LEVEL2™

EXPLORING NON-*SACCHAROMYCES* UNIVERSE

LALLEMAND

LALLEMAND OENOLOGY

Original **by culture**

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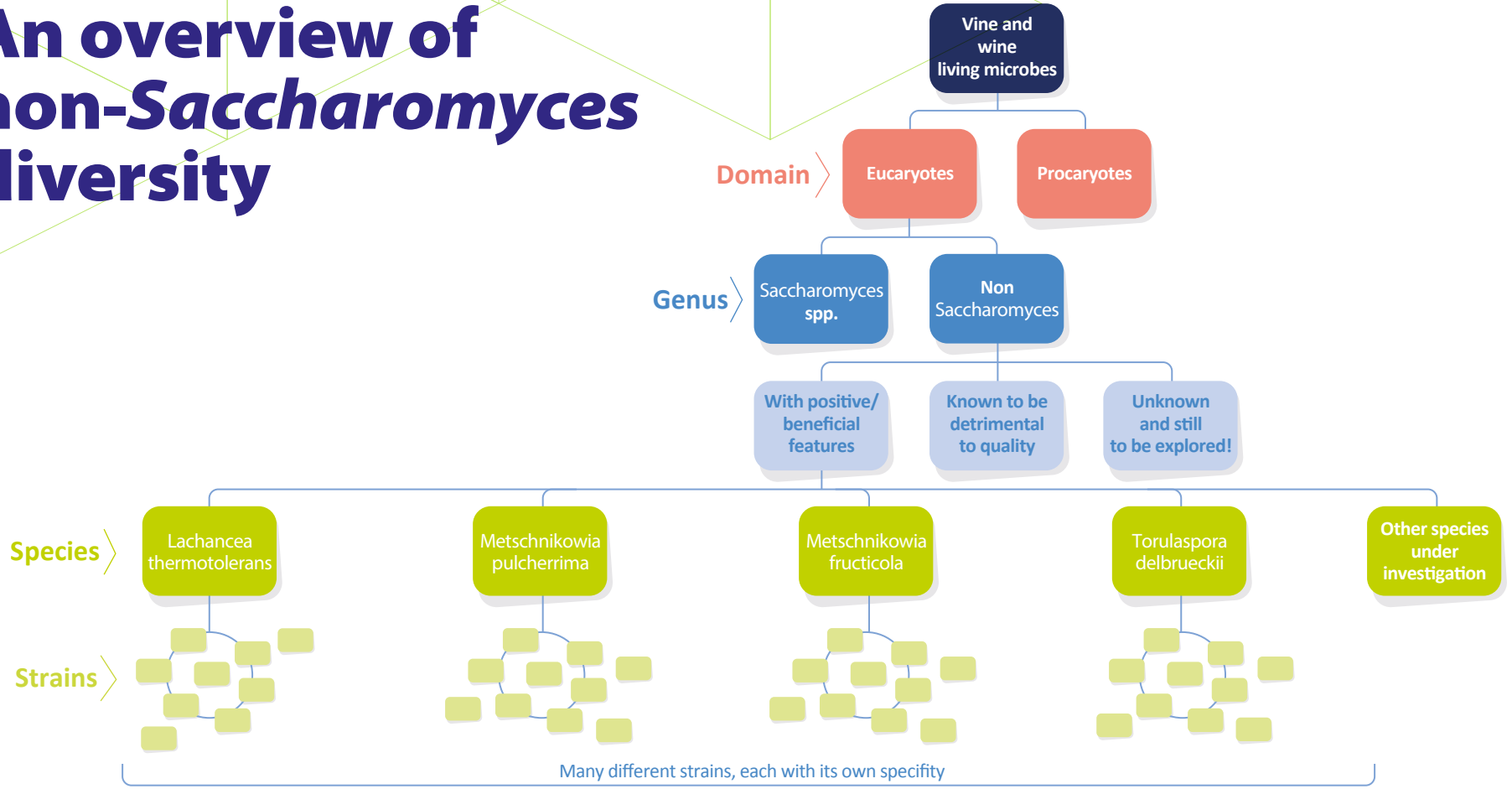
An overview of non-*Saccharomyces* diversity



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.

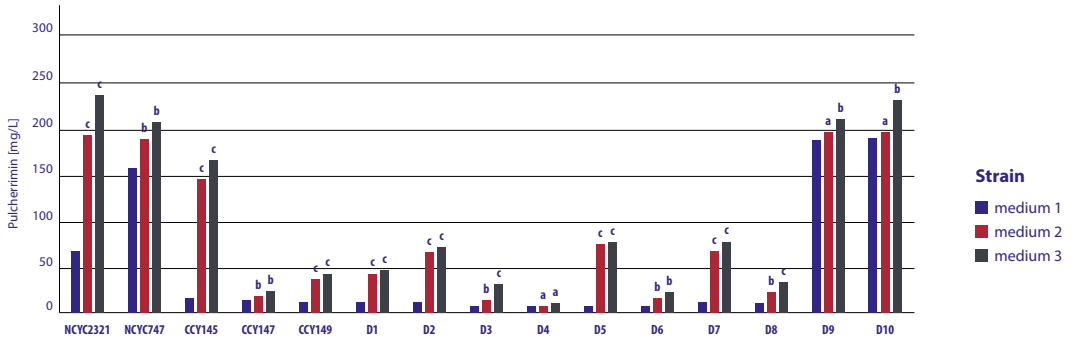
Not all non-*Saccharomyces* are equal

The way non-*Saccharomyces* are used in winemaking is highly specific, either at the species or the strain level. For example, *Lachancea thermotolerans* is mainly used for bioacidification because its original metabolism leads to production of lactic acid and ethanol from sugar. Hranilovic et al. (2018) studied the phenotypes of 94 *Lachancea thermotolerans* from different ecological niches and geographical areas in Chardonnay grape juice. Under the same conditions, they obtained lactic acid production from 1.8 g/L to 12 g/L, highlighting the difference between strains and their intraspecific variation of this trait.

Metschnikowia spp. is a good candidate for biocontrol as it produces pulcherriminic acid which binds iron from the medium through a spontaneous reaction to form an iron-bidding red pigment called pulcherrimin, leading to an iron deprivation[(1)].

This is a type of biocontrol preventing the indigenous flora development.

This genetic trait is species dependent, as demonstrated by Pawlikowska et al. (2020) who measured productions of pulcherriminic acid varying from 10 mg/L to 240 mg/L.



Pulcherrimin production of different *Metschnikowia spp.* strains in several culture media

Our bold R&D: trailblazing for innovative non-*Saccharomyces* solutions

Increasingly studied over the last 15 years, the vine and must ecosystem represents a real reservoir of biodiversity, beyond the *Saccharomyces cerevisiae* species.

Non-*Saccharomyces* yeasts make up a vast and extremely diverse group, with numerous species and a wide range of phenotypes.
The diversity and originality of their technological properties, which differ from those of *Saccharomyces cerevisiae*, have aroused a great deal of interest in the scientific community, among microbiologists and yeast producers, and among winemakers.

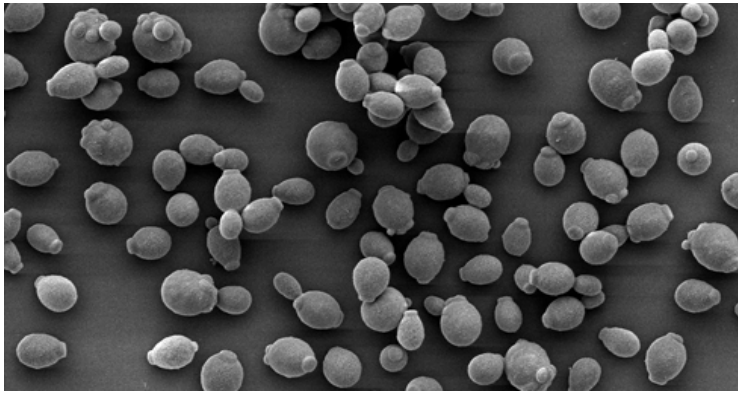
These species play a major role in the pre-fermentation stages of white, rosé and red winemaking, and are the subject of numerous studies carried out by our R&D teams in partnership with researchers from all over the world (Stellenbosch Institute in South Africa, INRAE SPO in France, University of Madrid, University of Palermo, etc.). New environmental challenges such as climate changes and market demands (reduction of chemical inputs, fresher wines, etc.) are at the heart of our concerns and drive our research programs. Bioprotection, improving freshness and diversifying the aromatic profiles of wines are all objectives that involve the study of specific non-*Saccharomyces* yeast species.

Optimal bioprotection for grapes and musts

▶ IOC GAÏA™

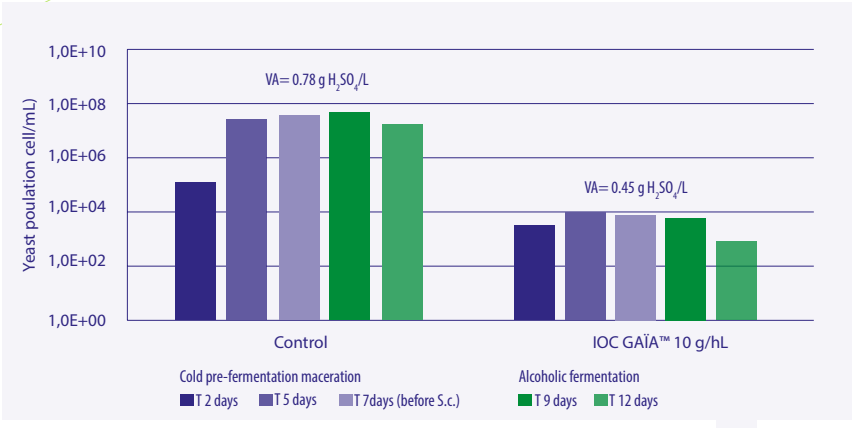
Bioprotection to control microbial contamination while reducing the use of SO_2 has been recently developed. Bioprotection can be achived by using a non-fermentative yeast, *Metschnikowia* species in the early stages of the winemaking process. It can be applied from the harvest until the selected *Saccharomyces cerevisiae* inoculation for alcoholic fermentation.

The Institut Français de la Vigne et du Vin (French Wine and Vine Institute) has selected IOC GAÏA™ , a *Metschnikowia fructicola* yeast with no fermenting power, to combat harmful flora. It fills an ecological niche by limiting deviations and the risk of triggering an excessively early alcoholic fermentation. IOC GAÏA™ is a major tool for limiting pre-fermentation sulphiting whether used during vatting or in harvesting trucks.
IOC GAÏA™ suppresses the growth and acetic acid production of native microorganisms like *Kloeckera apiculata* (also known as *Hanseniaspora uvarum*), acetic acid bacteria, and other microflora during the pre-fermentative stages.



***Metschnikowia fructicola* strain (Laboratoire R&D Lallemand Oenology, France).**

IOC GAÏA™ can be added directly to the grapes during harvest (with a sprayer or into each picking bin) or to red grapes at the beginning of cold soak.



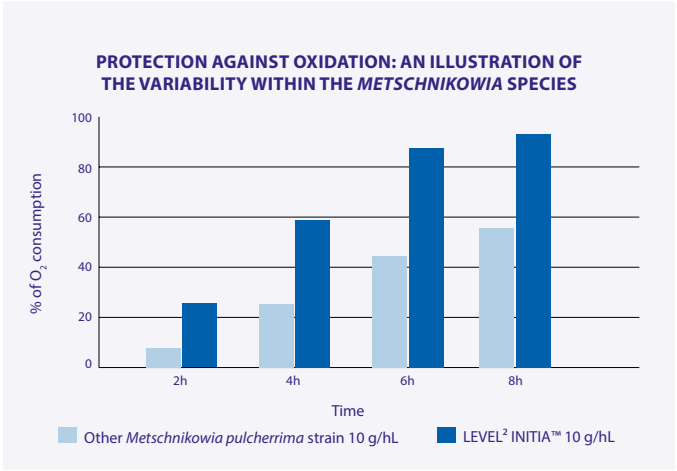
Evolution of Hanseniaspora uvarum populations and production of final acetic acid in the absence or presence of pre-fermentation bioprotection with IOC GAÏA™.
Pinot Noir (pasteurized must) pH 3.6, non SO_2 added - cold soak at 13 °C

The early colonisation and growth of IOC GAÏA™ 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.

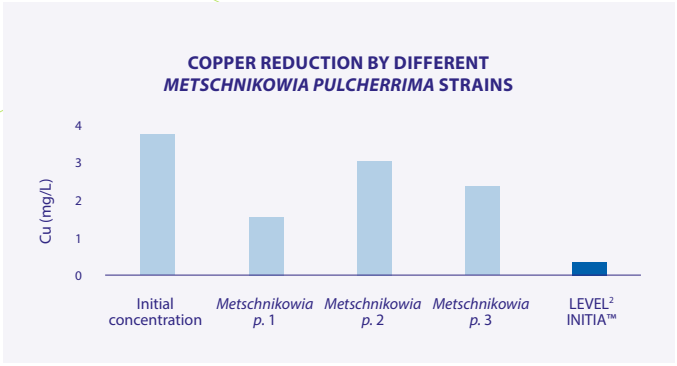
▶ LEVEL² INITIA™

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 a unique 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.



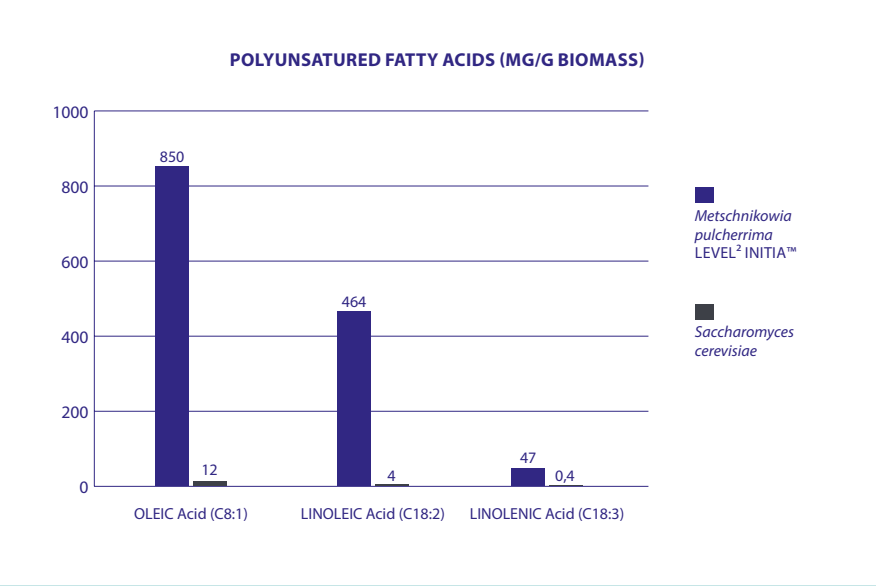
Oxygen consumption by two *Metschnikowia pulcherrima* strains inoculated at 10 g/hL in a Chardonnay must at 10 °C



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

Why does LEVEL² INITIA™ have this ability to consume oxygen so quickly?

LEVEL² INITIA™ has a low ability to assimilate grape fatty acids and phytosterols because they do not possess genes encoding for the corresponding transporters (*AUS1* and *PDR11*)[2]. During a PhD in partnership with Lallemand Oenology at Stellenbosch University (South Africa), Lethiwe Mbuyane demonstrated that this strain of *Metschnikowia pulcherrima* produces high amounts of polyunsaturated fatty acids (C18:2 + C18:3) while *Saccharomyces cerevisiae* only produces trace amounts. This synthesis requires substantial consumption of oxygen. For this property, there is a high intra-species variability and LEVEL² INITIA™ possesses a unique ability to quickly uptake dissolved oxygen in prefermentitive steps that explains its powerful bioprotection against must & wine oxidation.

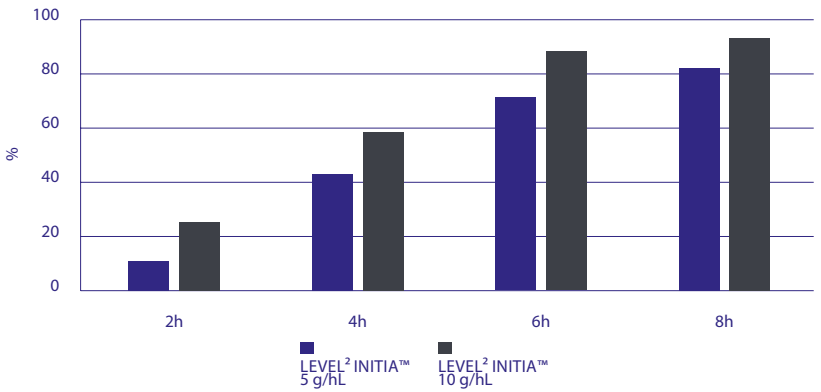


Polyunsaturated fatty acids composition of *Metschnikowia pulcherrima* LEVEL² INITIA™ versus *Saccharomyces cerevisiae*[3]

What is the optimal dosage for bioprotection non-*Saccharomyces* yeasts?

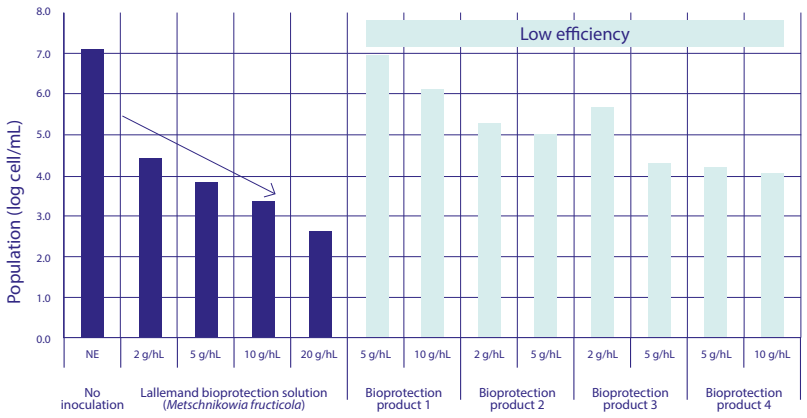
While 5 g/hL is the minimum recommended dosage by OIV (International Organisation of Vine and Wine), we demonstrated that higher inoculation rates can drastically improve efficiency of bioprotection and also dissolved oxygen consumption. 10 g/hL is our average optimal recommended dosage but we can go up to 25 g/hL depending on the risks and the prefermentative steps management (temperature, SO_2 level, pH, sanitary status of the grapes, etc.). At lower dosage, LEVEL² INITIA™ and IOC GAÏA™ will still have a positive impact and a better efficiency than other strains at similar dosage, but our recommendation to optimise their benefits remains 5 to 25 g/hL.

% OF O_2 CONSUMPTION



Oxygen consumption by LEVEL² INITIA inoculated at 5 g/hL or 10 g/hL in a Chardonnay must at 10°C

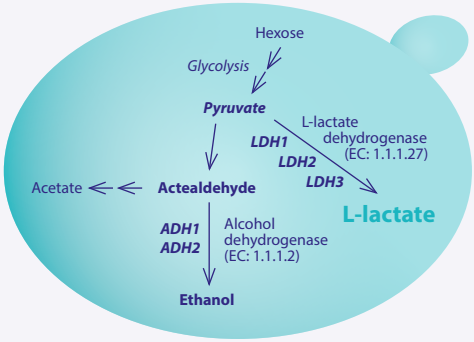
IMPACT OF BIOPROTECTION DOSE AND PRODUCT ON POPULATION OF *HANSENIASPORA UVARUM* DURING COLD SOAK (RESULTS OBTAINED IN COLLABORATION WITH IFV)



Bioacidification: naturally balancing climate changes effects

▶ LEVEL² LAKTIA™

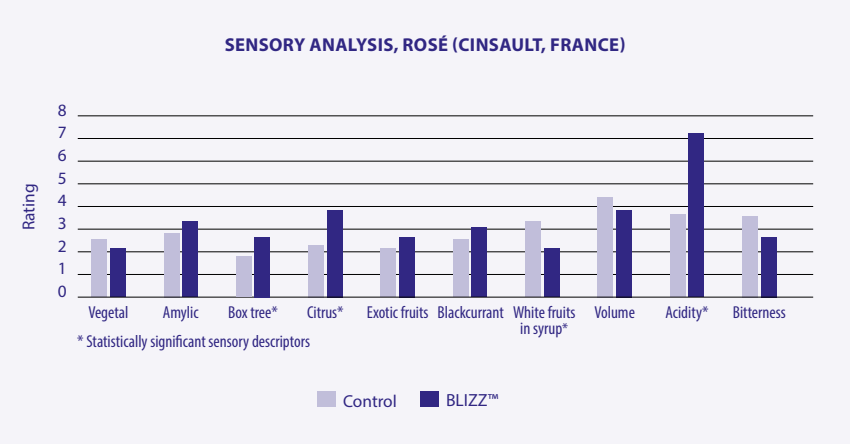
With the increase in alcohol levels and loss of acidity and freshness due to climate change, it is common practice to acidify wines with different acids of chemical origin.
This acidification has some limitations in terms of legislation, labelling in some countries, and in terms of impact (from an acidification 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 "sharp" and more appreciated, compared to the authorised organic acids usually added (tartaric, lactic, malic).

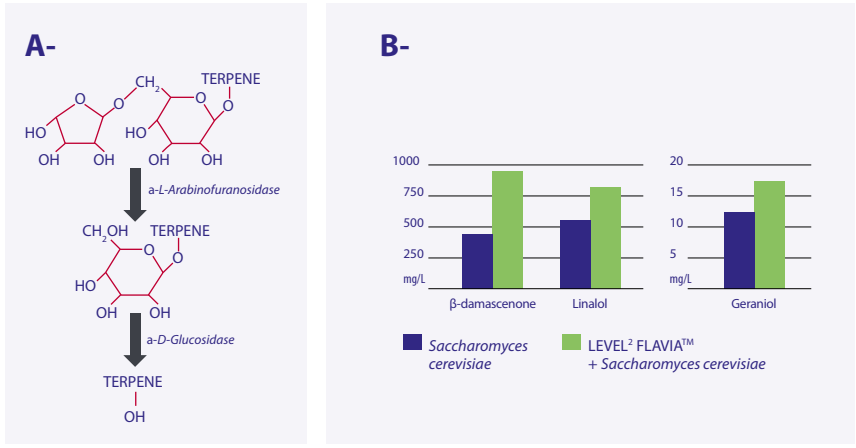


Sensory analysis led by ICV group on a rosé Cinsault (South of France) with BLIZZ™ compared to a control with no *Lachancea thermotolerans*

Enhanced complexity and aromas

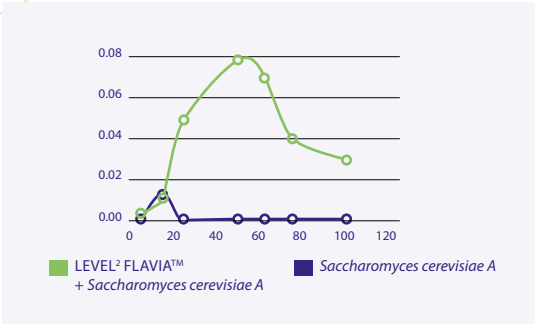
▶ LEVEL² FLAVIA™

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 Universitad 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.

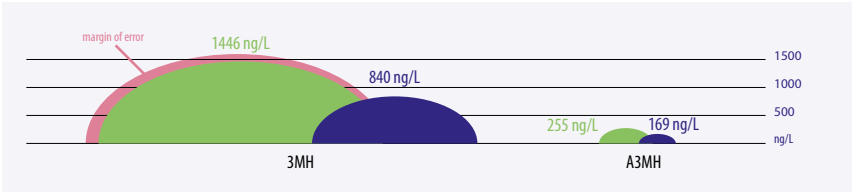


Impact of LEVEL² FLAVIA™ on the release of terpenes and norisoprenoids. Enzymatic synthesis pathway (A) and illustration on Muscat (France) inoculated or not with LEVEL² FLAVIA™

A collaboration between Lallemand Oenology and Institut National de Recherche pour l'Agriculture et l'Environnement[8] has established that LEVEL² FLAVIA™ possesses a significant β-lyase activity, responsible for the release of volatile thiols such as 3MH and 4MMP.

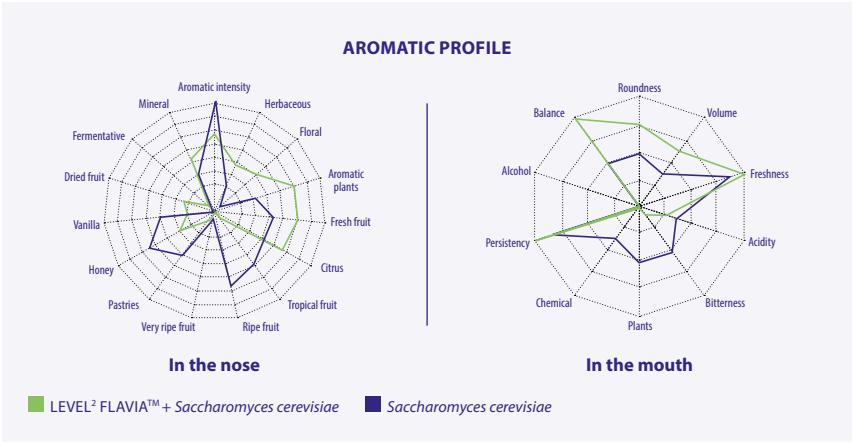


β-lyase activity (U/mL) of LEVEL² FLAVIA™ in comparison with *Saccharomyces cerevisiae*



Impact of LEVEL² FLAVIA™ on the release of thiols on Colombard (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.

A-

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

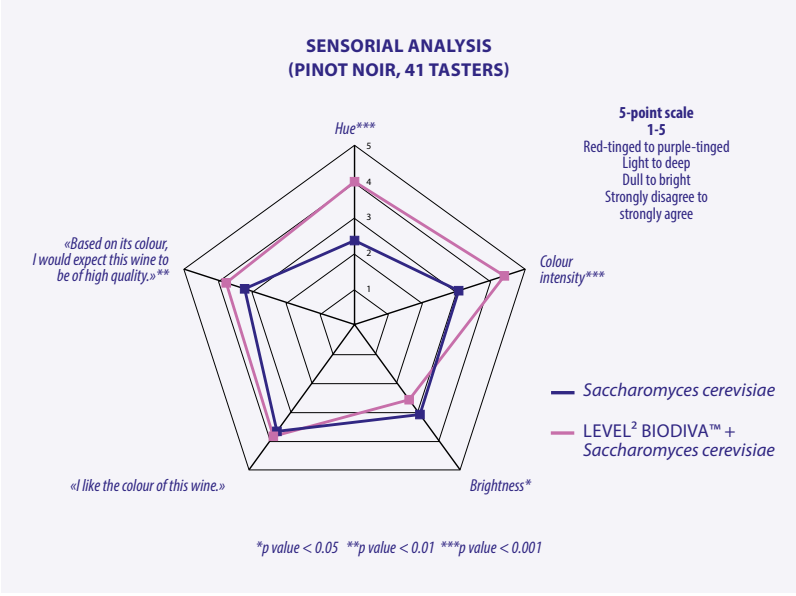
B-

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 in premium red wines. For some varietals such as Pinot noir colour stability is a challenge due to the anthocyanin type and availability. McCollough *et. al.*[9] (University of Auckland) demonstrated that wines made using LEVEL² BIODIVA™ and *Saccharomyces cerevisiae* resulted in greater density (at 420 and 520nm) than wines fermented with *Saccharomyces cerevisiae* alone. This can be explained by the initial binding of colour pigments onto the cell wall polysaccharides of LEVEL² BIODIVA™ which are subsequently released and stabilised during the aging process.

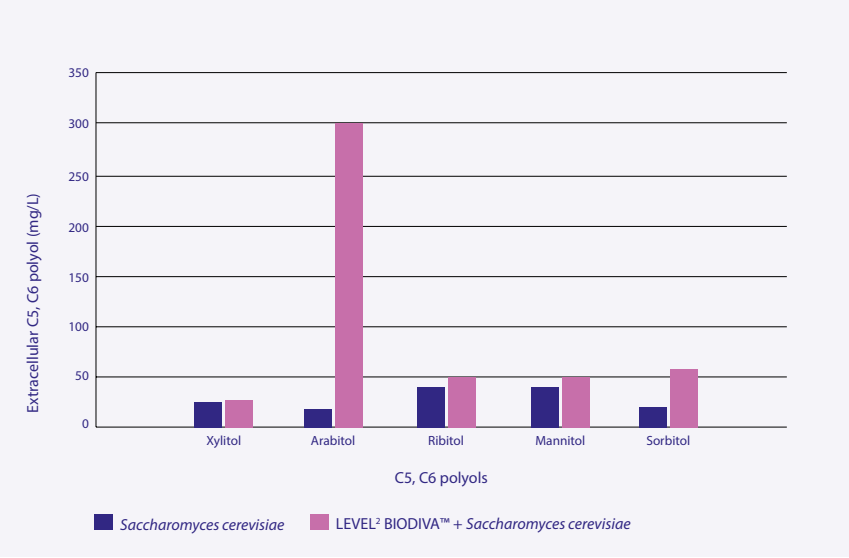


LEVEL² BIODIVA™ contributes to a better quality with a higher colour intensity with more purple in tone (Pinot noir, New Zealand).

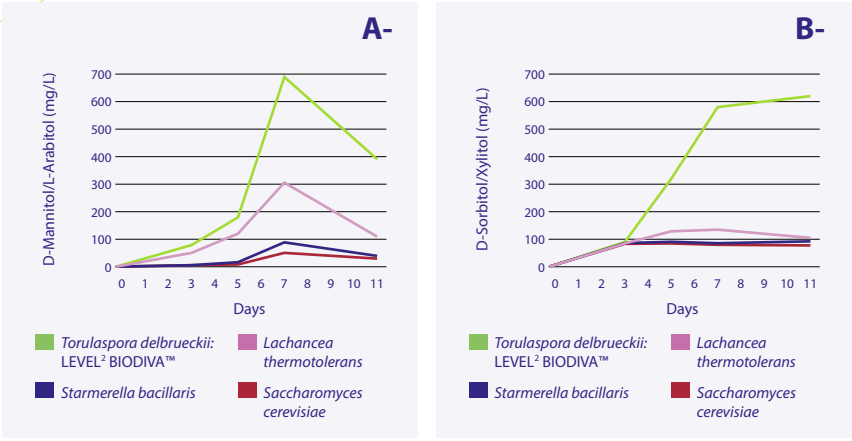
Improved mouthfeel and texture

▶ LEVEL² BIODIVA™

LEVEL² BIODIVA™, a unique strain of *Torulaspora delbrueckii* produces significant concentrations of C5 and C6 polyols under wine fermentation conditions (Mbuyane et al., 2018, Stellenbosch University[10]). 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)

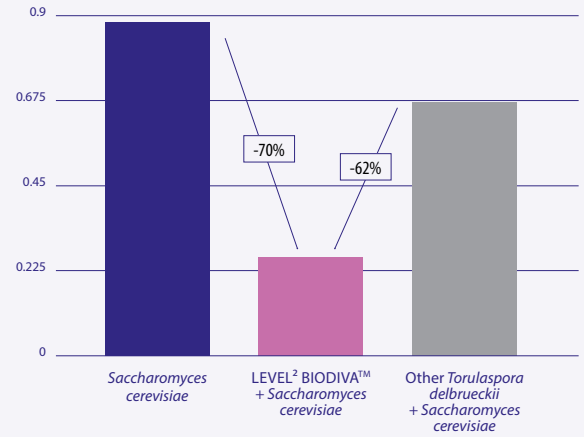
Better management of volatile acidity

▶ LEVEL² BIODIVA™

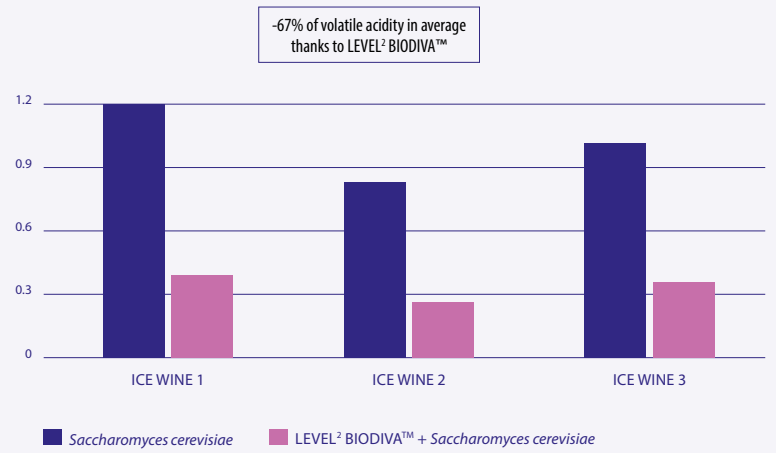
In prefermentative stages, the contaminating flora, particularly *Hanseniaspora uvarum* can lead to very high production of volatile acidity. LEVEL² INITIA™, because of its powerful antimicrobial action, lead to lower levels of volatile acidity in final wines.

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 H_2SO_4) IN MUST WITH HIGH LEVELS OF SUGAR



VOLATILE ACIDITY (g/L H_2SO_4) 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 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 process adapted to each strain has allowed Lallemand Oenology to gain an unparalleled expertise in the production of non-*Saccharomyces* yeasts.

WINEMAKING APPLICATION AND BENEFITS	LALLEMAND OENOLOGY SOLUTIONS	MAIN ACTIVITY AND WAY OF ACTION
Protects red grapes against microbial spoilage during transportation or cold soak	**IOC GAÏA™** *Metschnikowia fructicola*	Outcompetes VA-producing native microflora via early colonization and microbial crowding.
Specific bioprotection for white and rosé must combining protection from oxidative damage and microbial spoilage	**LEVEL² INITIA™** *Metschnikowia pulcherrima*	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™** *Lachancea thermotolerans*	Consistent and reliable lactic acid production from sugar.
Enhances complexity and aromatics	**LEVEL² FLAVIA™** *Metschnikowia pulcherrima*	High expression of unique enzymes that cleave aroma precursors to reveal terpenes and thiols.
Improves colour density in red wines	**LEVEL² BIODIVA™** *Torulaspora delbrueckii*	Release of polysaccharides improving colour stability.
Managing volatile acidity in high sugar musts	**LEVEL² BIODIVA™** *Torulaspora delbrueckii*	Overcomes osmotic pressure through a specific metabolism non producing acetic acid.
Improving mouthfeel and texture	**LEVEL² BIODIVA™** *Torulaspora delbrueckii*	High production of polyols and mannoproteins.

References

(1) - Oro, Ciani et Comitini 2014; Sipiczki, 2006
(2) - Tesnière et al., 2021
(3) - Lethiwe Mbuyane PhD thesis - Stellenbosch University and Lallemand Oenology
(4) - Sgouros et al, 2020
(5) - Comitini et al. 2011; Gobbi et al., 2013; Morata et al. 2019; Sgouros et al. 2020; Hranilovic et al. 2021
(6) - Günata et al., 1988
(7) - Vilanova & Sieiro, 2006
(8) - Pauline Seguinot PhD thesis - INRAE SPO and Lallemand Oenology
(9) - McCullough et al., 2023
(10) - Mbuyane et al., 2018

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