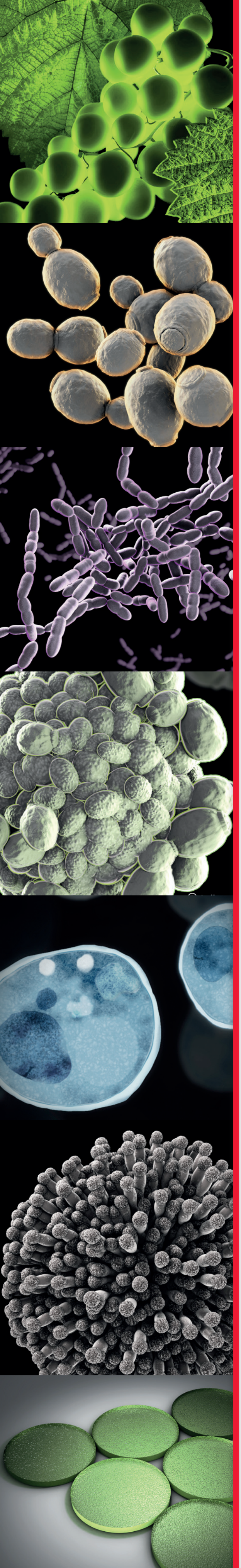

Active bioprotection in red wines with *Metschnikowia pulcherrima* LEVEL² GUARDIA™

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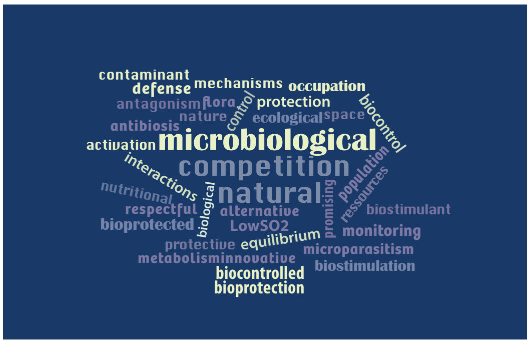
More and more, winemakers reduce their use of sulites in wine to respond to consumer demands. Alternative biological solutions to control microbial contamination while reducing the use of SO_2 have been recently developped as bioprotection. One of the principles of bioprotection is based on the management of detrimental microbial populations more than their eradication. Moreover, having an alternative such as microbiological bioprotection 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, an particular attention has been on the selection of non-*Saccharomyces* species/strains for, amongst other thing, the natural bioprotection abilities against spoilage yeasts or bacteria.

One of the non-*Saccharomyces* studied is *Metschnikowia pulcherrima*. This paper will focus on the antagonistic activity of a specific strain *M. pulcherrima* ***LEVEL² Guardia™*** on other wine yeast species for bioprotection applications.

LEVEL² Guardia™: powerful antimicrobial action in red wines

LEVEL² Guardia™ is the latest *Metschnikowia pulcherrima* yeast in our portfolio and was selected by the Institut Français de la Vigne et du Vin in Burgundy, France for its properties suitable during the prefermentative steps in red winemaking as well as its high ability to control other contaminating microorganisms.

In wine must, ***LEVEL² Guardia™*** can implement itself very efficiently and multiply, and by doing so, occupied the must environment to displace other species, even at low temperature. As shown in Figure 1, a Pinot noir 2020 (IFV Beaune, Burgundy, France), ***LEVEL² Guardia™*** was able to multiply during a cold soak of 5 days at 10°C. Consequently, at the end of this prefermentative step, a reduction of the spoilage yeast *Hanseniaspora uvarum* and other contaminating yeasts in comparison with a control with SO_2 addition was seen.

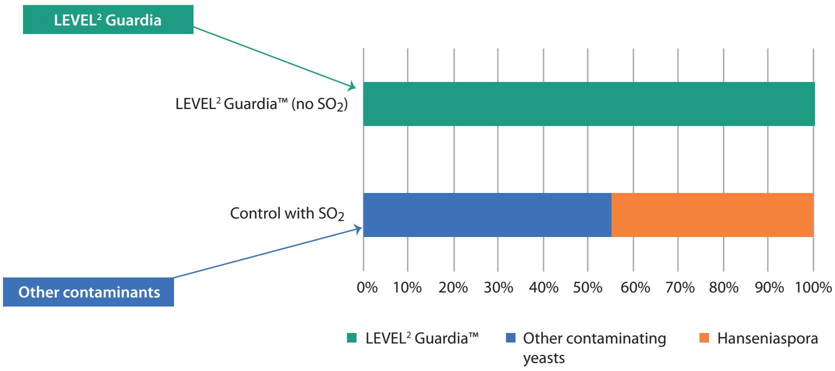


Figure 1. Yeast count after a 5 days cold soak at 10°C in a Pinot Noir (IFV Beaune, France, 2020). Trial comparing *LEVEL² Guardia™* added at 10 g/hL to a control with SO_2 addition at 2.5 g / 100 kg.

Another trial led 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 5 days at 10°C. Results during the cold soak showed a good implantation of ***LEVEL² Guardia™*** and other contaminating species such as *Hanseniaspora* numbers are 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 bioprotected wine (Figure 3)

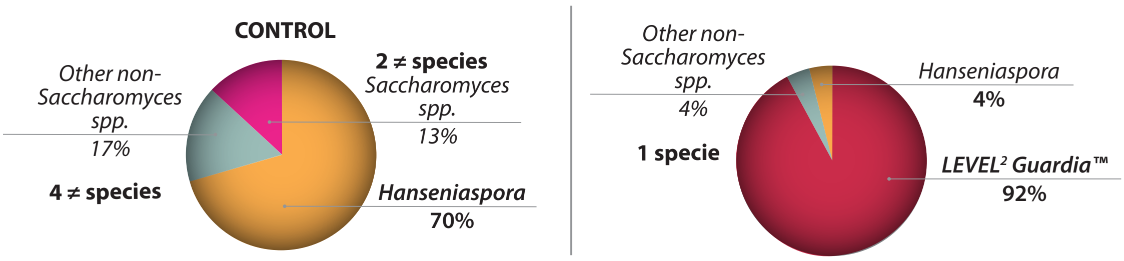


Figure 2. Implantation control done during a 5 days cold soak at 10°C in a Grenache (INCAVI, Spain, 2020). Trial comparing *LEVEL² Guardia*™ added at 10 g/hL to a control without bioprotection. No sulfites were added in both cases.

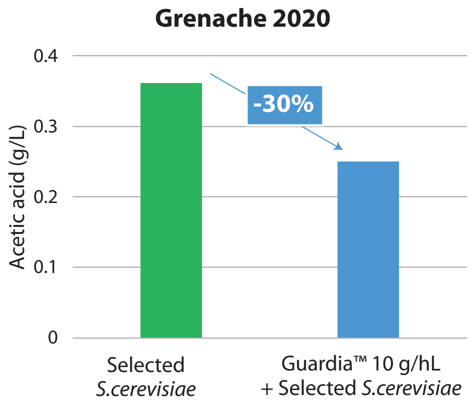


Figure 3. Volatile acidity in Grenache wines (INCAVI, Spain, 2020). Trial comparing *LEVEL² Guardia*™ added at 10 g/hL to a control without bioprotection. No sulfites were added in both cases.

Why is *LEVEL² Guardia*™ such a powerful bioprotection agent?

Metchnikowia pulcherimma 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.pulcherimma* 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 to the free iron, and chelate it (Figure 4).

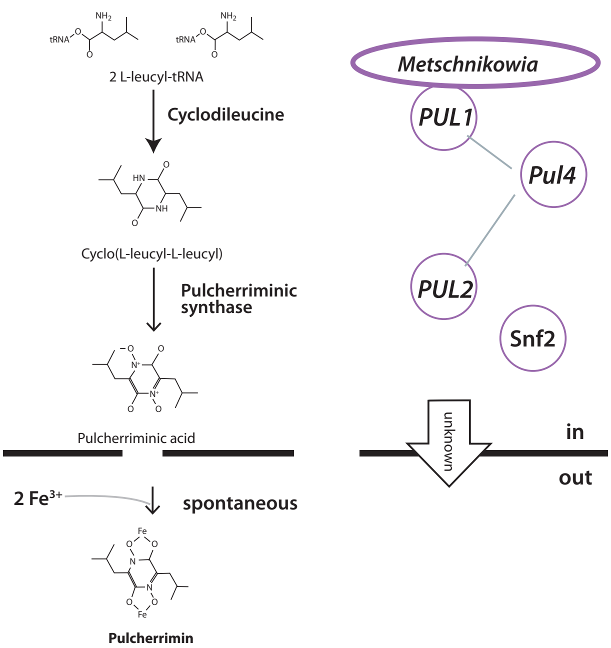


Figure 4. Pulcherrimin biosynthesis of *M. pulcherrima* and its iron scavenging ability (Sipiczki, 2020).

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* among which ***LEVEL² Guardia™*** and a selected *Saccharomyces cerevisiae*, were used.

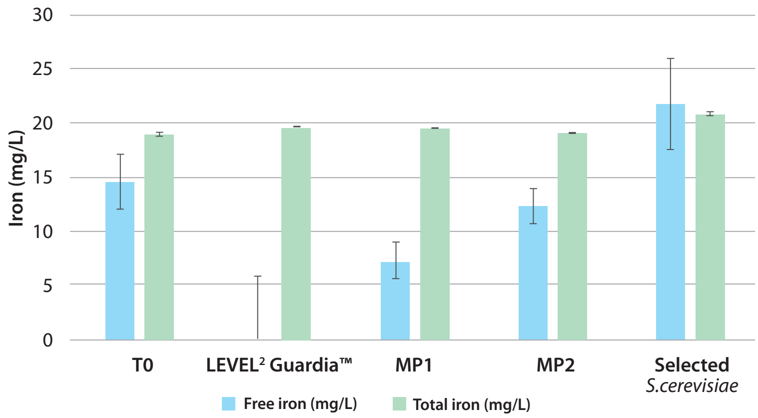


Figure 5. Free and Total iron concentration in must with different *M.pulcherrima* and a *S. cerevisiae*

Phenotypically speaking, 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 (Figure 6).

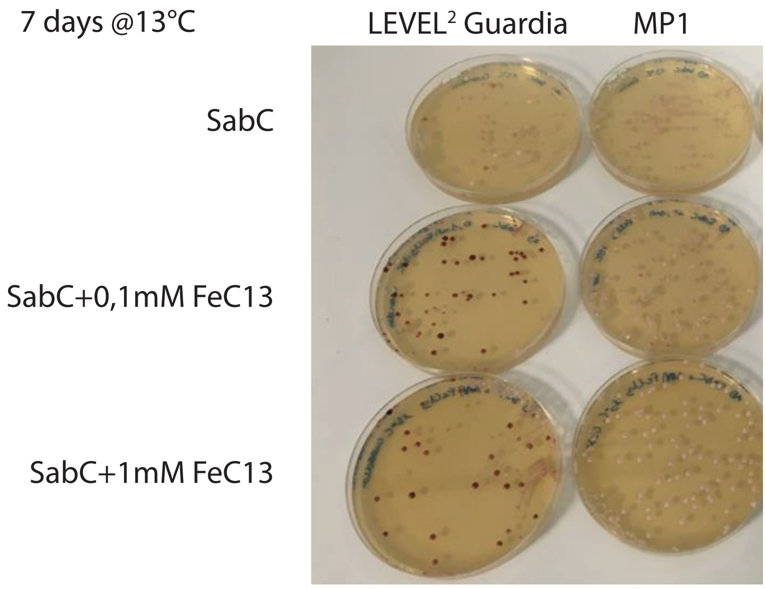


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Figure 6. *LEVEL² Guardia™* grown 7 days at 13°C on specific media with iron and compared to other *M.pulcherrima* (Laboratoire R&D Lallemand, France)

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 specie, it would be assumed that the essential *S. cerevisiae* needed to complete fermentation could suffer from its pulcherrimin formation (leading to the must depletion in iron). 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 (Krause et al, 2018), 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 7, probably because of the strong limitation of contaminant flora.

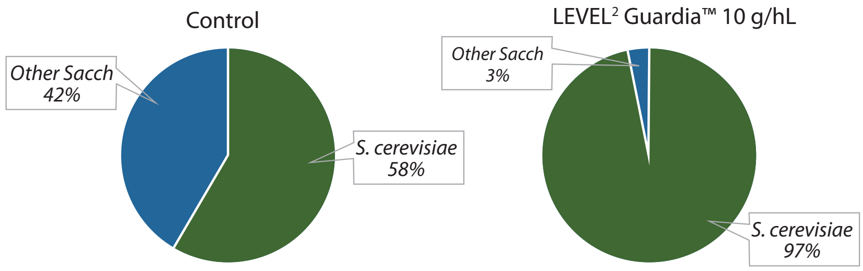

Figure 7. Implantation control done halfway through alcoholic fermentation in a Grenache (INCAVI, Spain, 2020). Trial comparing *LEVEL² Guardia™* added at 10 g/hL to a control without bioprotection. No sulfites were added in both cases.

Conclusion

During pre-fermentation, the must is vulnerable to the development of undesirable microorganisms, and protection of the must is necessary to avoid sensory deviation right at the onset of the winemaking itinerary. 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.