Active bio protection in red wines with *Metschnikowia pulcherrima*

SHUTTERSTOCK.

ONE OF THE NON-SACCHAROMYCES YEASTS STUDIED IS *METSCHNIKOWIA PULCHERRIMA*. THIS ARTICLE WILL FOCUS ON THE ANTAGONISTIC ACTIVITY OF A SPECIFIC STRAIN, *M. PULCHERRIMA* LEVEL^2 GUARDIA™, ON OTHER WINE YEAST SPECIES FOR BIO PROTECTION APPLICATIONS.

BY **AMANDINE DEROITE**[1]

MORE AND MORE WINEMAKERS ARE REDUCING their use of sulphites in wine in order to respond to consumer demands. Alternative biological solutions to control microbial contamination, while reducing the use of SO_2, have recently been developed as bio protection. One of the principles of bio protection is based on the management of detrimental microbial populations more than their eradication. Moreover, having an alternative such as microbiological bio protection can be an interesting option, especially in the context of global warming where the increase in pH renders SO_2 less efficient.

With the continuous interest in the selection of new *Saccharomyces cerevisiae* and *Oenoccocus oeni* strains, particular attention has been on the selection of non-*Saccharomyces* species/strains for, amongst other things, the natural bio protection abilities against spoilage yeasts or bacteria.

LEVEL^2 GUARDIA™ – POWERFUL ANTIMICROBIAL ACTION IN RED WINES

LEVEL^2 Guardia™ is the latest *Metschnikowia pulcherrima* yeast in the Lallemand portfolio. It was selected by the Institut Français de la Vigne et du Vin in Burgundy, France, for its suitable properties during the pre-fermentative steps in red winemaking, as well as its high ability to control other contaminating microorganisms.

In wine must, LEVEL^2 Guardia™ can implement itself very efficiently and multiply, and by doing so, occupy the must environment to displace other species, even at low temperatures. As shown in Figure 1, a Pinot noir 2020 (IFV Beaune, Burgundy, France), LEVEL^2 Guardia™ was able to multiply during a cold soak of five days at 10°C. Consequently, at the end of this pre-fermentative step a reduction of the

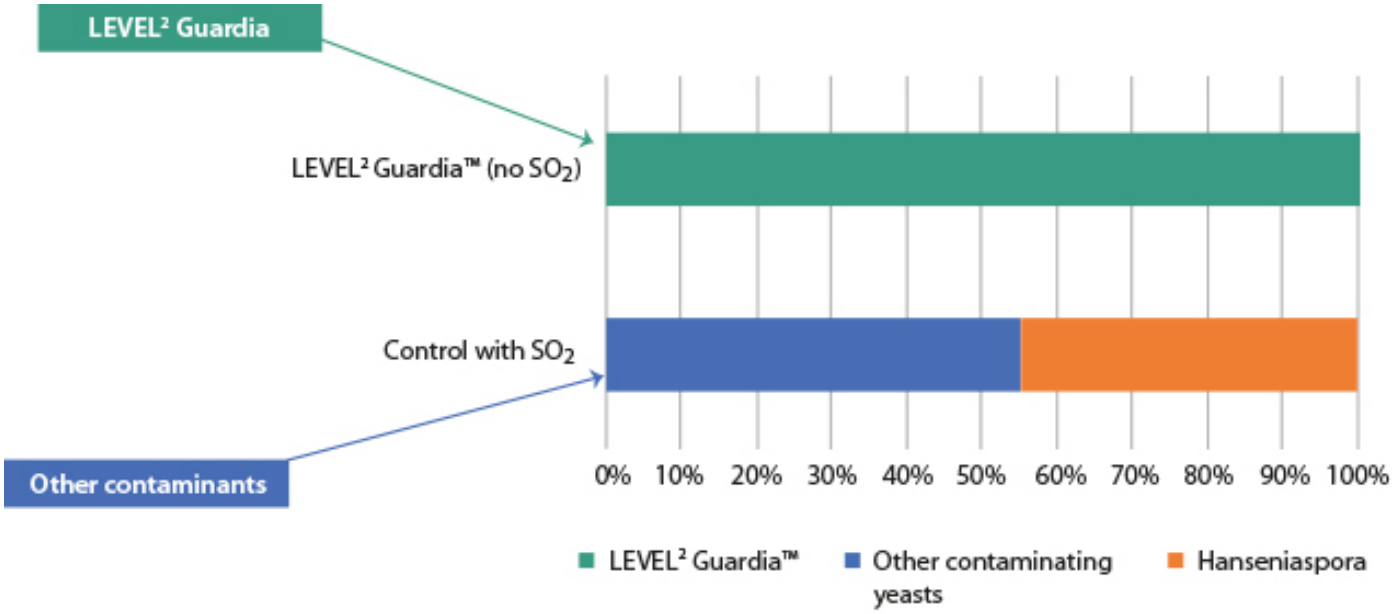

FIGURE 1. Yeast count after a five day cold soak at 10°C in a Pinot noir (IFV Beaune, France, 2020). Trial comparing LEVEL² Guardia™ added at 10 g/hL to a control with SO_2 addition at 2.5 g/100 kg.

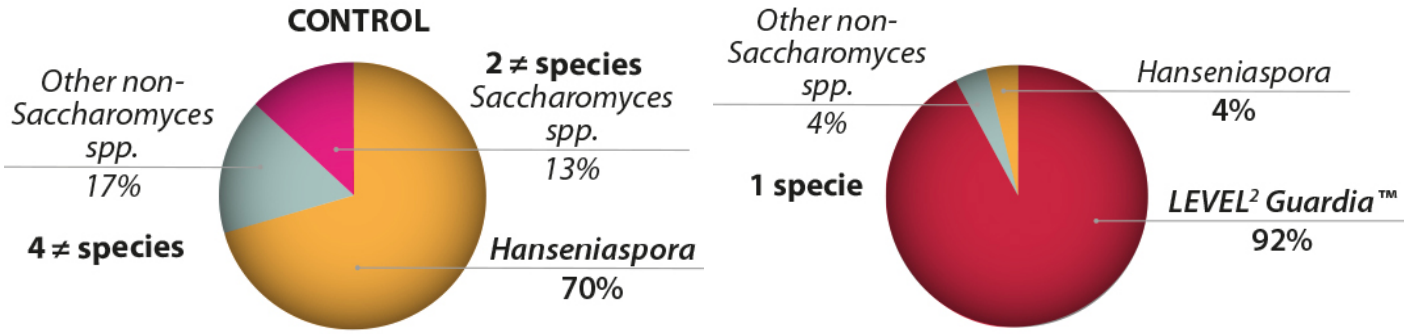

FIGURE 2. Implantation control done during a five day cold soak at 10°C in a Grenache (INCAVI, Spain, 2020). Trial comparing LEVEL² Guardia™ added at 10 g/hL to a control without bio protection. No sulphites added in both cases.

spoilage yeast *Hanseniaspora uvarum* and other contaminating yeasts, in comparison with a control with SO_2 addition, was seen.

Another trial on a Grenache 2020 (INCAVI, Spain) also illustrates the good implantation of LEVEL² Guardia™ at low temperature, as well as its high antimicrobial action against different microbial populations. As with the previous trial, LEVEL² Guardia™ inoculation was measured against SO_2 addition during cold soak of five days at 10°C. Results during the cold soak showed a good implantation of LEVEL² Guardia™ and other contaminating species, such as *Hanseniaspora* numbers, were significantly reduced (Figure 2). Both tanks were then inoculated with the same *Saccharomyces cerevisiae*. Volatile acidity measured at the end of the alcoholic fermentation was significantly lower for the bio protected wine (Figure 3).

WHY IS LEVEL² GUARDIA™ SUCH A POWERFUL BIO PROTECTION AGENT?

Metschnikowia pulcherrima is an interesting microorganism found in the must flora. As with *Saccharomyces cerevisiae*, within the specie, there are many different strains behaving differently from one another, hence the importance of selecting the right yeast for a specific application.

The mechanism of action, quite unique to this strain of *M. pulcherrima*, is its ability to secrete pulcherimmic acid. Pulcherimmic acid is a natural acid with no sensory impact, produced by some yeast species, especially *M. pulcherrima* who possesses the genes (PUL1, PUL2, PUL4, snf2) which enables its synthesis. When pulcherrimic acid is produced by the yeast, once excreted into the media, it will have a strong affinity for the free iron and subsequently chelate it (Figure 4).

Pulcherrimin is then formed. The iron present in the must is depleted and the growth of contaminating species (for example, *Hanseniaspora*, etc.) will be reduced as free iron is a necessary element for their growth. Figure 5 shows the different free and total iron concentration in a must where different *M. pulcherrima* strains, among which LEVEL² Guardia™ and a selected *Saccharomyces cerevisiae* strain, were used.

THE POSITIVE ASSOCIATION OF LEVEL² GUARDIA™ AND *SACCHAROMYCES CEREVISIAE* WINE YEAST

While LEVEL² Guardia™ is exceedingly efficient at chelating free iron from the must environment and thus reduce the growth of other yeast species, it could be assumed that it can also affect the growth

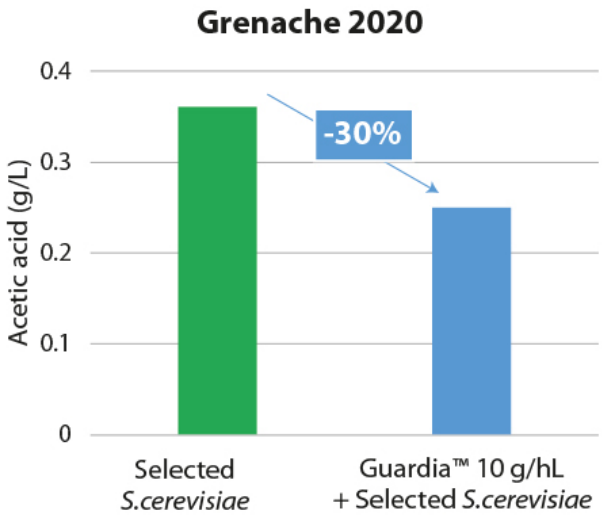

FIGURE 3. Volatile acidity in Grenache wines (INCAVI, Spain, 2020). Trial comparing LEVEL² Guardia™ added at 10 g/hL to a control without bio protection. No sulphites added in both cases.

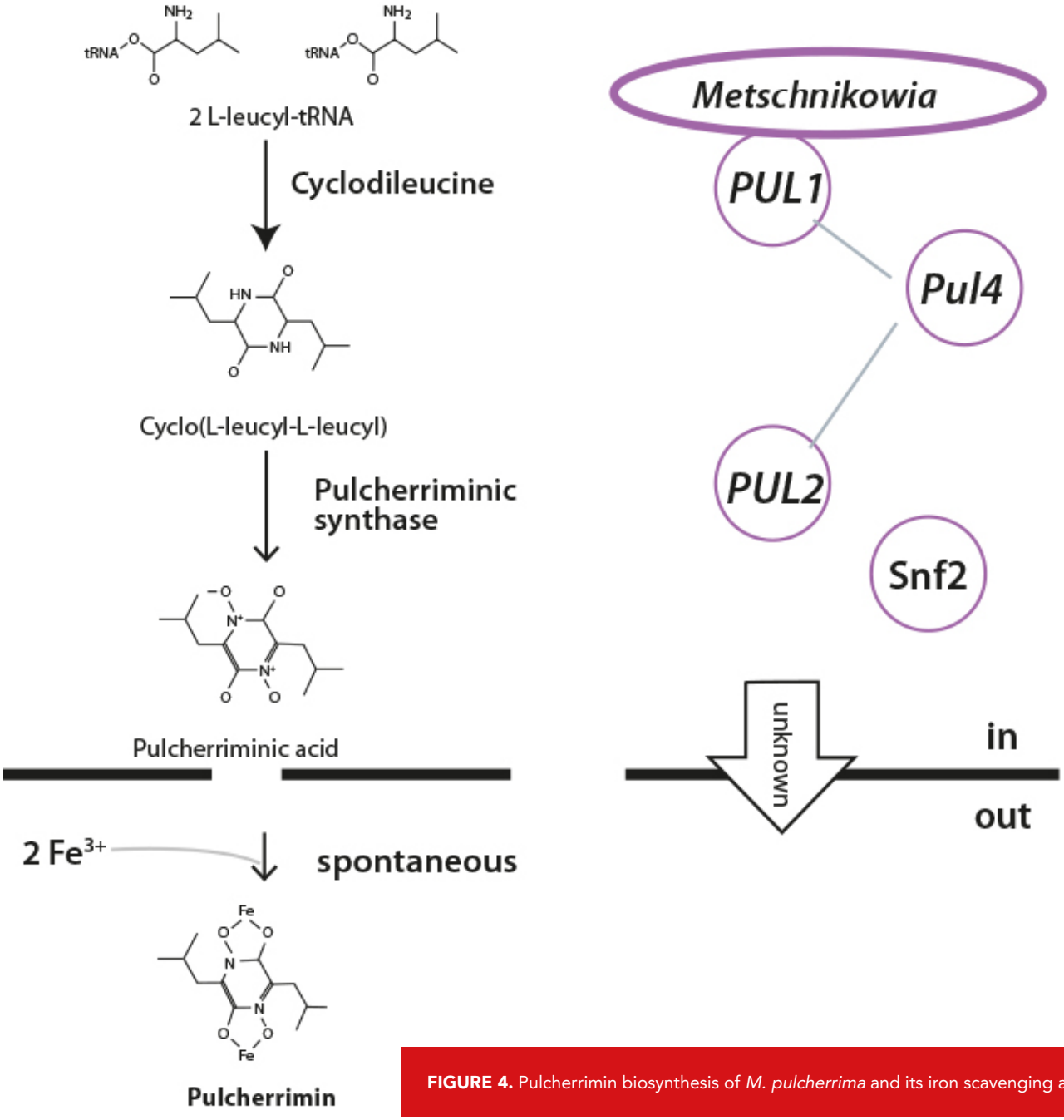

FIGURE 4. Pulcherrimin biosynthesis of *M. pulcherrima* and its iron scavenging ability.

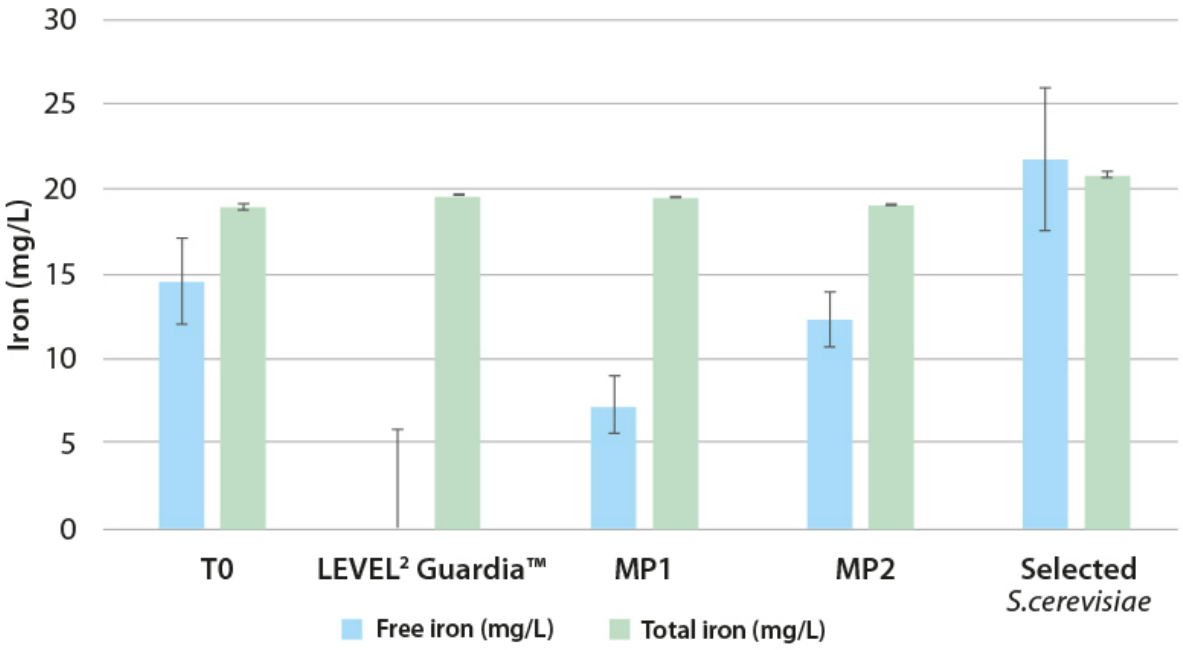

FIGURE 5. Free and total iron concentration in must with different *M. pulcherrima* strains and a *S. cerevisiae*.

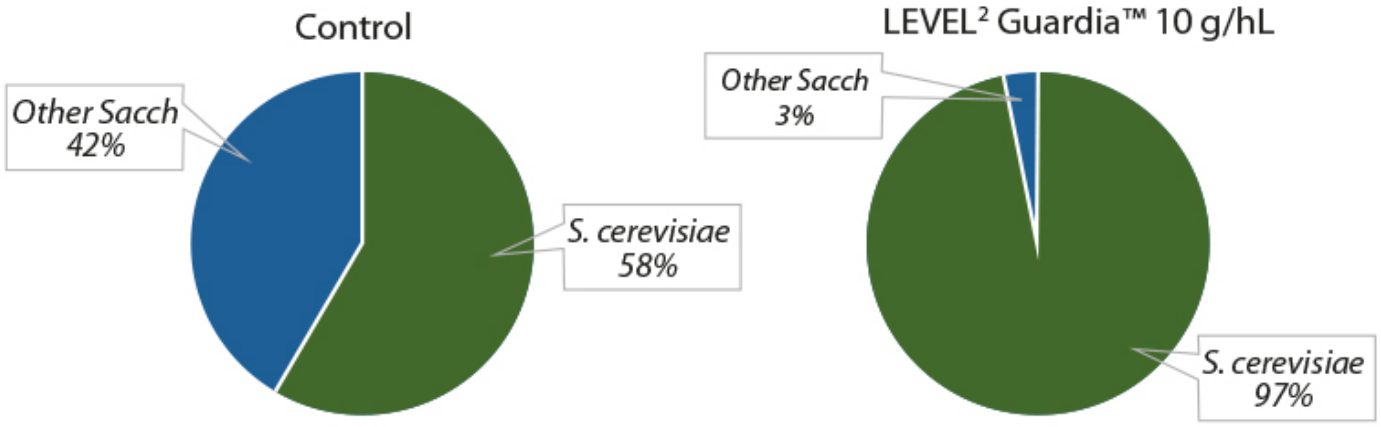

FIGURE 6. Implantation control done halfway through alcoholic fermentation in a Grenache (INCAVI, Spain, 2020). Trial comparing LEVEL² Guardia™ added at 10 g/hL to a control without bio protection. No sulphites added in both cases.

of the essential *S. cerevisiae* needed to complete the fermentation. However, the wine yeast *S. cerevisiae* has the ability to scavenge back the iron bound to pulcherrimic acid and use it for its metabolic functions. Thanks to the presence of the PUL3 and PUL4 genes within its genome, selected wine yeast *S. cerevisiae* can be inoculated following the use of LEVEL² Guardia™.

Moreover, the implantation of the selected *S. cerevisiae* was shown to be even more efficient when LEVEL² Guardia™ has been used prior to fermentation as shown in Figure 6, probably because of the strong limitation of contaminant flora.

CONCLUSION

During pre-fermentation, the must is susceptible to the development of undesirable microorganisms and protection of the must is necessary to avoid sensory deviation right at the onset of the winemaking process. The use of LEVEL² Guardia™, for example during cold soak of red grapes, is an efficient alternative to SO_2 to control a wide range of contaminants.

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Note: Article previously published in *Revue des Oenologues*.

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