Using the wine yeast *Saccharomyces cerevisiae* for acidity management in wine

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LALLEMAND OENOLOGY

INTRODUCTION

Over recent decades and in line with global warming, we have been seeing changes in the maturity levels of the grape berries that impact the sensory qualities of wines. Indeed, sugar levels have progressively increased, directly resulting in rises in the alcohol levels of wines, which can reach an additional 2-3%. In parallel, a reduction in the acidity of wines has also been observed, giving rise to heavier, less balanced wines (Figure 1). These developments run contrary to consumer trends and public health recommendations. Moreover, some countries have imposed taxes on wines with an alcohol content of more than 14%.

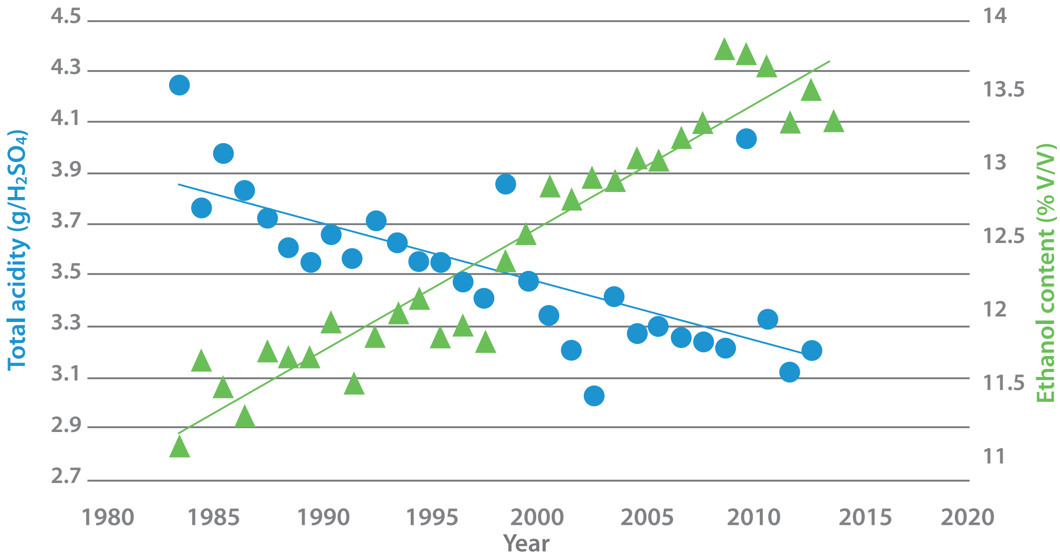

Figure 1. Evolution of the alcohol and acidity content in wines: a trend is confirmed.
(Source: *DUBERNET laboratories* – analyses of around 1,500 wines/year)

Wine yeasts convert the sugars of grape must (glucose and fructose) into ethanol and CO_2 (around 92% of sugars), into biomass, organic acids and aroma compounds, as well as into glycerol (around 3%). The yield of ethanol production during alcoholic fermentation is, on average, 0.47 g ethanol per g of sugar. To obtain 1° v/v alcohol, 16.8 g sugar must be consumed. This yield from the conversion of sugars into ethanol is only slightly variable within the *Saccharomyces cerevisiae* species and the different yeasts on the market all have very similar yields (Figure 2).

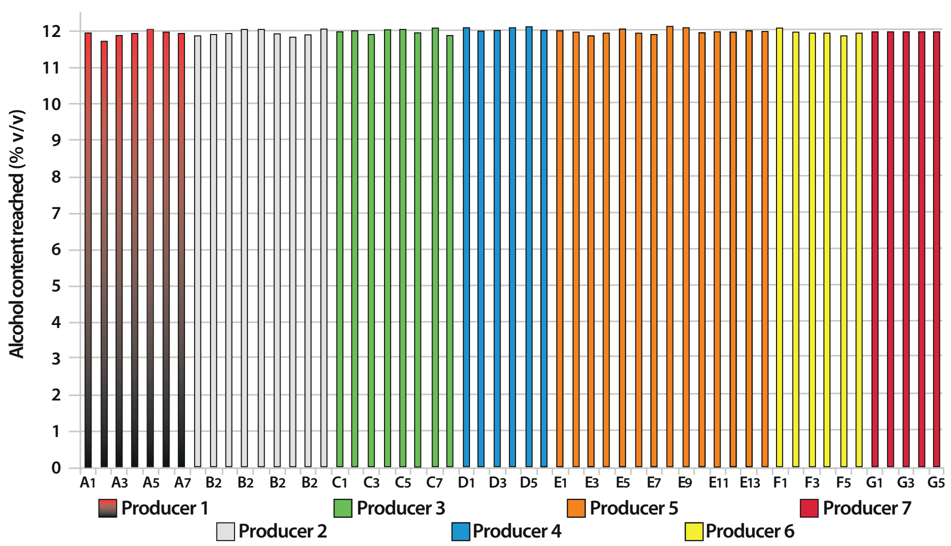

Figure 2. Alcohol content (% v/v) reached after inoculation with 56 strains of *Saccharomyces cerevisiae* in white wines, whose sugars were fermented in their totality (Palacios et al., 2007).

AN ORIGINAL APPROACH OF EVOLUTIONARY ADAPTATION

Lallemand, in collaboration with the Sciences for Enology laboratory of INRA in Montpellier, has therefore implemented an innovative selection process in order to obtain a non-GMO yeast whose metabolism would be diverted towards production of glycerol and organic acids, at the expense of ethanol production [1]. Glycerol is a colourless, odourless compound, often associated with taste sensations of volume depending on its concentration in wine.

The strategy adopted to obtain a new yeast meeting these criteria was that of evolutionary adaptation. This approach is perfectly suited to the context, making it possible to naturally select yeasts with specific properties, beyond the phenotypes of already-known strains. The principle consists of simply encouraging cells to adapt their metabolism to stressful conditions and then selecting those that meet the criteria.

Yeast cells were placed in culture under conditions of high osmotic stress. To combat these difficult conditions, they can respond by activating a metabolic pathway, the HOG (High Osmolarity Glycerol) pathway. This pathway allows for the overproduction of glycerol, which plays the role of osmoprotectant of cells. The yeasts are subcultured many times in this medium with high osmolarity, until several hundreds of generations are created. Over these generations, some cells will undergo spontaneous mutation that will give them a selective advantage and allow them to better resist the conditions of the medium. At the end of the process, the culture will be considerably enriched with these new, better adapted, glycerol-overproducing yeasts, which will then be easy to select (Figure 3).

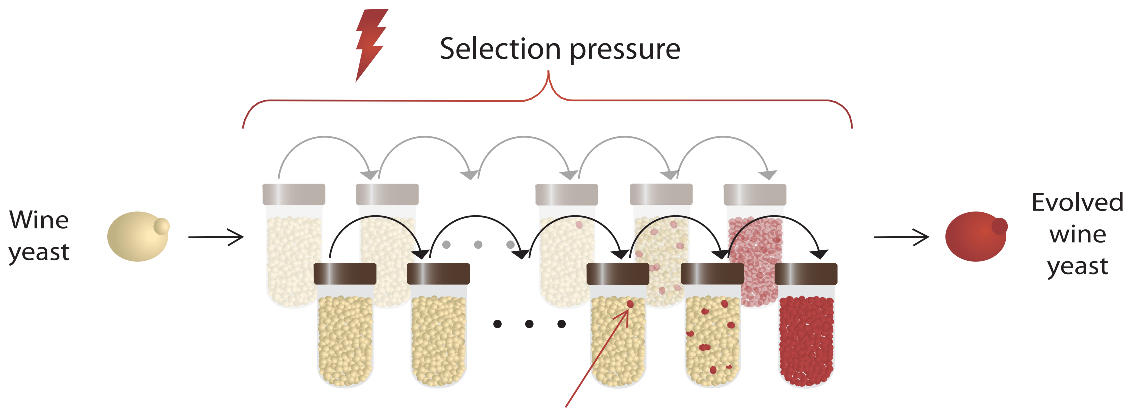

Figure 3. Schematic representation of the evolutionary adaptation approach

The process implemented led to the selection of an initial yeast, which was then further optimised by crossing to obtain a second-generation yeast that is overproductive of glycerol and has a reduced yield from sugar-to-alcohol conversion [2].

ETHANOL REDUCTION THROUGH INCREASED GLYCEROL AND THE IMPACT ON ACIDITY

This new yeast was first tested in laboratory trials on several types of synthetic and natural musts. These trials made it possible to validate the overproduction of glycerol and the reduction in ethanol production (Figure 4). More surprisingly, these trials demonstrated very low production of volatile acidity contrasting with a reduction in pH and an increase in total acidity.

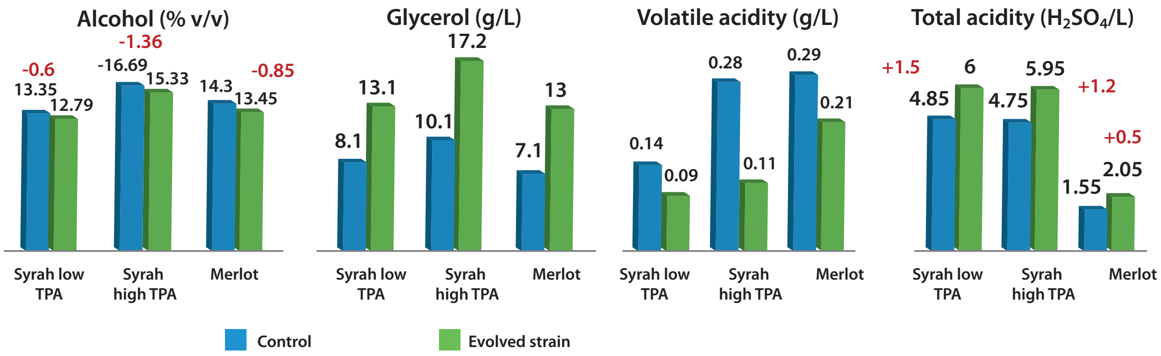

Figure 4. Production of ethanol, glycerol, volatile acidity and total acidity by the parent strain and the evolved strain on three natural musts (Syrah with low potential alcohol content, Syrah with high potential alcohol content and Merlot), fermented at 25°C.

REDIRECTED CARBON FLOWS

The increase in the total acidity is explained by the levels of the different organic acids present in the wines, these levels having been influenced by the metabolism of the new selected yeast. Several organic acids – such as succinic, α-ketoglutaric and malic acid – have been shown to be involved in this phenotype, with their combination leading to a noteworthy increase in the total acidity. Indeed, metabolic analyses on finished wines have demonstrated a significant increase in the succinate content and a marked increase in α-ketoglutarate, with pyruvate remaining relatively stable. Measurements of malic acid have also shown an increase between the initial and final content, suggesting production by the yeast during fermentation, or at least a positive production/consumption balance. The metabolism of these acids correlates since they all form part of the Krebs cycle (or tricarboxylic acid cycle).

An in-depth study of the metabolism and carbon balance was conducted for the new selected yeast and its parent yeast, enabling the calculation of the intracellular carbon flows in the different metabolic pathways. It appears that the flows are highest in the two branches of the tricarboxylic acid cycle, and also towards glycerol for our new yeast compared with a classic wine yeast (Figure 5). Inversely, they are lower towards acetate and ethanol. Variations in the number of moles of carbon per 100 moles of sugar consumed can go from -8% for acetate to +116% for glycerol. Variations in percentage relate to the metabolic flows and not to the final concentration in the compounds involved.

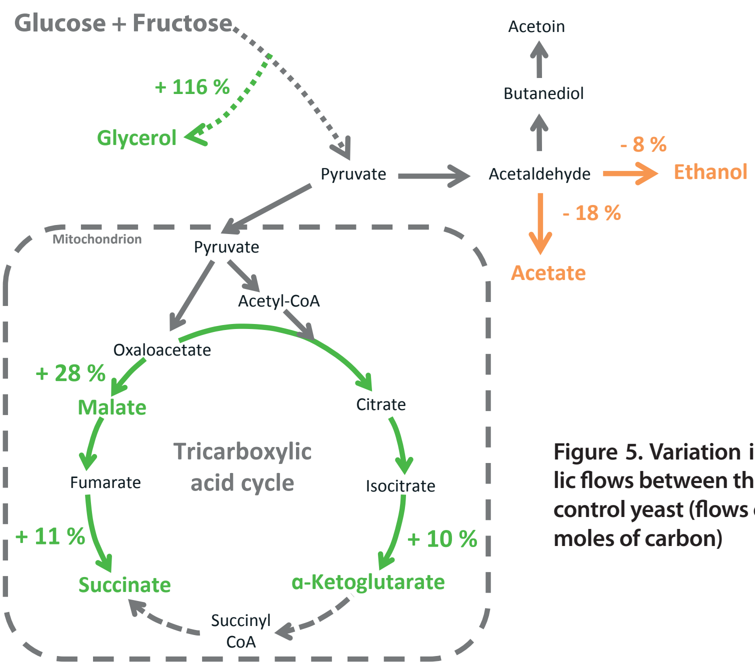

Figure 5. Variation in intracellular metabolic flows between the IONYS™ yeast and the control yeast (flows expressed in number of moles of carbon)

WINERY TRIALS

Three years of validation with a large number of winery trials carried out in France, Spain, Italy, California and Australia on a variety of musts (Syrah, Cabernet Sauvignon, Tempranillo, Pinot Noir, Grenache Noir, Touriga Nacional, Zinfandel, Petit Verdot, Mourvedre, Touriga Franca...) have confirmed a strong acidification power with a pH reduction of up to 0.2, a rise in the total acidity of 0.5-1.2, a reduction in ethanol of 0.3-0.8% under winery conditions, very low volatile acidity production and overproduction of glycerol (up to 17 g/L compared with 8 g/L for the control).

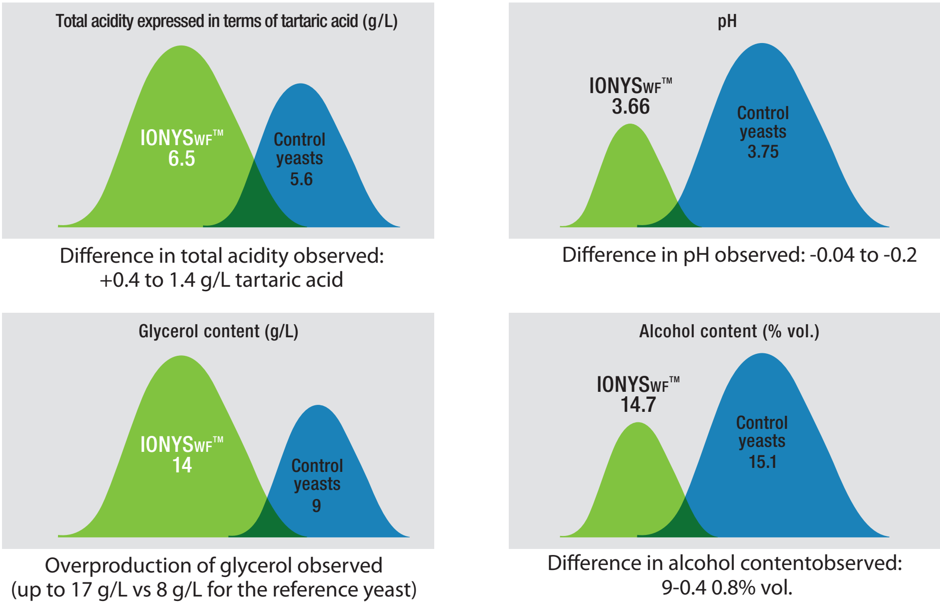

Figure 6. Measurement of different parameters after alcoholic fermentation with IONYS™

RECOMMENDED FOR USE ON RED WINES

Following our characterisation studies, it has been shown that the optimal temperature range for fermentation is between 25 and 28°C. This yeast therefore seems best adapted to conditions for making red wine, however other trials are being carried out on white.

A trial conducted on a Tempranillo (La Rioja, Spain) in 2017 demonstrated a significant impact on the wine. These wines were fermented with the IONYS™ yeast in one case, and the Uvaferm BC™ yeast in another. They were rehydrated according to protocol with Go-Ferm Protect Evolution™, and Fermaid O™ and Fermaid E™ were used as nutrients. We can see differences in terms of the levels of alcohol, total acidity, pH and glycerol. The wines fermented with the IONYS™ yeast had a lower level of alcohol (0.60°), yet the impact was the most significant mainly regarding the total acidity (which was higher).

arameters (bottled wine)	Control	Ionys
Alcohol (% vol.)	14.55	13.95
Total acidity (g/L TH2)	4.7	7.37
pH	3.81	3.45
Glu+Fru (g/L)	0.10	0.10
Volatile acidity (g/L)	0.45	0.20
Free SO_2 (g/L)	7	10
Total SO_2 (g/L)	15	28
Colour index	17.14	20.02
Glycerol (g/L)	10.3	14.3

Table 1. Analysis of different parameters of the Tempranillo (La Rioja) 2017

During a tasting by a professional jury (journalists, Masters of Wine, wine buyers) on a Syrah wine (Languedoc) fermented with the IONYS™ wine yeast, an increased perception of freshness was evident compared with a control (Figure 7). The wine was also overwhelmingly preferred over the control (Figure 8).

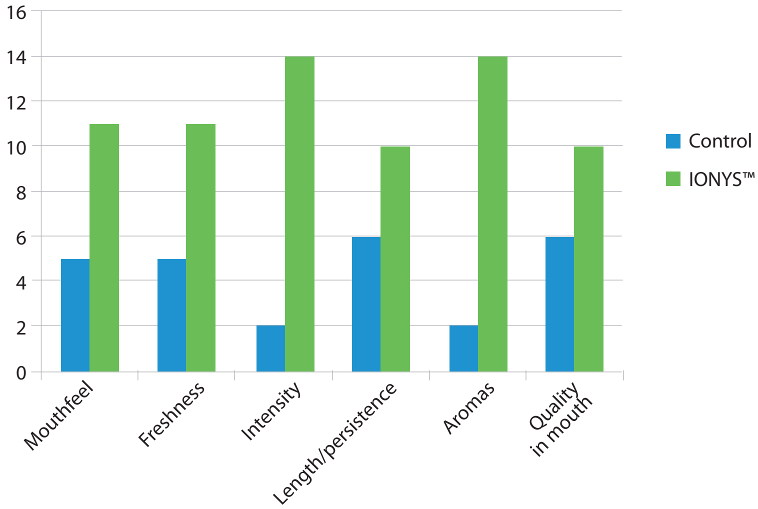

Figure 7. Evaluation of the sensory profile (in the mouth) of a Syrah (2013 – Languedoc).

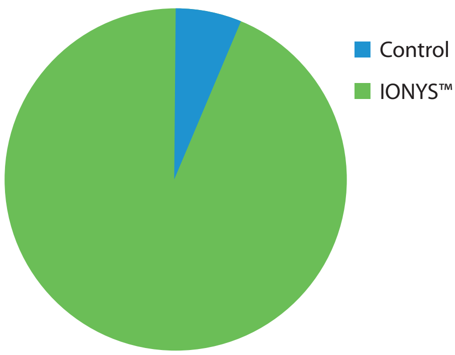

Figure 8. Syrah 2013 (Languedoc) preference test.

CONCLUSION

Adaptive evolution and crossing are powerful non-GMO approaches for selecting yeast strains with properties of interest, making it possible to go beyond classic phenotypes. In the case of IONYS™, these strategies made it possible to obtain a new yeast with a lower yield in terms of the conversion of sugars to ethanol and with overproduction of organic acids thanks to a redirection of carbon flows towards glycerol and the tricarboxylic acid cycle.

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