Nº3 NON-*SACCHAROMYCES*: BIODIVERSITY UNDER CONTROL

The initial microbial population present in grape must is very diverse. During the early stages of alcoholic fermentation, *Saccharomyces cerevisiae* is not the dominant specie and other species (non-*Saccharomyces*) are present. Non-*Saccharomyces* yeasts are part of the must microflora and represent an important reservoir of the wine sensory potential. While some are detrimental to sensory quality, others have the potential to add to wine complexity or bring about a real benefit with the right selected *Saccharomyces* yeast. Three species are presented: *Torulaspora delbrueckii*, *Lachancea thermotolerans* and two different strains of *Metschnikowia pulcherrima*.

PROTECTION FROM OXIDATION DURING PRE-FERMENTATION

During pre-fermentation, the must is vulnerable to the development of undesirable microorganisms and is susceptible to oxidation. The use of SO_2 is efficient to control both problems. In collaboration with Institut Français de la Vigne (Beaune, Burgundy, France), a *Metschnikowia pulcherrima* (Level² Initia™) was selected by Vincent Gerbaux and his team. This naturally-occurring wine yeast has the extraordinary capacity to consume oxygen during its growth. Level² Initia™ consumes O_2 a necessary cofactor to synthesize unsaturated fatty acids (UFA), an essential component for the integrity of the plasma membrane. This feature gives Level² Initia™ an unique advantage during white and rosé pre-fermentation to protect the must from oxidation and preserve the quality of the wine. As figure 1 shows in a trial in Sauvignon blanc, the dissolved oxygen remains almost zero with Level² Initia™ which will also protects the aromatic compounds sensitives to oxygen (thiols) and the wine color. Following the inoculation of Level² Initia™, inoculation with a selected *S.cerevisiae* is needed to complete fermentation.

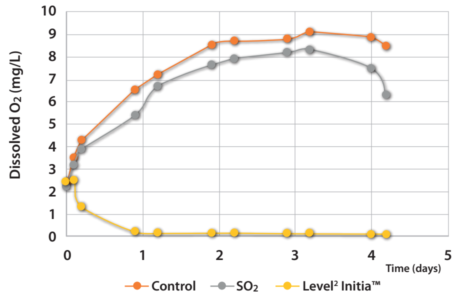

Figure 1. Dissolved O_2 measured in a Sauvignon blanc over several days; Control versus SO_2 at 50 mg/L or Level² Initia™.

SENSORY CONTRIBUTION – AROMATIC

Terpene aroma compounds are bound to sugars, preventing them from being aroma active. Most of the sugars accompanying the aroma precursors are often arabinose and glucoside (Yanai and Sato, 2000). Enzymes with α-arabinofuranosidase and β-glucosidase activity are extremely important in the release of those volatile aromatic compounds. For varietals thiols such as 4MMP, 3MH, and 3MHA, they are not present in grapes as free thiols and are released during alcoholic fermentation from grape-derived non-volatile precursors. Some thiols like 4MMP and 3MH are released by wine yeast β-lyase activity.

Level² Flavia™ is a *Metschnikowia pulcherrima* strain that was isolated from the Maule region of Chile. It secretes an enzyme with α-arabinofuranosidase activity to release the bound terpenes (Ganga et al, 2014). It has also recently been shown to possess a very active β-lyase which will boost the cleavage of cysteinylated precursors to release aroma active thiols. When used before inoculating with a selected *S.cerevisiae* yeast, the wine can develop a complex aromatic profile.

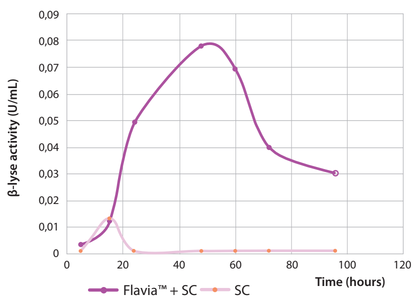

Figure 2. β-lyase activity of Level² Flavia™ during sequential inoculation with *S.cerevisiae* versus *S.cerevisiae* alone.

Figure 2 shows that Level² Flavia™ has a very high and unique β-lyase activity. The resulting wines have more aromas of boxwood, passionfruit, grapefruit and other thiol-related aromas. *(Seguinot P. et al., 2018, Lallemand PhD – INRAE SPO)*

MANAGEMENT OF ACIDITY

In the context of global warming, the lack of acidity in wine can be a major issue and create unbalanced wines. Chemical acidification was initially the only option but it is now possible to use biological alternative to reestablish the wine acidity. The non-*Saccharomyces* wine yeast, Level² Laktia™ (*Lachancea thermotolerans*) was selected in La Rioja, Spain in 2016. Level² Laktia™ 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. *S. cerevisiae* is inoculated 24 to 72 hours later depending on the lactate production objective (the longer the delay is the higher lactate production will be). It is used mainly in reds as it brings freshness and red fruit aromas (Figure 3) but also in white and rosé, however, ensure that initial SO_2 level is as low as possible.

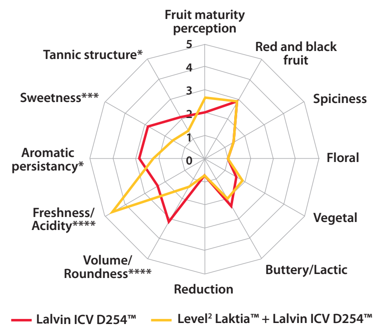

Figure 3. Sensory evaluation of Merlot (France 2018) with and without Level² Laktia™

SENSORY CONTRIBUTION – AROMATIC AND MOUTHFEEL

Level² Biodiva™, a *Torulaspora delbrueckii* wine yeast, has a uniquely high polyols production. Polyols are sugar alcohols, naturally produced by yeast during fermentation. The best known compound is glycerol but also includes arabitol, ribitol, sorbitol, mannitol and xylitol (C5 and C6 polyols). Their main functions are osmoprotection, redox balancing, and pathway signaling for a reduced acetic acid production. Those compounds are known for their sweetening perception. Wine inoculated with Level² Biodiva™ and *S. cerevisiae* versus a wine only inoculated with *Saccharomyces*, will have higher glycerol as well as higher C5 and C6 polyols. At the end of the fermentation, ribitol, arabitol, mannitol and sorbitol were twice as much when Level² Biodiva™ was used. In the case of arabitol, its concentration could be 18 times higher (Figure 4). The production of those polyols, provides a natural and efficient solution to improve wine's sensory properties by enhancing mouthfeel and sweetness perception. *(Collaboration Lallemand & B. Erasmus, B. Divol, 2018)*

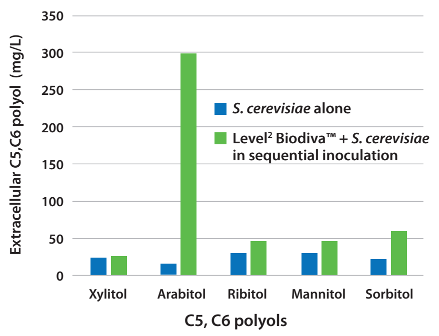

Figure 4. Production of polyols from Level² Biodiva™/*Saccharomyces cerevisiae* compared to only *S. cerevisiae*.

Level² Biodiva™ has a unique metabolism in terms of osmotic shock response, it is also often used for the reduction of the volatile acidity in high brix juice.

HOW ARE NON-SACCHAROMYCES USED ?

The use of selected non-*Saccharomyces* yeast can help winemakers to achieve several benefits, such as better wine complexity and quality. Rigourous quality controls of these microrganisms (added to must or before inoculation with selected *S.cerevisiae)* minimizes the risks associated with uncontrolled fermentation. Nutrition strategies are particularly important as non-*Saccharomyces* will consume nitrogen, as much as 60-80 mg/L in the first three days following inoculation, and should be used in musts containing at least 150 mg/L of assimilable nitrogen or with appropriate nutrition management. They are inoculated in a very similar way to a *Saccharomyces*, except rehydration is at approximately 30°C rather than at 37°C, after which it is added to the must.