



LEVEL²TM

EXPLORING
NON-SACCHAROMYCES
UNIVERSE



LALLEMAND OENOLOGY
Original **by culture**

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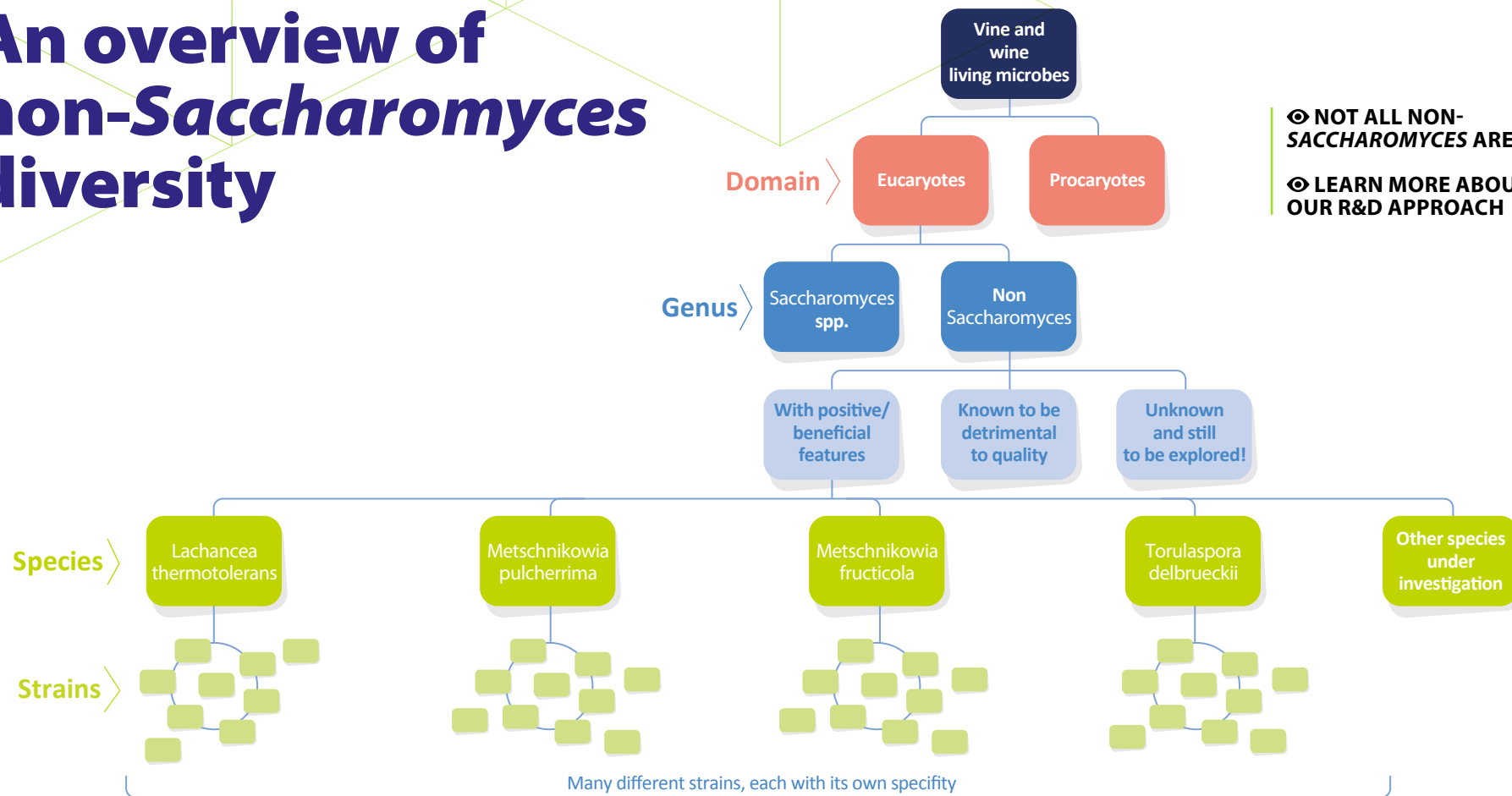
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**A SUMMARY
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An overview of non-Saccharomyces diversity



👁️ NOT ALL NON-SACCHAROMYCES ARE EQUAL
 👁️ LEARN MORE ABOUT OUR R&D APPROACH

Non-*Saccharomyces* yeasts, found on grapes and in grape musts, were originally considered as sources of microbial contamination in wine fermentation. They were thought to be responsible for stuck fermentations and sensory deviations. However, for the last 20 years, the scientific community has been studying their diversity and discovered the potential of their metabolism and enzymatic activities. In fact, some of them bring positive benefits different than the ones known with *Saccharomyces cerevisiae* to the wine industry.

Non-*Saccharomyces* can impact the wine sensory properties positively (aromas, mouthfeel, colour) and participate in bioacidification, bioprotection, biocontrol and lowering alcohol content, and most likely many other roles not yet discovered.

The word itself of “non-Saccharomyces” hides a tremendous diversity of species, and of strains inside each species.

Let’s dive into the fascinating world of non-*Saccharomyces* and explore the various oenological benefits of the LEVEL^{2™}

range, the Lallemand Oenology non-*Saccharomyces* natural selected wine yeasts. Unlike the well-known *Saccharomyces cerevisiae*, which dominates alcoholic fermentation, **these non-Saccharomyces species mainly play roles in the early stages of wine fermentation and can bring various benefits to wines.**

Our unique expertise in non-*Saccharomyces* wine yeast production

Lallemand Oenology has started this journey of exploring the non-*Saccharomyces* potential more than 20 years ago and was one of the first ones to rise the challenge of offering non-*Saccharomyces* strains in a dry active form, as a result of a flexible and patient approach, both from the R&D and the expertise in production side.

For decades, Lallemand Oenology has dedicated its efforts to continuous improvement in production and quality and has gained an unparalleled experience in producing a huge diversity of wine yeast strains. Lallemand Oenology took advantage of this in-depth knowledge in various wine yeast strains production to develop a specific and unique expertise in producing non-*Saccharomyces* in active dry form, for a reliable, stable and practical experience for winemakers.

The challenges with non-*Saccharomyces* production are very different and much greater from those encountered in most of the *Saccharomyces cerevisiae* strains production: higher risks of contamination, slower growth rate, higher risk of Crabtree effect, very high oxygen demand, specific nutrient and growth media requirements, different enzymatic activities, smaller cells size, more challenging downstream processing (filtration, extrusion and drying), etc. In addition to those specificities, each species and each strain within each species behave in a different way, so every single step in production has to be adapted and customised for each strain, in order to offer the best quality, the best efficiency and to provide an optimal customer experience.



Optimal bioprotection for grapes and musts

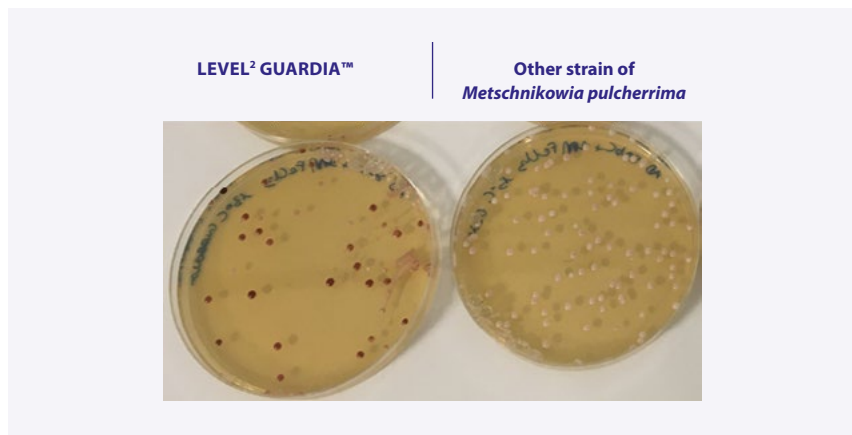
▶ LEVEL² GUARDIA™



Bioprotection to control microbial contamination while reducing the use of SO₂ has been recently developed. Bioprotection can be obtained (or done) by using a non-fermentative yeast, *Metschnikowia* species in the early stages of the winemaking process. It can be applied from the harvest until the selected *Saccharomyces cerevisiae* inoculation for alcoholic fermentation. *Metschnikowia* species produce pulcherrimic acid, a strong iron chelating agent. This iron depletion renders the environment unsuitable for the excessive development of contaminating species. This feature is strain dependent and LEVEL² GUARDIA™ is a *Metschnikowia pulcherrima* strain specifically selected from nature by IFV (*Institut Français de la Vigne et du Vin*, France) for its high pulcherrimic acid production leading to a fast iron depletion.

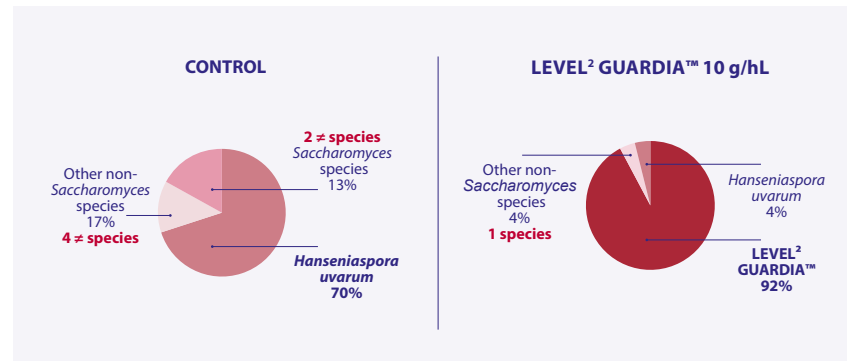
This unique property can be visually seen when LEVEL² GUARDIA™ is grown on specific media and the resulting colonies are pink since pulcherrimin has a red pigment.

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Intraspecific variability of pulcherrimic acid production. LEVEL² GUARDIA™ grown 7 days at 13 °C on specific media with iron and compared to another strain of *Metschnikowia pulcherrima* strain (Laboratoire R&D Lallemand Oenology, France).

The early colonisation and growth of LEVEL² GUARDIA™ lead to a powerful antimicrobial action. It facilitates the implantation of the selected *Saccharomyces cerevisiae* and has a positive impact on wine quality by avoiding sensory deviation.



Implantation control after 5 days of cold soak at 10°C in a Grenache (INCAVI, Spain). Trial comparing LEVEL² GUARDIA™ at 10 g/hL to a control without bioprotection. No SO₂ addition in both cases.



▶ LEVEL² INITIA™

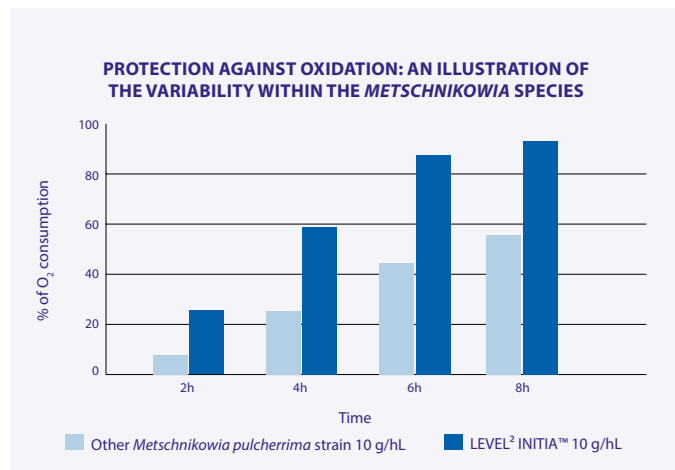


A natural protection against oxidation and spoilage microorganisms. LEVEL² INITIA™ can also decrease copper content in must. Oxygen plays a key role for enzymatic oxidation and for chemical oxidation, and copper is a catalyst for these reactions. By reducing both dissolved oxygen and copper content, LEVEL² INITIA™ makes an excellent bioprotection tool to limit these oxidation phenomena.

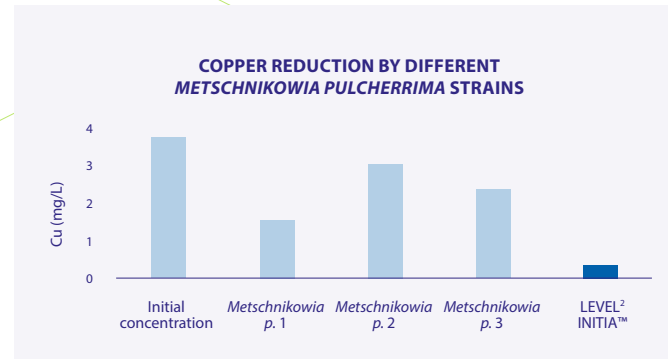
Protecting wines from oxidation is another layer of bioprotection, which is important in white and rosé wines. LEVEL² INITIA™ is another strain of a *Metschnikowia pulcherrima* isolated by IFV Beaune (*Institut Français de la Vigne et du Vin*, France) from a collection of more than 100 strains.

It has low to no fermentation activity, a very good capacity to grow at very low temperatures, and has a unique capacity to rapidly consume dissolved oxygen.

🗨️ WHY DOES LEVEL² INITIA™ HAVE THIS HABILITY TO CONSUME OXYGEN SO QUICKLY?



Oxygen consumption by two *Metschnikowia pulcherrima* strains inoculated at 10 g/hL in a Chardonnay must at 10 °C



Residual copper in a Chardonnay juice with different strains of *Metschnikowia pulcherrima* inoculated at 10 g/hL

🗨️ WHAT IS THE OPTIMAL DOSAGE FOR BIOPROTECTION NON-SACCHAROMYCES YEASTS?



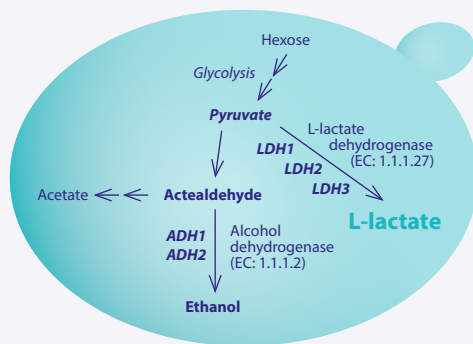
Bioacidification: naturally balancing climate changes effects

► LEVEL² LAKTIA™



With the increase in alcohol levels and loss of acidity and freshness due to climate change, it is common practice to acidify wines with different acids of chemical origin. This acidification has some limitations in terms of legislation, labelling in some countries, and in terms of impact (from an acidification point of view and from a sensory one).

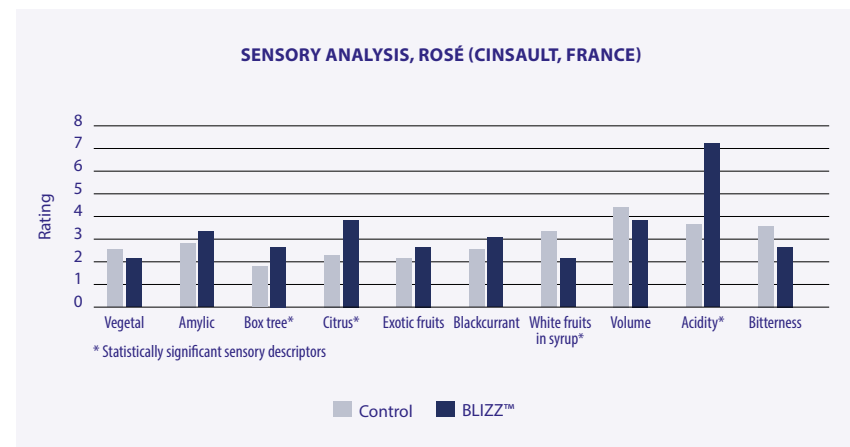
An innovative and natural way to increase freshness and acidity is possible with *Lachancea thermotolerans*. This species can produce lactic acid from pyruvate through a reaction catalysed by three lactic acid dehydrogenase LDH1, 2 and 3.



Fermentative metabolism scheme of sugar in *Lachancea thermotolerans*⁽⁴⁾

Besides acidification, certain strains of *Lachancea thermotolerans* species can positively impact the sensory profiles of the wine by producing aromatic compounds (esters, 2-phenyl ethanol, linalool, etc.) and glycerol⁽⁵⁾. There is a remarkable phenotypic diversity found within this species. LEVEL² LAKTIA™ is a *Lachancea thermotolerans* selected from Tempranillo in the Rioja region in Spain. Characterisation was done at lab, pilot and winery scale to better understand the environmental factors impacting lactic acid production. More than 70 trials were conducted worldwide on 16 different grape varieties. Compared to other strains, LEVEL² LAKTIA™ shows a remarkable consistency in terms of results.

Regarding the sensory impact, the acidity was better integrated and balanced with LEVEL² LAKTIA™ compared to tartaric acid addition. The wines were generally more complex, fruitier and less bitter. Another *Lachancea thermotolerans* strain has been selected by the “Universidad Politécnica de Madrid” (UPM, Spain) and validated by ICV (Institut Coopératif du Vin, France) for its unique sensory impact on white and rosé wines and exceptional properties for bioacidification. BLIZZ™ *Lachancea thermotolerans* reveals notes of citrus, fresh and exotic fruit, even on white and rosé matrix known as “low aromatic” varieties. The acidity provided by BLIZZ™ is always perceived as more complex, less “squeaky” and more appreciated, compared to the authorised organic acids usually added (tartaric, lactic, malic).



Sensory analysis led by ICV group on a rosé Cinsault (South of France) with BLIZZ™ compared to a control with no *Lachancea thermotolerans*

Enhanced complexity and aromas

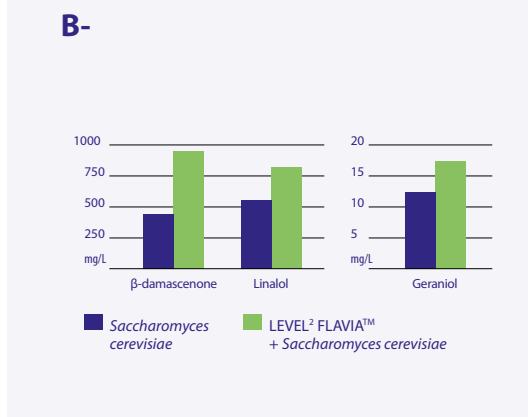
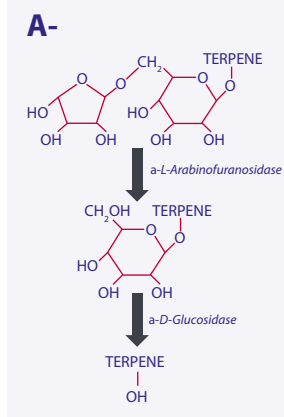
► LEVEL² FLAVIA™



Other strains of *Metschnikowia pulcherrima* can have a major positive sensory impact, by enhancing intensity and complexity of the wines. LEVEL² FLAVIA™, a specific strain of *Metschnikowia pulcherrima*, was selected by the Universidad de Santiago de Chile (USACH) for its unique capacity to release varietal aromas from precursors present in grape must. LEVEL² FLAVIA™ shows a very high enzymatic activities expression level, of α-arabinofuranosidase, β-glucosidase and β-lyase involved in varietal aromas release.

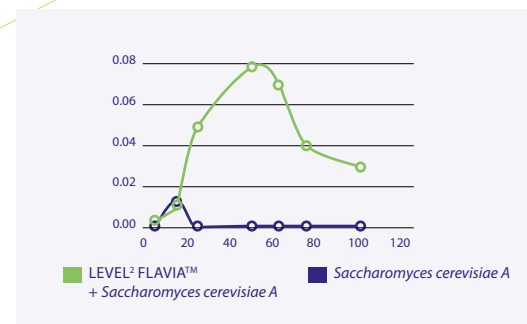
Terpenes (such as linalool, geraniol, nerol) participate in the aromatic profile of wines⁽⁶⁾, responsible for floral and fruity aromas⁽⁷⁾. In grapes and musts, they are linked to sugars and form glycosidic compounds that are non-aromatic.

They can be released from their sugar moiety with the strong α-arabinofuranosidase and β-glucosidase activities of LEVEL² FLAVIA™, and become aroma active and contribute to the typicity and aromatic profile of the wines.



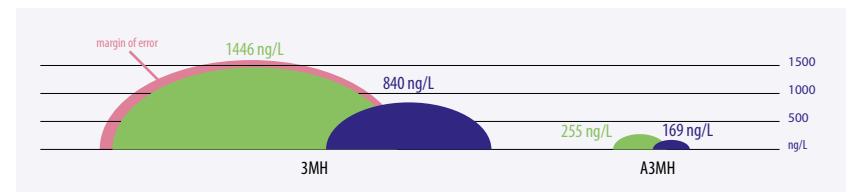
Impact of LEVEL² FLAVIA™ on the release of terpenes and norisoprenoids. Enzymatic synthesis pathway (A) and illustration on Muscat (France) inoculated or not with LEVEL² FLAVIA™

A collaboration between Lallemand Oenology and INRAE⁽⁸⁾ has established that LEVEL² FLAVIA™ possesses a significant β-lyase activity, responsible for the release of volatile thiols such as 3MH and 4MMP.

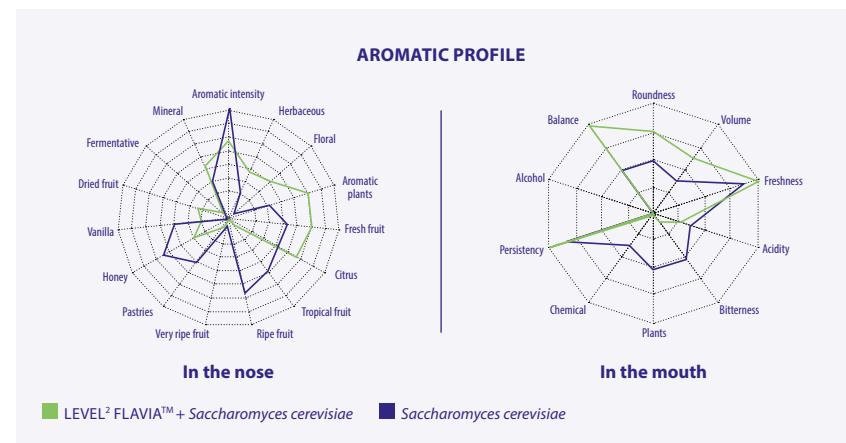


β-lyase activity (U/mL) of LEVEL² FLAVIA™ in comparison with *Saccharomyces cerevisiae*

Impact of LEVEL² FLAVIA™ on the release of thiols on Colombar (France)



These specifically high activities, with LEVEL² FLAVIA™, contribute to the expression of the intense and complex aromatic profile of the wines. Used in sequential fermentation with a *Saccharomyces cerevisiae*, LEVEL² FLAVIA fully reveals the varietal aromas potential.



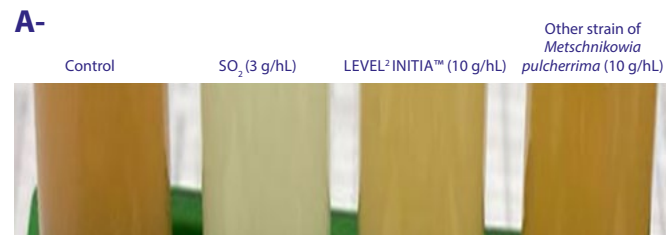
Sensory analysis led by a panel of professional tasters, Alvarinho (Portugal)

Improved colour stability in white wines

▶ LEVEL² INITIA™



LEVEL2 INITIA™ is a unique strain of *Metschnikowia pulcherrima* isolated in Burgundy by IFV (Institut Francais de la Vigne et du Vin, France) for its **unique capacity to rapidly consume dissolved oxygen**. This feature, associated with its ability to remove copper (an oxidation catalyser), leads to a better preservation of white and rosé colour, limiting browning.



Chardonnay must (France) after pressing. Comparison of different solutions of protection against oxydation.



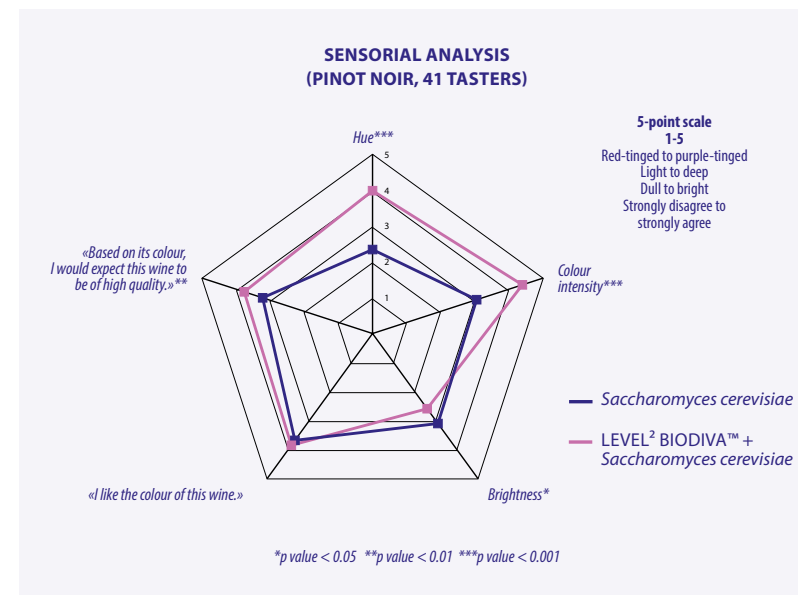
Sauvignon blanc after juice transport with or without LEVEL² INITIA™ at 10 g/hL from the vineyard to the winery (Uruguay).

Improved colour density of red wines

▶ LEVEL² BIODIVA™



Colour density is an important quality parameter for red wines as consumers prefer deeper colour. For some varieties such as Pinot noir, the skin is thinner, making more difficult for anthocyanins extraction and stabilisation. McCullough et al (New Zealand University) demonstrated that LEVEL² BIODIVA™ displayed high sedimentation rates, and as a result led to higher colour intensity, more purple tone and lower concentration of total and monomeric anthocyanins.



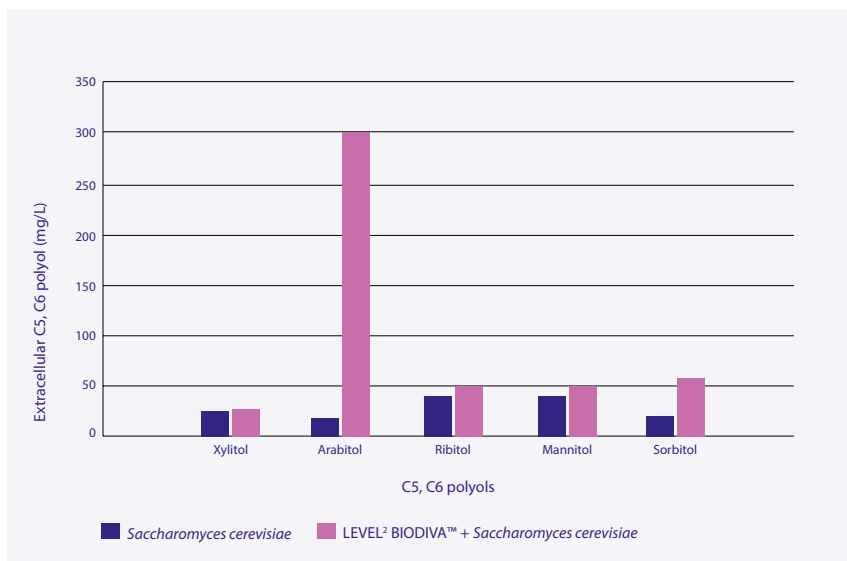
LEVEL² BIODIVA™ contributes to a better quality with a higher colour intensity with more purple in tone (Pinot noir, New Zealand).

Improved mouthfeel and texture

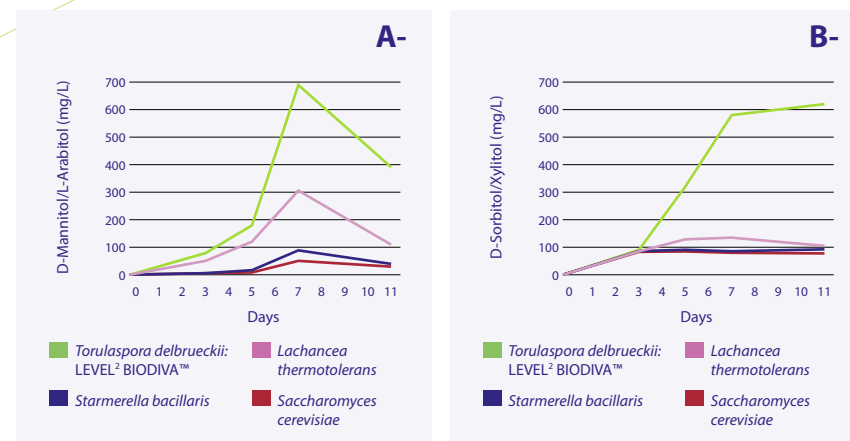
► LEVEL² BIODIVA™



LEVEL² BIODIVA™, a unique strain of *Torulaspota delbrueckii* produces significant concentrations of C5 and C6 polyols under wine fermentation conditions (Mbuyane et al., 2018, Stellenbosch University). In particular, D-arabitol, D-sorbitol and D-mannitol increase when LEVEL² BIODIVA™ is used prior to *Saccharomyces cerevisiae* inoculation.



Production of polyols from LEVEL² BIODIVA™ + *Saccharomyces cerevisiae* compared to *Saccharomyces cerevisiae*



Polyol production in synthetic grape juice-like medium containing 230 g/L of sugar⁽⁹⁾

The release of those polyols into the wine during fermentation significantly increases sweetness and volume perceptions.



Impact of LEVEL² BIODIVA™ on sensory profile. Sensorial analysis led on Syrah (Rhône Valley, France, 27 tasters)

Better management of volatile acidity

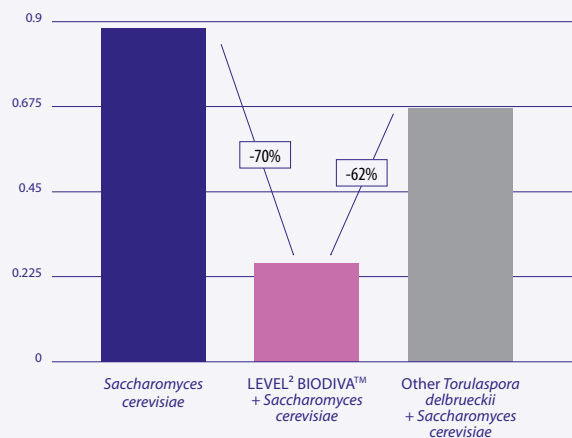
▶ LEVEL² BIODIVA™



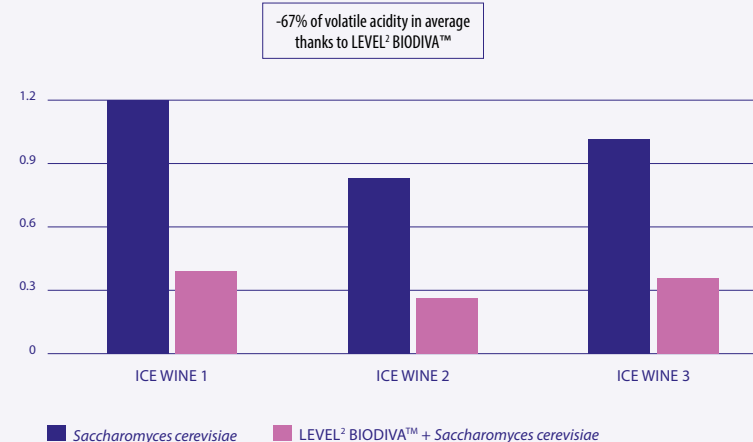
In **prefermentative stages**, the contaminating flora, particularly *Hanseniaspora uvarum* can lead to very high production of volatile acidity. LEVEL² GUARDIA™ and LEVEL² INITIA™, because of their powerful antimicrobial action, lead to lower levels of volatile acidity in final wines.

In musts with extreme initial levels of sugar (natural sweet wines, ice wines, etc.), the osmotic pressure on the yeast cell is high and prevents the yeasts from growing properly. To counterbalance this external pressure, yeasts produce and accumulate intracellular glycerol. For *Saccharomyces cerevisiae*, one of the inevitable by-products of this accumulation is acetic acid. Because of a specific metabolism related to osmotic pressure regulation, *Torulaspora delbrueckii* species produce high level of polyols (glycerol, etc.) and it results in a much lower production of volatile acidity. There is a variability within the species and LEVEL² BIODIVA™ is suitable to decrease volatile acidity in these type of conditions.

VOLATILE ACIDITY (g/L H₂SO₄) IN MUST WITH HIGH LEVELS OF SUGAR



VOLATILE ACIDITY (g/L H₂SO₄) IN ICE WINES



A summary of our non-*Saccharomyces* benefits and specificities

Behind the word “non-*Saccharomyces*” there is an amazing diversity of species and strains within each species, with unique characteristics and features for each one. Exploring and understanding their individual potential has been and still is one of the main focuses of Lallemand Oenology R&D as they can bring valuable and diverse oenological benefits for winemakers.

Apart from exploring and selecting new strains from this exciting microbiome universe, mastering their production requires a unique know-how and is a mandatory prerequisite to give access to winemakers to this fascinating microbial diversity. Being one of the first ones to dedicate continuously its efforts in developing specific production processes adapted to each strain has allowed Lallemand Oenology to gain an unparalleled expertise in the production of non-*Saccharomyces* yeasts.

WINEMAKING APPLICATION AND BENEFITS	LEVEL ² SOLUTIONS	MAIN ACTIVITY AND WAY OF ACTION
Flexible and efficient bioprotection for grapes and musts	LEVEL² GUARDIA™ <i>Metschnikowia pulcherrima</i>	High pulcherriminic acid production, a strong iron chelating agent. Outcompetes contaminant microflora via early colonisation and growth.
Specific bioprotection for white and rosé must combining protection from oxidative damage and microbial spoilage	LEVEL² INITIA™ <i>Metschnikowia pulcherrima</i>	Very fast and complete oxygen consumption. Excellent capacity to survive and grow even at low temperature. Ability to decrease copper content in prefermentative steps.
Bioacidification and freshness	LEVEL² LAKTIA™ <i>Lachancea thermotolerans</i>	Consistent and reliable lactic acid production from sugar.
Enhances complexity and aromatics	LEVEL² FLAVIA™ <i>Metschnikowia pulcherrima</i>	High expression of unique enzymes that cleave aroma precursors to reveal terpenes and thiols.
Improves colour density in red wines	LEVEL² BIODIVA™ <i>Torulaspora delbrueckii</i>	Release of polysaccharides improving colour stability.
Managing volatile acidity in high sugar musts	LEVEL² BIODIVA™ <i>Torulaspora delbrueckii</i>	Overcomes osmotic pressure through a specific metabolism non producing acetic acid.
Improving mouthfeel and texture	LEVEL² BIODIVA™ <i>Torulaspora delbrueckii</i>	High production of polyols and mannoproteins.

[VIEW OUR SCIENTIFIC REFERENCES](#)



Being original is key to your success

At Lallemand Oenology, we apply our passion for innovation, maximise our skill in production and share our expertise, to select and develop natural microbiological solutions. Dedicated to the individuality of your wine, we support your originality, we cultivate our own.

www.lallemandwine.com



WINE
YEASTS



WINE
BACTERIA



NUTRIENTS
/PROTECTORS



SPECIFIC
YEAST DERIVATIVES



ENZYMES



CHITOSAN



VINEYARD
SOLUTIONS



LALLEMAND OENOLOGY

Original **by culture**