Wine Bacteria under investigation

Nº4

BIOCONTROL AGENTS AGAINST *BRETTANOMYCES*

1. *BRETTANOMYCES* : A ROBUST CONTAMINANT

The contaminating yeast *Brettanomyces* is a problem, notably for red wines. This yeast is very opportunistic and can survive and multiply in difficult conditions throughout the life of the wine. Hygienic conditions and microbiological controls can inhibit its growth, but will not eliminate it. The goal then is to limit its development, which will in turn limit the production of volatile phenols. The use of SO_2 is the preferred method to control its development, however there is a recent trend to reduce the use of SO_2 in wine, as well as a general increase in wine pH, which reduces its efficacy. Moreover, there is a great variability in the resistance of SO_2 among different *Brettanomyces* yeasts. The inoculation with our selected bacteria is a good option to protect the wine during the fermentation process, and new studies also show their potential to protect wine during the ageing steps against *Brettanomyces* re-contamination.

2. CO-INOCULATION AS PREVENTIVE TOOL

Previous studies have shown the clear impact of early inoculation of selected wine bacteria on the reduction in final volatile phenols levels. In 2014, the OIV recognized that co-inoculation of selected lactic bacteria could help to reduce the phase between alcoholic fermentation (AF) and malolactic fermentation (MLF) and consequently limit the development of *Brettanomyces*. Recent studies in collaboration with IFV (France) show that some selected bacteria can have a direct inhibition on *Brettanomyces* growth.

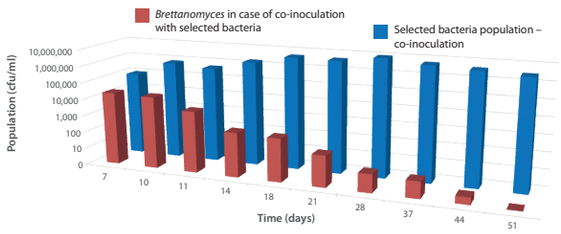

Figure 1. *Brettanomyces* growth during co-inoculation with wine bacteria in Pinot Noir (Burgundy, France)

Yeast and bacteria populations were monitored in *Brettanomyces* contaminated wine inoculated for MLF with a selected bacteria (Figure 1) or spontaneous MLF (indigenous bacteria) (Figure 2) to demonstrate how lactic acid bacteria can affect the growth of *Brettanomyces*. When inoculated with wine bacteria, there is no growth of *Brettanomyces* (even with a high contamination) and moreover, the Brett levels decrease when the population of the selected bacteria increases. In contrast, where spontaneous MLF occurs, *Brettanomyces* population maintains a high level until the 11th day (date of racking) and there is a regrowth due to the slow development of spontaneous bacteria population. Final Brett levels are significantly different between the wines in co-inoculation and the control: there are 10 times more *Brettanomyces* in the control than the co-inoculated wines. These results confirm the strong competition between our selected bacteria and *Brettanomyces*, due to the early dominance and an excellent survivability of those bacteria.

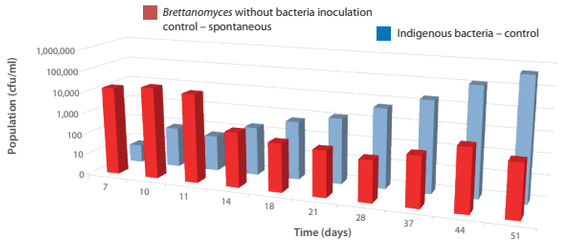

Figure 2. *Brettanomyces* growth during spontaneous MLF in Pinot Noir (Burgundy, France)

BIOCONTROL IS OPTIMIZED WITH OUR HIGH QUALITY WINE BACTERIA

3. BIOCONTROL AFTER ALCOHOLIC FERMENTATION

For a variety of reasons, it may not be possible to co-inoculate wines, however sequential inoculation, at the end of AF, can also help reduce the risk of *Brettanomyces* development. A study done (no SO_2 addition at the end of MLF) with IFV (France) showed that even if the wine, when you inoculate with our wine bacteria, has *Brettanomyces* contamination (100 cfu/mL) after AF, the growth of our selected bacteria is not affected and significantly limits the development of *Brettanomyces*. Final populations of *Brettanomyces* in the presence of selected bacteria was equivalent to the initial population of Brett (around 100 cfu/mL) whereas in the control with spontaneous MLF, final level of *Brettanomyces* is much higher (100,000 cfu/mL) with a peak at 1,000, 000 cfu/mL, with these wines showing notable bretty aromas. The control over the contaminants lasted for at least 2 months after the end of MLF.

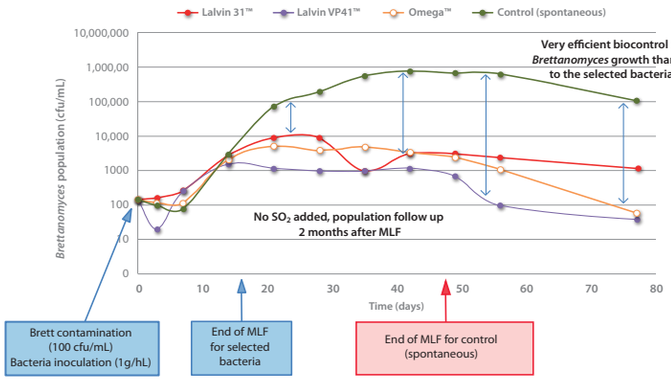

Figure 3. Biocontrol of *Brettanomyces* population with various selected wine bacteria.

4. PROTECTING WINES AGAINST BRETT RE-CONTAMINATION AFTER MLF

Recent findings from the IFV (France) had shown that maintaining a living population of selected wine bacteria, after MLF, can prevent *Brettanomyces* re-contamination under certain wine conditions. It was shown in 2017 Pinot Noir wine (pH 3.5, 18°C, no residual sugars), inoculated with wine bacteria after AF, being better protected from re-contamination by *Brettanomyces* compared to non-inoculated wines. If the level of contamination is low (70 cfu/mL), wine bacteria will reduce the *Brettanomyces* population to insignificant levels (figure 4). More than one month after the end of MLF, the volatile phenols were undetectable, whereas the uninoculated wine had volatile phenols above detection threshold. Without stabilisation, VA remaind low as well, at 0,4 g/L. It was concluded that our selected bacteria remaining viable after the end of MLF has a protective action against Brett re-contamination during ageing for wines with no residual sugars. This a good strategy to reduce the use of SO_2 during winemaking in function of wine conditions (no residual sugars, pH < 3,7) and to monitor the volatile acidity.

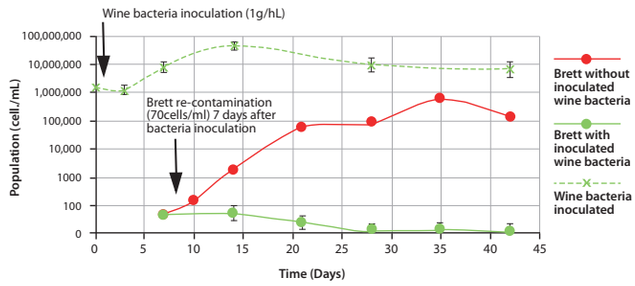

Figure 4. Evolution of *Brettanomyces* at low level of contamination with or without inoculated selected wine bacteria, without SO_2 addition, at the end of MLF