

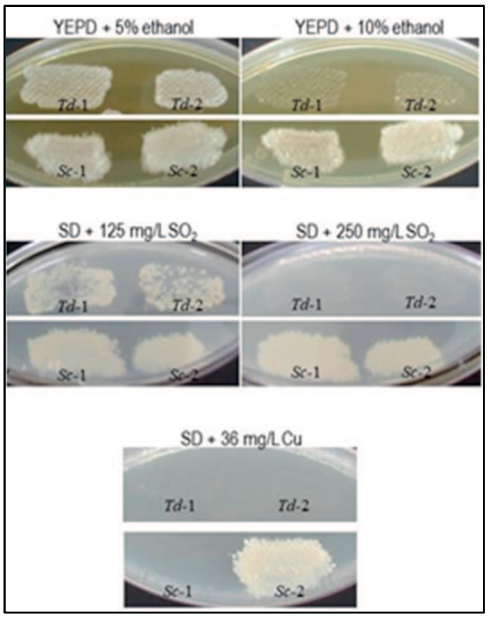

Figure 2. Resistance of T. delbrueckii (Td) and S. cerevisiae (Sc) to ethanol, SO_2 and Cu. Two strains of each yeast species are shown (Td-1 and Td-2, and Sc-1 and Sc-2, respectively). YEPD: rich medium. SD: minimal medium.

Based on our experience, some strains of *T. delbrueckii* can fully dominate and complete crushed grape fermentation to reach above 14º GL, which is a very high alcohol content for *non-Saccharomyces* yeast fermentation (Fig. 3A). However, this achievement occurs only rarely,

under certain favourable environmental conditions: presence of low amounts of competitor *Saccharomyces* yeasts, a large inoculum of healthy *T. delbrueckii* cells, frequent agitation to provide extra oxygen, addition of extra nutrients and low amounts of toxic compounds (SO_2, Cu, pesticides, etc.). Even under these favourable conditions, *T. delbrueckii*-dominated fermentation took much longer to complete than *S. cerevisiae*-dominated fermentation, because fermentation vigour and cell viability declined more quickly in *T. delbrueckii* than *in S. cerevisiae* after the wine reached about 5% alcohol (Fig. 3B).

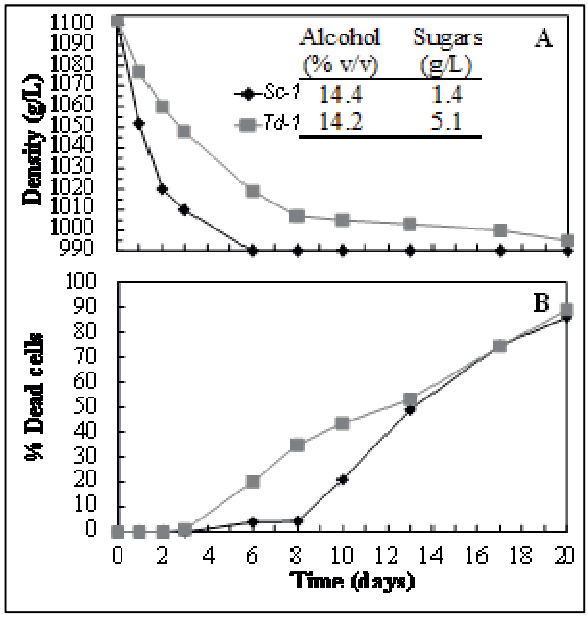

Figure 3. Must fermentation kinetics (A) and proportion of dead cells (B) of Garnacha grape fermentation inoculated with S. cerevisiae (Sc) and T. delbrueckii (Td). The concentrations of ethanol and residual sugars in the wines are given.

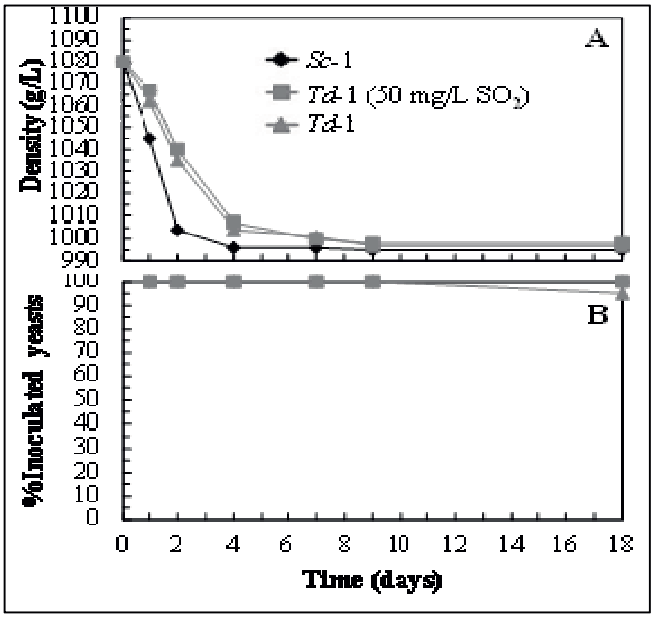

Figure 4. Must fermentation kinetics (A) and yeast population dynamics (B) of Pinot Noir grape fermentation inoculated with S. cerevisiae (Sc) and T. delbrueckii (Td) in the absence and presence of 50 mg / L SO_2.

The differences in ethanol resistance between the two yeast species can be seen on YEPD agar plates supplemented with different ethanol concentration (Fig. 2). Similarly, while *T. delbrueckii* is able to complete fermentation in the presence of 50 mg/L SO_2 (Fig. 4), although a partial lethal effect cannot be ruled out, it is clearly less resistant to this compound than *S. cerevisiae*.

CONCLUSIONS

In order to obtain the required improvement of wine quality, we would propose for the time being to use single inoculation with *T. delbrueckii* under appropriate conditions to achieve its full dominance, and then to mix the resulting wines with other *S. cerevisiae* wines.

REFERENCES

(1) Benito, S. The impact of *Torulaspora delbrueckii* yeast in winemaking. Appl. Microbiol. Biotechnol. 2018, 102, 3081–3094.
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ABSTRACT

Torulaspora delbrueckii is probably the most suitable *non-Saccharomyces* yeast for winemaking. This is because it has a good fermentation performance compared to other *non-Saccharomyces* yeasts that might be considered for winemaking, such as *Hanseniaspora uvarum*, *H. vineae*, *Candida zemplinina*, *C. pulcherrima*, *C. stellata*, *Schizosaccharomyces pombe*, *Hansenula anomala*, *Metschnikowia pulcherrima*, *Lachancea thermotolerans*, *Pichia fermentants*, *P. kluyveri*, and *Kazachstania aerobia*. Additionally, it has been claimed that *T. delbrueckii* can be used to optimize some wine parameters with respect to usual *S. cerevisiae* wines. However, this option entails additional complications and economic costs in controlling must fermentation with respect to the use of conventional selected strains of *S. cerevisiae*, which is undoubtedly the most reliable yeast species for this purpose. Some of the complications of using *T. delbrueckii* in commercial wineries come from its physiological properties under the stressing conditions that are usual in the winemaking process. In this work, we review these specific properties, with a focus on their relevance for some applied aspects of winemaking. Many of the claimed advantages for *Torulaspora* are based on results from different research studies that are often contradictory, or non-reproducible. The easiest explanation is to attribute these discrepancies to possible differences in behavior of the different strains of *T. delbrueckii* used in different researches. However, it is quite possible that the different results found in the literature are due to the variable or unpredictable behavior of *T. delbrueckii* depending on these environmental conditions. Given this current situation, in order to obtain the required improvement of wine quality, we would propose for the time being to use single inoculation with *T. delbrueckii* under appropriate conditions to achieve its full dominance, and then to mix the resulting wines with other *S. cerevisiae* wines.

ACKNOWLEDGMENT

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