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# Editorial

## 'Domesticating' Yeast

Bread, wine and cheese are in integral part of French culture. These three agricultural products are microbiologically linked; the same yeast is used - *Saccharomyces cerevisiae*. When it comes to bread, most of us don't even think of asking if a selected yeast has been used (it is in the vast majority of cases) or if the baker made his or her own sourdough starter. In the world of viticulture and wine production, however, this issue is currently much debated and even unleashes passionate arguments. And yet both selected and indigenous wine yeast have resulted from a shared past – extending back to 5,000 years B.C. – of environmental pressures in this creation.

An international team led by researchers from the French National Institute for Agricultural Research (INRA), in collaboration with the French Alternative Energies and Atomic Energy Commission (CEA), has compared the genomes of 82 *Saccharomyces cerevisiae* yeasts. The results (published in the journal *Molecular Biology and Evolution* on 8 May 2018) identified groups of yeasts in wine, flor, rum, bread, cheese (and fermented milk) and oak trees. Each of these groups has specific genomic traits, providing clues about their ecological specialization. Although they probably share the same genetic origin, wine and cheese yeasts have very specific specializations. For example, yeast found in cheese is very effective at metabolising galactose (from the hydrolysis of milk lactose by other micro-organisms during cheese making), but is very inefficient at fermenting sugars (glucose and fructose) in grape must. Conversely, wine yeast is much better at fermenting grape must and grows more slowly on galactose than yeasts found in cheese. *Saccharomyces cerevisiae* has therefore shaped its genome to adapt to the cheese/fermented milk environment. Furthermore, in domesticating yeast for wine fermentation, humans have empirically selected genomic variations and gene exchange between genetically distant species. The use of selected wine yeast could therefore be regarded as continuing the work that started several thousand years ago...

What's more, this research has only just begun! To date, just 10% of the world's micro-organisms are known, with yeast forming part of this amazing biodiversity. In addition to *Saccharomyces cerevisiae*, non-*Saccharomyces* yeast are opening up a vast field of investigation and new applications! Not to mention all the potential by-products, where both the selected yeast and it's inactivated form (inactive yeast) can be exploited, giving rise to many application options during winemaking.

# Improving the Colour and Texture of Red Wine with a Natural Product: OPTI-MUM RED™

Recent research has led to the development of more detailed characterisation tools for wine yeasts and their by-products. This knowledge has enabled Lallemand to develop a new yeast autolysate with unique properties and of particular interest for red winemaking.

## The Unique Properties of a Wine Yeast and its Autolysate

### An especially interesting yeast, high in mannoproteins

Recent research by Lallemand, in partnership with INSA's teams in Toulouse, has led to a new level of yeast biochemical and biophysical characterisation. By using atomic force microscopy (AFM), the architecture of yeast cell walls and their surface properties has been extensively characterised (Schiavone *et al.*, 2014). Among the wine yeasts preselected for their high mannoprotein production, these AFM results have demonstrated the unique properties of a *Saccharomyces cerevisiae* wine yeast. This yeast has specific adhesive characteristics and longer mannoprotein chains on its cell surface.

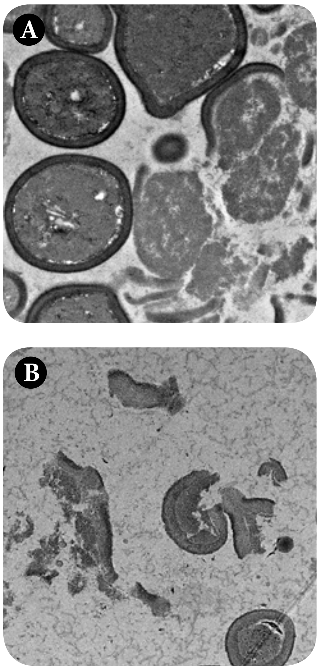


Figure 1. TEM microscopy observations of heat (**A**) and physico-chemical (**B**) inactivation processes on the same interesting *Saccharomyces cerevisiae* strain

### Combination of an Specific Inactivation Process for an Innovative Result

Various autolysis conditions and inactivation processes involving heat or physico-chemical treatment were applied to this yeast with unique properties. Figure 1 shows the impact of these processes on the product's appearance under a transmission electron microscope (TEM). The products obtained had very different appearances.

- After heat treatment, the yeast retained some degree of cell integrity and remained over 60% insoluble (figure 1 A).
- After physico-chemical treatment, there was a loss of cell integrity and a high level of solubility (around 80%), resulting in an autolysate with superior winemaking properties (figure 1 B).

This new yeast autolysate – OPTI-MUM RED™ – has unique properties, which depend on the unprecedented combination of a very specific *Saccharomyces cerevisiae* and a specific inactivation process.

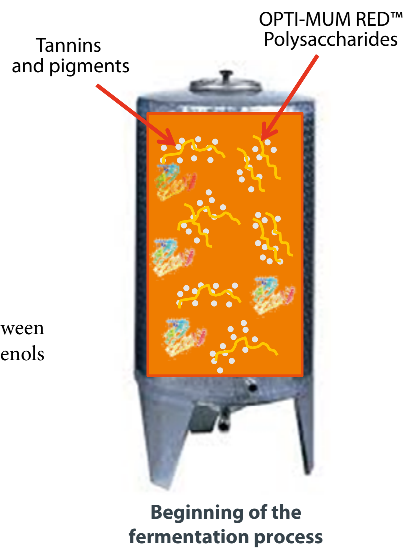


Figure 2. Interactions between polysaccharides and polyphenols

## Impact on Wine Sensor – Wine colour, Palate structure and Texture

### Better understanding of mechanisms of action

Our research has demonstrated the role of OPTI-MUM RED™ macromolecules, especially on colour stabilization and reduction of astringency in red wines. The soluble fraction of OPTI-MUM RED™ is high in polysaccharides and the active component, which interacts more readily with polyphenols, consists of high-molecular weight mannoproteins. Greater pigment stability and lower interaction of tannins with saliva proteins – which reduces the perception of astringency (figure 2) – is explained by the formation of stable, soluble complexes.

## Improved Palate Texture and Structure

We have shown that using OPTI-MUM RED™ has an impact on red wine's astringency, volume in the mouth, and structure perception.

Figure 3 illustrates the impact of OPTI-MUM RED™ on the wine sensory profile. The red wines are more opulent, while also preserving freshness.

When OPTI-MUM RED™ was compared to oak chips, (figure 4) the wine made with OPTI-MUM RED™ was seen as having a higher score for aroma intensity, length on the palate, tannin quality, balance and volume. There was also less perception of bitterness and acidity in these wines.

## More Intense Colour

Many pilot-scale (1 hL) and winery (50-200 hL) trials were conducted on various varietals and in several wine-producing regions to assess the impact of OPTI-MUM RED™. Each trial compared standard winery red winemaking (control) with 30 g/hL dose of OPTI-MUM RED™ added at the start of alcoholic fermentation (AF). In many cases, an OPTI-MUM RED™ addition was observed to have a positive impact on colour stabilisation.

Trials were also carried out to assess the impact of OPTI-MUM RED™ compared to the oenological tannins usually added for this purpose. Figure 5 shows the trial carried on Pinot Noir in Burgundy (2017) to measure wine colour (parameter L). Wine produced from fermentation with OPTI-MUM RED™ had a darker colour than the control at the end of AF. This difference persisted up to bottling (Δ E = 2.5, indicating a colour difference visible to the naked eye). We also note that the impact on colour was comparable (slightly higher) to that of oenological tannins.

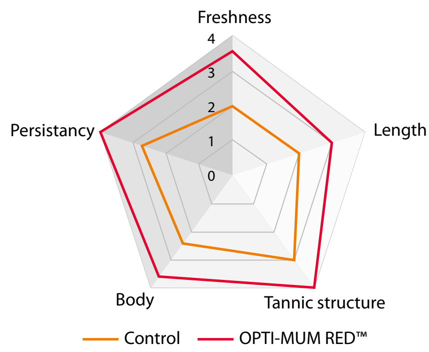


Figure 3. Palate Impact – Cabernet Sauvignon (Paso Robles, California 2016) – Control vs OPTI-MUM RED™ (30 g/hL) added at the start of alcoholic fermentation.

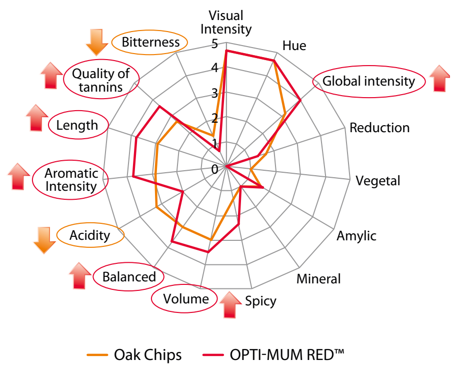


Figure 4. Colour Impact – Cabernet Sauvignon/Merlot (Bordeaux 2016) – fresh oak chips (2 g/L) vs OPTI-MUM RED™ (30 g/hL), both added at the start of alcoholic fermentation.

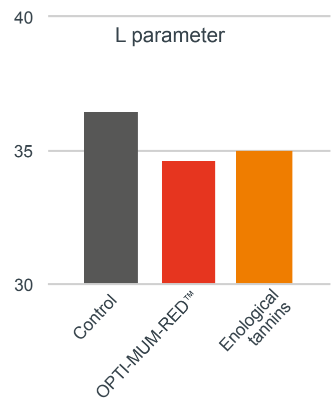


Figure 5. Wine Colour Analysis - Pinot Noir (Burgundy, 2017): CIELAB L analyses at bottling.

# Gaïa™ - New Bioprotection Applications for the Pre-Fermentation Stages

*Metschnikowia fructicola* Gaïa™ is a yeast providing natural protection for must and harvested grapes during pre-fermentation. In addition to its biocontrol action against *Hanseniaspora uvarum*, it has been shown to inhibit the development of acetic acid bacteria in red grape must. Applications in musts during cold maceration have been demonstrated.

### Limiting the development and production of acetic acid by *Hanseniaspora uvarum*

Micro-organisms responsible for acetic spoilage and the start of unwanted fermentation can proliferate rampantly from the grape harvest right up to the fermentation vats. The yeast flora of healthy grapes is dominated by *Hanseniaspora uvarum*, a species of moderately fermentation power and producing large amounts of acetic acid and ethyl acetate (Blondin, 2011; Gerbaux and Thomas, 2017). In the first phase of winemaking – during the pre-fermentation stage and the beginning of the fermentation stage – *Hanseniaspora* yeast can quickly spoil the wine. This risk increases when the level of sulphites is reduced, when there is a *Botrytis* contamination, and when the temperature is too high. Biocontrol with the non-fermentative yeast *Metschnikowia fructicola* Gaïa™ is an effective way to prevent this undesirable flora.

Trials carried out by the IFV on Pinot Noir/Chardonnay musts confirmed this bioprotective effect. Must was deliberately contaminated with *Hanseniaspora uvarum* before undergoing a 7-days pre-fermentation stage at 13°C under three different conditions: a control without bioprotection and two tanks inoculated with different doses of Gaïa™ (10 g/hL and 40 g/hL). In the absence of bioprotection, *Hanseniaspora* increased from 100 to 100 million cells/mL in 5 days, despite the cool temperature (figure 1). Gaïa™ inhibited the growth of *Hanseniaspora*, which did not exceed the level of 10,000 cells/mL. A dose of Gaïa™ at 10 g/hL is enough to maintain the population below a dangerous level, while a 40 g/hL dose slightly improves this inhibition. In the absence of Gaïa™, *Hanseniaspora*'s ability to develop and survive meant it maintained its population even after 5 days into alcoholic fermentation (AF). At the end of AF, this was expressed as a 0.3 g/L difference in acetic acid concentration between the treatments with and without bioprotection.

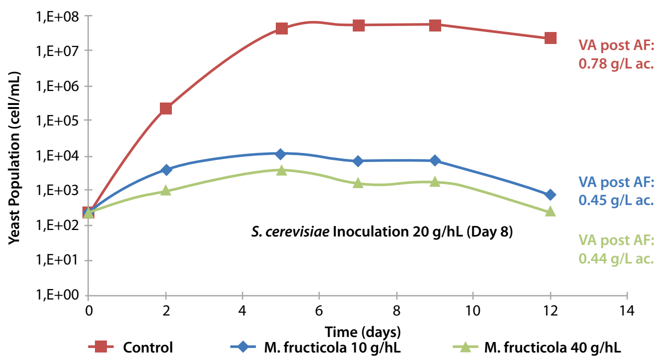


Figure 1. Population monitoring of *Hanseniaspora uvarum* during a pre-fermentation stage at 13°C.

### Inhibiting the growth of acetic acid bacteria

Other trials were also carried out, contaminating Pinot Noir/Chardonnay musts with *Acetobacter* or *Gluconobacter* under the same modalities (control without bioprotection, inoculation with 10 g/hL or 40 g/hL of Gaïa™). A 7 days pre-fermentation stage at two temperature conditions (10°C and 16°C) was undertaken. In the absence of bioprotection, the population of acetic acid bacteria developed faster and more extensively at 16°C (the population reached 1 million) than at 10°C (the population plateaued at 100,000 cells/mL). In contrast, when Gaïa™ was present the population of acetic acid bacteria did not increase and even started to decrease, regardless of the pre-fermentation temperature. The Gaïa™ 'dose' effect was only seen in the wine contaminated with *Acetobacter* and kept at 10°C. This effect virtually disappeared when the pre-fermentation temperature was increased, probably due to Gaïa™ growing more at this temperature.

The volatile acidity concentrations after 14 days are representative of these population differences. In the control, regardless of incubation temperature, acetic acid levels were especially high, whereas Gaïa™ maintained acetic acid at acceptable levels despite a high initial contamination (figure 2).

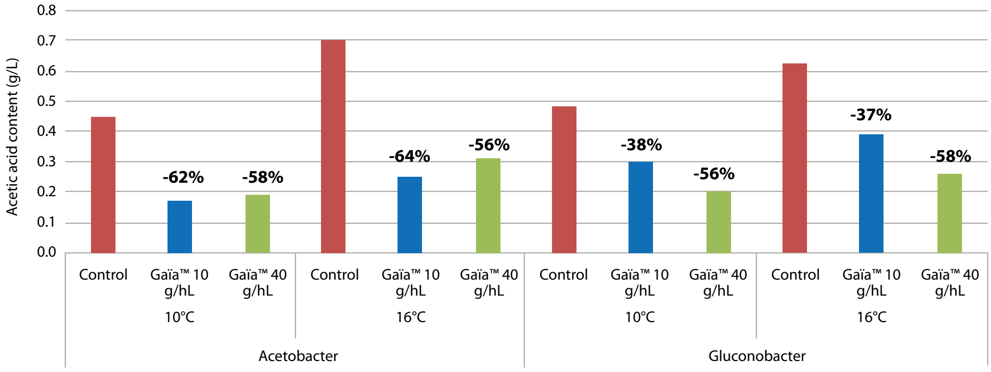


Figure 2. Acetic acid concentrations after 14 days in Pinot noir/Chardonnay juice contaminated with acetic acid bacteria, with and without addition of Gaia.

### Avoiding early fermentation starts

When cold maceration is required for red winemaking, it is sometimes difficult to carry through. It can be hard to maintain a low temperature and/or fermentation may start spontaneously, forcing AF to be started early. Even in the absence of $SO_2$, Gaïa™ is an effective tool for cold maceration and for delaying the start of fermentation in white and rosé juice. Recent results have also shown the benefit of Gaïa™ in juices kept cold for several months.

As well as requiring a lot of energy to store wine at cold at temperatures (below 0°C), this process often requires many filtrations to reduce the yeast population, which is liable to trigger AF too early. During the R&D trialling stage, the ability of Gaïa™ to survive at such low temperatures was checked in the laboratory. Muscat juice stored at 0°C was inoculated with 10 g/hL of Gaïa™. Note; Gaïa™ was rehydrated in water at 20°C before cold must was gradually added to progressively lower the culture temperature. The population monitoring showed that Gaïa™ had an excellent survival rate during the 80 days trial.

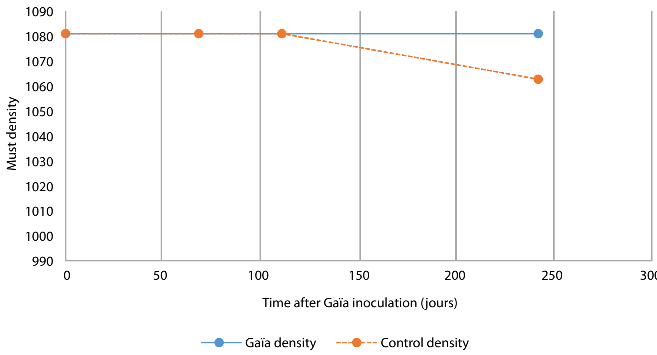


Figure 3. Monitoring the start of AF – Savignon Blanc juice kept at 0-2°C.

A trial was then carried out in two 300 hL tanks of a Sauvignon Blanc juice kept at 0°C. The non-inoculated control was compared to inoculation with a 10 g/hL dose of Gaïa™. Three months later, a slight gas production was observed in the inoculated wine (explained by Gaïa™'s respiration) but no fermentation started. Seven and 7.5 months later, this gas production was still observed but the density remained unchanged, unlike the control must where fermentation eventually started spontaneously (figure 3).

Recent results enable wine's pre-fermentation stages to be considered in a new light. $SO_2$, which is used extensively in these early stages to manage undesirable flora, is no longer the only option to be considered. The impact of Gaïa™ for biocontrol, to complement or even completely replace the anti-microbial role of $SO_2$, has been confirmed.

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## Did you know?

By genetically modifying yeast cells, a US company is now preparing to launch a material resembling leather onto the market.

Modern Meadow – a company employing around sixty people – developed this new material in order to present it to several companies in the clothing, furniture and automotive sectors.

To produce its 'leather', Modern Meadow began by genetically modifying a yeast strain so that it would make a protein identical to bovine collagen. Collagen is the main structural protein in animal bodies and gives skin its resistance and elasticity.

Will biotechnology soon be seen in fashion shows?

W news

**80% of Australian bulk wine volumes are taken by the traditional British market. Chinese purchases are increasing exponentially, with Australian exports now averaging 32% in volume per year.**

## LalVigne Academy – a 2nd Successful Year

After the success of the 1st year in 2017, the 2018 LalVigne® Academy took place in Madrid and brought together 80 people from over 15 countries. The purpose of the programme was to present the latest scientific advances relating to LalVigne®. Its impact on the thickness of berry skin, as well as for increasing concentrations of extractable anthocyanins and grape skin tannins, is now known. A few results were cited before explaining the action mechanisms, especially the impact of LalVigne® MATURE on the expression of genes directly involved in synthesising phenolic compounds, in particular anthocyanins.

Many tasting sessions were also organized, demonstrating the organoleptic impact of LalVigne® AROMA and LalVigne® MATURE on white and red wines made from vine varieties throughout the world.