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 SO2 is the preferred method to control its development, however there is a recent trend to reduce the use of SO2 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 SO2 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)](image)

Yeast and bacteria populations were monitored 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)](image)

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 SO2 addition at the end of MLF) with IFV (France) showed that even if the wine after AF has a high level of Brettanomyces contamination (1000 cfu/mL), the growth of our selected bacteria after AF significantly limits the development of Brettanomyces. Final levels of Brettanomyces in the presence of selected bacteria was equivalent as the initial level (between 100 to 1,000 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 Brett aromas. The control over the contaminants lasted for at least 2 months after the end of MLF.

4. **PROTECTING THE WINE 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. It was shown in 2017 Pinot Noir wine (pH 3.5, 18°C), 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 (50 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 remain 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. This a good strategy to reduce the use of SO2 during winemaking.

![Figure 3. Biocontrol of Brettanomyces population with various selected wine bacteria.](image)

![Figure 4. Evolution of Brettanomyces at low level of contamination with or without inoculated selected wine bacteria, without SO2 addition, at the end of MLF](image)