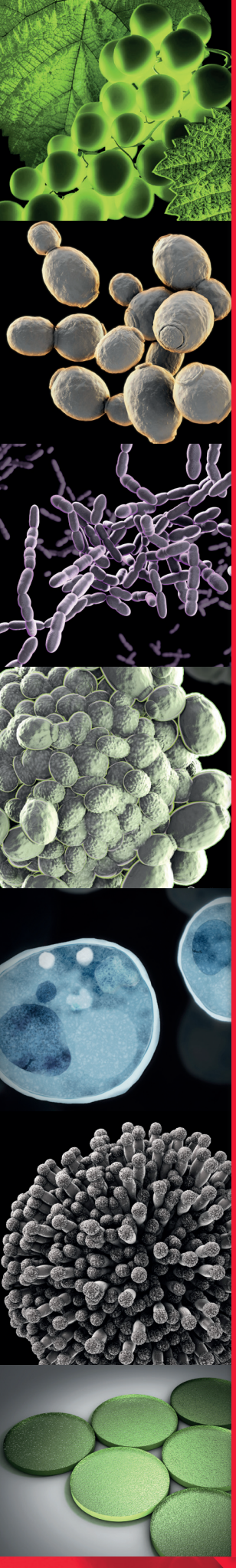

WHAT'S SO SPECIAL ABOUT SPECIALTY WINE YEAST ?

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For more than 50 years now, selected wine yeasts have been used by winemakers successfully in active dry yeast form. It has been longer than that, since Pasteur discovered that *Saccharomyces cerevisiae* was responsible for alcoholic fermentation, that winemakers have intuitively selected the best wine yeasts. Now, with dedicated selection program, winemakers have access to the best of the best in terms of wine yeasts. Why is there so many available and what sets them apart from one another? These are all the ways, selected wine yeast are special.

MORE THAN JUST ALCOHOLIC FERMENTATION

Initially, wine yeasts were selected for their ability to complete alcoholic fermentation (AF). This is well-demonstrated as some of the earliest selected wine yeasts were 'work horses' and could pummel through any fermentation (LALVIN EC-1118™ for example). The discovery of the "killer factor" in wine yeast led to the selection of the Lalvin ICV K1™. This yeast strain has a strong killer factor (against other sensitive *S.cerevisiae*) and can lead to a better implantation during the AF. Since its discovery, this killer factor is one of the characterization features measured for each wine yeast. Its relevance is sometimes questioned, as some wine yeast strains, despite being killer factor sensitive, are excellent fermentors and show very good implantation capacities. But this discovery was one of the first step for the «specialty» yeasts.

More and more, and with the advance in selection and characterization techniques, we began to understand that beyond AF, some strains were more adapted than others to certain winemaking parameters and/or must conditions. We discovered also that there was a real contribution to the sensory profile of the wine (flavors, color, mouthfeel,) or to have a precise technological advantage (alcohol tolerance, acidification, bioprotection, low SO_2/H_2S/acetaldehyde, low VA production, etc.). Moreover, there is a large genetic diversity amongst wine yeast, and that leads us to selections that are more precise to find the appropriate yeast for winemaker's need. The diversity of the wine yeast ecology is also further explored with more and more non-*Saccharomyces* yeasts being selected to use in conjunction with specific *Saccharomyces* yeasts. As seen in Figure 1, the genetic diversity is large, and along with environmental factors, it drives the expression of the phenotypic properties of each yeast strains. Those phenotypic properties, as well as the must/juice conditions, participate in the different yeast metabolisms, which is what matters to the winemakers, as it will be reflected in the fermentation performance and sensory contribution.

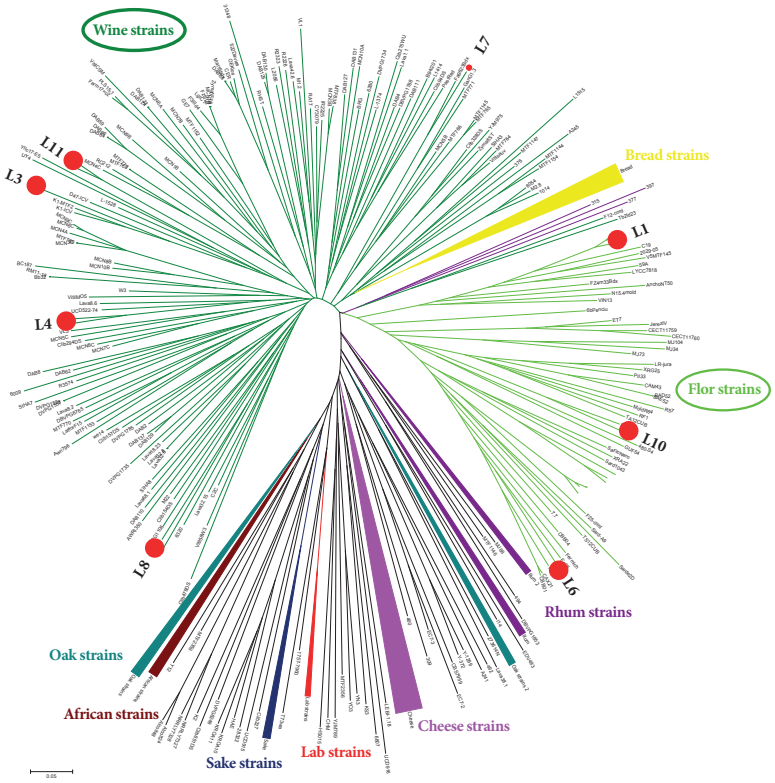

Figure 1. Phylogenetic tree representing the genetic diversity of yeasts.

THE KEY DIFFERENCES

Key characterization is always done when a new yeast is selected. Alcohol tolerance, lag phase, optimum temperature of fermentation, nutritional needs, production of SO_2, volatile acidity, are some of the parameters measured under different conditions. This characterization helps winemakers select the right wine yeast that fits its juice or must conditions. As seen in the Figure 2 as an example, SO_2 production varies with different wine yeasts.

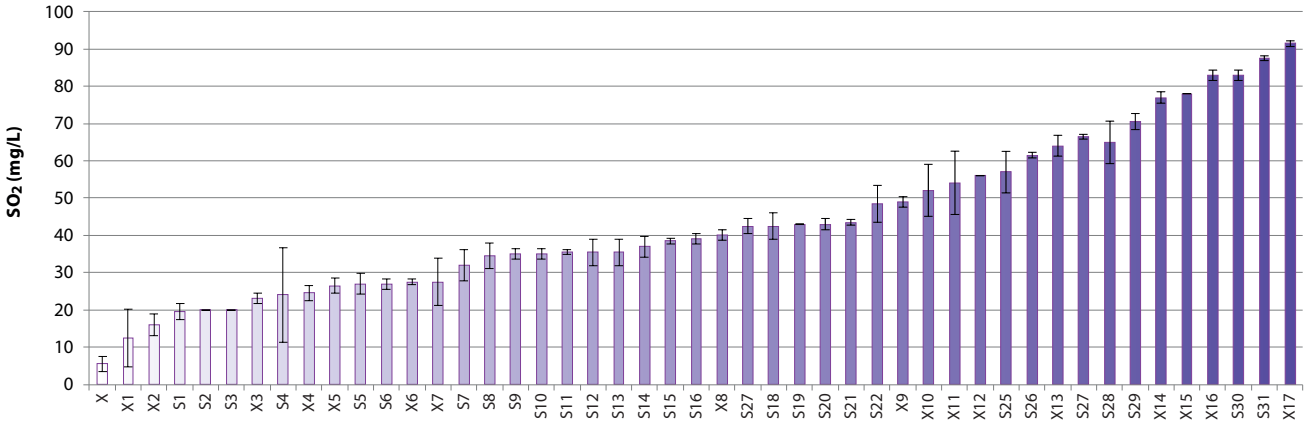

Figure 2. Total SO_2 production by different wine yeasts

Another example is characterizing the nutritional needs by wine yeast which are also a key property that are important to winemakers. As the YAN (Yeast Assimilable Nitrogen) level can vary greatly, not all wine yeasts are able to ferment under lower nitrogen levels. This information (Figure 3) can help winemakers in their fermentation management with proper nutrition to have a regular and complete fermentation, and avoid faults related to nitrogen stress.

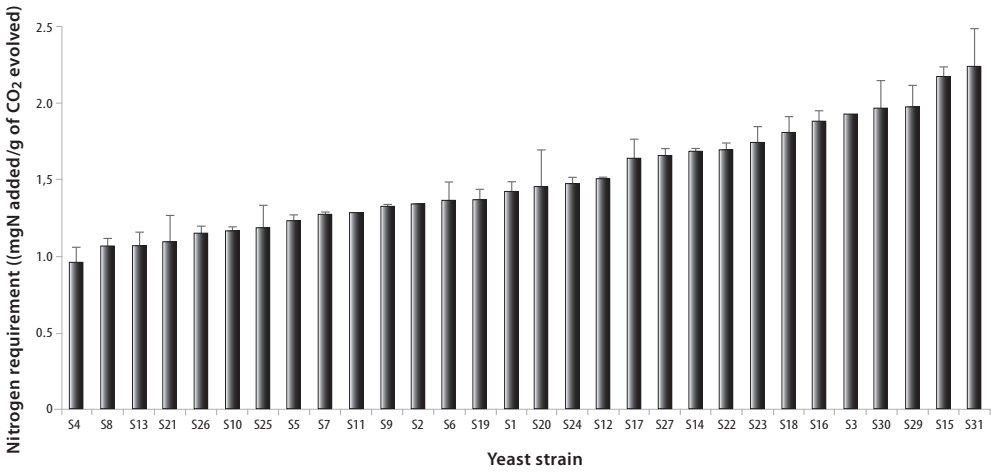

Figure 3. Comparison of nitrogen requirements of wine yeast strains.

FRUCTOPHIC CHARACTER

Glucose and fructose are the main fermentable sugars in wine must. Grape musts contain equal amounts of glucose and fructose, and their total concentrations typically range from 160 to 300 g/L. *Saccharomyces cerevisiae* is a glucophilic yeast, preferring glucose to fructose. During fermentation, glucose is consumed at a higher rate than fructose, and the proportion of residual fructose increases as fermentation progresses. This can lead to imbalances in the wines, and under the stressful conditions found at the end of fermentation, make it more difficult for wine yeast to utilize this non-preferred sugar. But different strains of *Saccharomyces cerevisiae* differ their capacity to consume fructose and it can have an important impact on the fermentation performance, especially under difficult conditions. Researchers have identified genes coding for the hexose transporters in yeast and showed that under oenological conditions, several genes are involved in sugar

transport, which is regulated by a large, multi-gene family called HXT. There are 20 HXT genes. It has been shown that Hxt3 gene has the highest capacity to support fermentation (Luyten et al., 2002) and studies have also identified that this gene is indeed responsible for the capacity for consuming fructose among certain yeasts (Guillaume et al., 2007). In a measure named the fructophilic index, we can evaluate the affinity of wine yeast for fructose. The wine yeast Uvaferm 43 Restart™ is the champion (Figure 4) in this category and has an unmatched capacity to utilize fructose and restart stuck or sluggish fermentations.

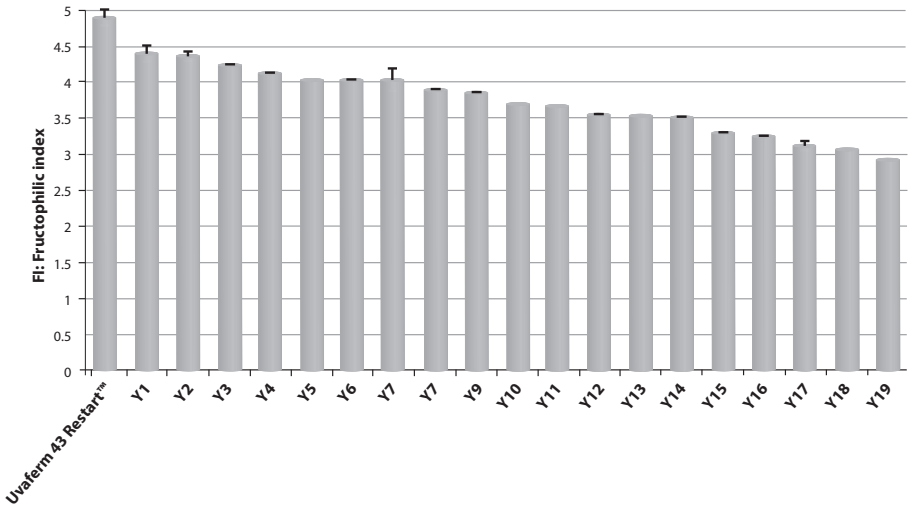

Figure 4. Fructophilic index of different selected wine yeast.

MAKING BUBBLES – THE IMPORTANCE OF A STRONG AND RESISTANT YEAST

Having a healthy fermentation is a priority in sparkling wine production. Selected wine yeasts are able to ferment reliably to dryness in conditions of high acidity/low pH, typically found in sparkling wine production. As pronounced aromas are generally not sought after in autolytic styles of traditional method of sparklings are usually made using yeast with a low to moderate sensorial impact and some of the first yeast selections were yeasts selected from the Champagne terroirs.

For the second fermentation, the stressful wine conditions need to be considered when choosing a yeast strain. During the prise de mousse step, the yeast has to be able to ferment in high alcohol conditions (10% for example), low pH, low temperatures, high pressure and with poor YAN. Rapid autolysis and flocculation are also desirable in traditional method wines. LALVIN EC1118™ and LALVIN DV10™ as well as IOC 18-2007™ and Levuline CHP™ are recognized worldwide as their low sensorial impact character enables the subtle primary fruit to be expressed and can withstand the difficult secondary fermentation conditions in the traditional method and also in the Charmat (or «cuve close») method.

UNDERSTANDING SENSORY CONTRIBUTION FROM WINE YEAST

Each wine yeast strain has a level of specific intracellular enzymatic expression, such as β-lyase, β-glucosidase, acetyltransferase 1 and 2 to name a few. These activities have different levels of expression which are determined by the unique genetic make-up of each *S.cerevisiae* strain, as well as the environmental conditions the yeast is in. These enzymatic activities have the power either to clive specific compounds called odorless precursors, naturally present in the grapes , revealing the odorant part, or to convert compounds in aromas such as esters. It is a mutualistic relationship between the must and the wine yeast strain that results in a unique combination. There are two main types of aroma compounds that *S.cerevisia*e will produce.

Fermentative aromas

Esters and higher alcohols are volatile aroma compounds that are sought-after for their very pleasant, fruity, floral contribution. Fruit-forward, early-release and primeur whites, rosés and reds all benefit from the sensory contribution of those compounds. With aromas of pineapple, rose, banana, melon, apple, those compounds bring positive descriptors to the sensory profile of the wine. Wine yeasts play a key role in the release of esters and higher alcohols as it is part of the fermentative metabolism during the sugar to alcohol conversion (Figure 5).

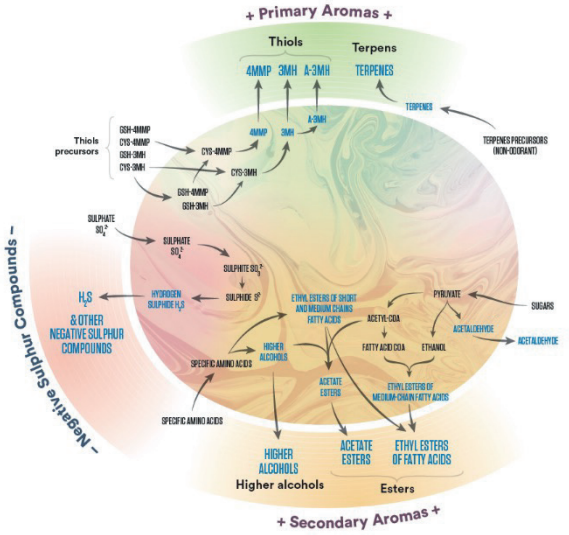

Figure 5. Illustration representing the metabolism of aroma compounds in a wine yeast cell.

Knowing a yeast's aptitude to reveal aromas through their unique metabolism, allows the winemaker to choose a well-characterized yeast to achieve the desired wine style. For example, the yeast LALVIN 71B™ is well-known for the production of esters and was one of the first yeast that was characterized with this feature. Now, many other selected wine yeasts are studied for their ability to reveal the ester potential, such as the Affinity ECA5 ™ which has been shown to produce esters and fruity aromas in wines. All the wine yeast that we study are characterized for their fermentative aroma contribution in different varieties and conditions as shown as an example in Figure 6.

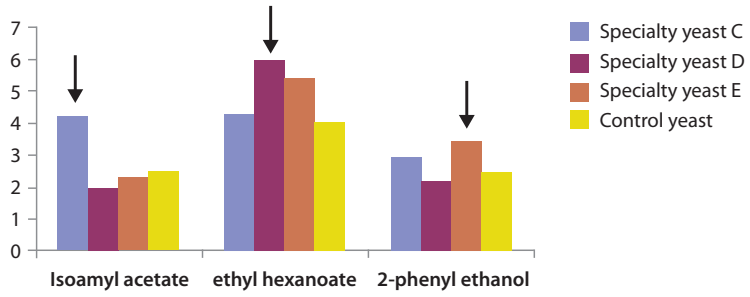

Figure 6. Analysis of aromatic compounds in Maccabeu wine fermented with different wine yeasts, 2005
Source: INRA

Varietal aromas

Varietal aroma compounds are found in grapes in the form of non-odorant precursors.With the unique metabolic activity of yeasts during fermentation, they are transformed into volatile aromas that gives the wine its signature sensory properties. When wine yeasts are selected, they are carefully characterized based on their impact on the sensory properties of the wine and their unique enzymatic metabolic activity. Some of those enzymatic activities in yeast are already known such as the activity of β-lyase for the release of thiols, or the activity of β-glucosidases for the release of terpenes.

One of the most studied impacts is for example how yeast can influence varietal thiols. These aromas are highly characteristic of some grape varieties, such as Sauvignon Blanc, Colombard or Verdejo and represent a huge part of their typicity. Understanding how different selected wine yeast reveal thiols helps winemakers choose the proper yeast based on the grape potential. More precise selection for the revelation of varietal aromas are now found with the help of cellular division for diversity exploration for wine yeast such as the SAUVY™. As seen in Figure 7, SAUVY™ wine yeast has a unique ability to release the varietal thiol 4MMP responsible for the typical gooseberry aromas in wines. While revealing those varietal aromas, the wine yeasts enhance the terroir, as can be seen in the New Zealand study where 2 different wine yeast (LALVIN MSB™ and SAUVY™), produced different concentrations of thiols in 4 different Marlborough region terroir (Figure 8).

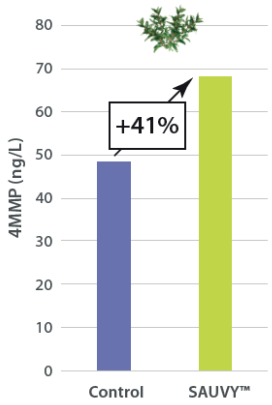

Figure 7. 4MMP released by SAUVY™ compared to a reference thiolic yeast (average of 7 trials performed in Sauvignon blanc 2019 from France, Germany, United States, New Zealand and Chile).

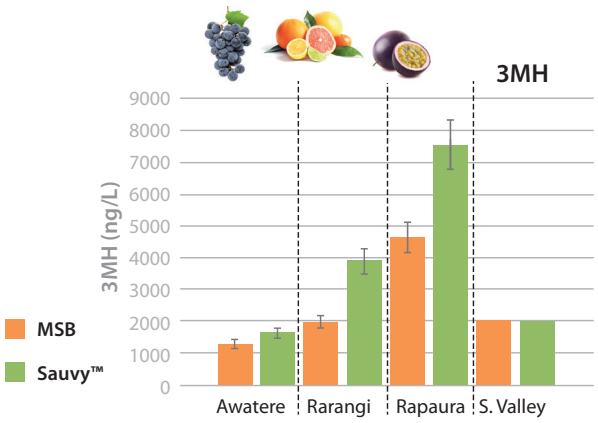

Figure 8. 3MH measured in Sauvignon blanc from 4 different regions in Marlborough, New Zealand, and fermented by 2 different wine yeasts.

We also know more about the importance of thiols in reds, and a selected yeast such as RUBY™ optimizes thiols release in reds, revealing aromas of blackberries and raspberries. The same can be said for the release of terpenes in Riesling and other aromatic varieties, where selected wine yeast play a positive role in their release. Some yeast, for example LALVIN QA23™, an isolate from Vinho Verde in Portugal, has shown high β-glucosidase activity which will enhance the expression of terpene.

Non-*Saccharomyces* selected yeasts also have unique enzymatic activities (such as the α-arabinofuranosidase & β-glucosidase) to release terpenes, thiol precursors and thiols.

MOUTHFEEL & COLOR

Wine yeasts release polysaccharides during alcoholic fermentation (Llauberes *et al*., 1987); and during aging on lees because of their cell autolysis (Feuillat *et al*., 1989). Different selected wine yeasts, and the composition of their cell wall, vary in the quantities and quality of polysaccharides they intrinsically have. Those polysaccharides complex with tannins and polyphenols, reducing the astringency, increasing mouthfeel, and stabilizing color in wines. Selected wine yeasts, for example, such as LALVIN ICV D254™, UVAFERM HPS™ (Figure 9) and LALVIN BM4x4™ are known selection to have this very positive impact in red wines. Some wine yeast are known for their ability to bring mouthfeel in white wines, like the LALVIN CY3079™.

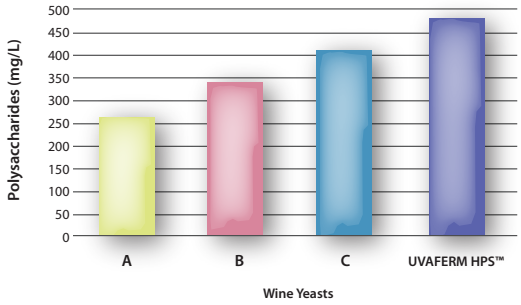

Figure 9. Polysaccharides release by 4 different wine yeasts in Tempranillo wine (Spain) with 14% ABV, pH 3.6, TA 5.5 g/L.

Another mechanism impacting mouthfeel and textural perception is seen with selected wine yeast, LEVEL^2 Biodiva™, a *Torulaspora delbrueckii* wine yeast with a unique high polyols production. Polyols are sugar alcohols, naturally produced by yeast during fermentation. The best-known compound is glycerol but it also includes arabitol, ribitol, sorbitol, mannitol and xylitol (C5 and C6 polyols). Those compounds are known for their sweetening perception. At the end of the fermentation, ribitol, arabitol, mannitol and sorbitol were twice as much when LEVEL^2 Biodiva™ was used. Polyols provides natural and efficient solution to improve wine's sensory properties by enhancing mouthfeel and sweetness perception. LEVEL^2 Biodiva™ ability to flocculate has been shown to absorb pigments during early fermentation, then release these later from the lees and eventually increase colour density (McCullough et al., 2023).

ACIDITY MANAGEMENT

Acidity is an important parameter for the quality of the wine and for the equilibrium between the different flavour sensations especially alcohol. Different acids are found in musts and wines, mainly L-malic, tartaric and citric. Acidity management is usually done via malolactic fermentation with selected wine bacteria, but wine yeast can also metabolize L-malic acid during alcoholic fermentation. The phenomenon, known as maloethanolic fermentation is wine yeast dependant, but also environmental factors will influence the process. Some wine yeasts can degrade malic acid up to 40%, such as the LALVIN 71B™ (Figure 10).

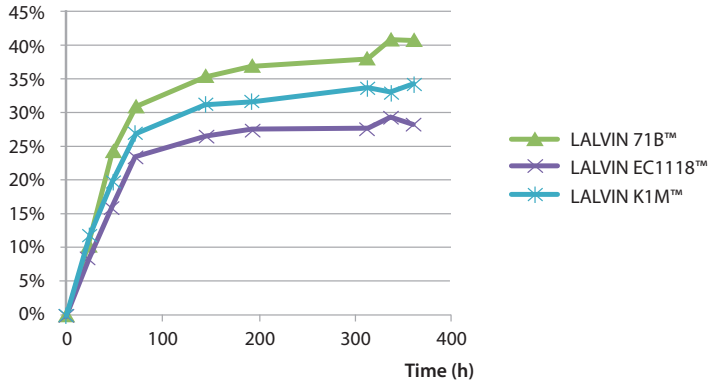

Figure 10. Percentage of L-malic acid consumption by different wine yeasts.

If acidification is desired, which is particularly important with the loss of freshness seen in wines due to climate change, then there are options for the winemakers with two selected wine yeast. IONYS™, is a selected *Saccharomyces cerevisiae* that can produce higher levels of succinic acid, α-ketoglutaric acid and malic acid (and lowering alcohol under certain conditions). By acidifying the juice, it can, for example, lower the pH as seen in Figure 11. The other option which can be complementary, is using LEVEL^2 Laktia™ (a specific selected strain of *Lachancea thermotolerans*) which converts glucose into lactic acid. It can produce 2-9 g/L of lactate depending on the conditions. It significantly increases total acidity and decreases the pH of wines in a very balanced and integrated way. This yeast is used in sequential inoculation, as with all non-*Saccharomyces* to complete AF.

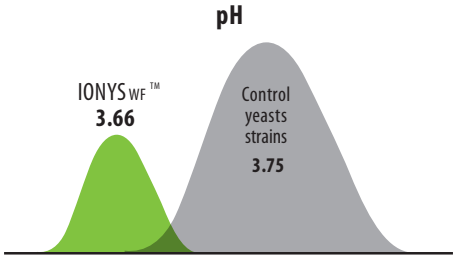

Figure 11. Difference in pH between IONYS™ and a control wine yeast.

LOW SO_2, H_2S, ACETALDEHYDE WINE YEASTS

Lowering SO_2, H_2S and acetaldehyde in wines is important for winemakers wishing to produce wines with lower concentration of those compounds. Consumer demand for low to no SO_2 wines is high and solutions can be found in bioprotection winemaking (Figure 12), as well as using the right wine yeast. New selection techniques based on hybridation and specific QTL, led to new selected wine yeasts that produce very low to none of those compounds. In white and rosé wines, where malolactic fermentation is not desired, avoiding the production of acetaldehyde is important, as if produced in high concentration, then more SO_2 must be added to stabilize the wine, as acetaldehyde binds SO_2, rendering it less efficient. Avoiding the production of H_2S is also important to obtain clean and fault free wines. Wine yeast such as LALVIN ICV Okay™, LALVIN ICV Opale 2.0™, LALVIN Persy™, Sensy™, have all been obtained through this patented selection technique, bringing a real technological advantage to winemaking.

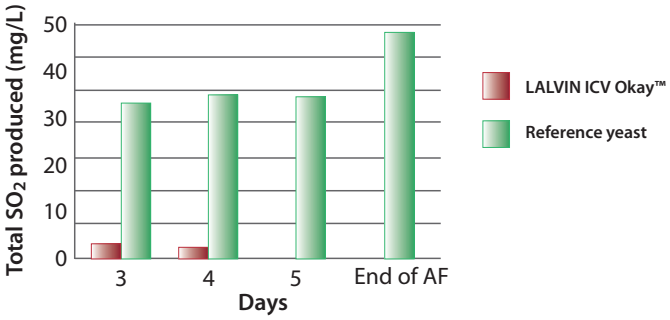

Figure 12. Total SO_2 produced by 2 different wine yeasts throught alcoholic fermentation.

BIOPROTECTION

Ultimately, the use of selected wine yeast, by securing fermentation, helps to control the development of contaminating microorganisms and off-flavors associated with their development (volatile acidity, ethyl acetate, volatile phenols). An important revolution in bioprotection has been our work on selected positive non-*Saccharomyces* such as *Metschnikowia fructicola* (Gaia™) and *Metchnikowia pulcherrima* (LEVEL^2 Initia™ and LEVEL^2 Guardia™) to biocontrol the development of contaminating microorganisms (Figure 13) and protecting the wine from oxidation (Figure 14) by scavenging oxygen during its growth during pre-fermentation with LEVEL^2 Initia™.

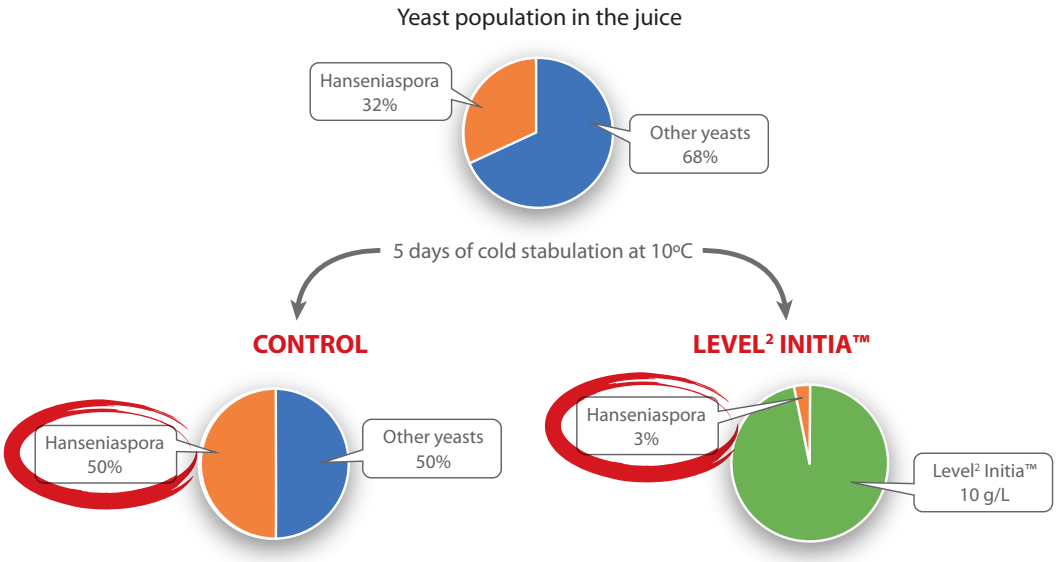

Figure 13. Microbiological bioprotection by LEVEL² Initia™ (Chardonnay no SO_2, Sicarex Beaujolais, France)

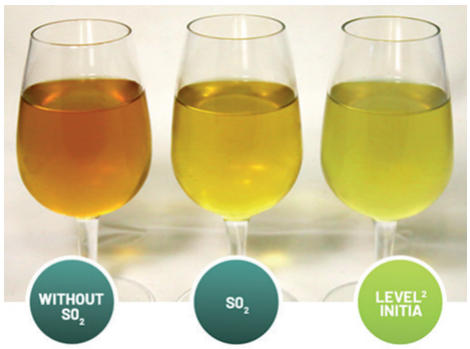

Figure 14. Protection from oxidation in Sauvignon blanc (Italy). LEVEL² Initia™ (without SO_2)

SUMMARY

We have been selecting specialty yeasts in collaboration with our academic/institute partners since the 1980s, based on different criteria and objectives in order to offer reliable wine yeasts with known characteristics and a great sensory diversity that goes far beyond the simple transformation of sugar into ethanol. With different selection techniques, the diversity within *Saccharomyces* and henceforth the non-*Saccharomyces*, winemakers have a wider choice distinctive option for wine quality and style, while respecting the sense of place of the wine. It also allows them to face different global issues such as used of natural products, bioprotection tools mitigate the impacts of climate change, reduce the incidence of wine faults and the production of SO_2, H_2S and acetaldehyde. The performance of selected wine yeast is only as good as how the fermentation management is handled. Right at the onset of wine yeast selection, it was shown that a proper yeast rehydration and nutrition were key in yeast performance and avoid fault production, allowing to maximize their functionalities and uniqueness to produce quality wine.