The Wine EXPERT
Practical Winemaking Information
LALLEMAND

CERTAIN SELECTED WINE BACTERIA ACT AS BIOCONTROL TOOL AGAINST *BRETTANOMYCES*

What is biocontrol?

The concept of biological control or protection also called « biocontrol » is a method known and applied since the beginning of agriculture even without knowing it. Agriculture development was necessarily linked with the need to protect cultures with various methods including biocontrol. With the development of chemistry applied to the agri-food sector biological control tools were put aside for many years. With a growing awareness of the environmental and health issues due to the use of chemicals, alternative methods such as biocontrol were studied again with improved knowledge and scientific approach. Biocontrol appears to be a great natural way to protect crops against pests and diseases.

Where does it originate?

The principle of biocontrol is founded on the management of the balance of negative populations more than their eradication. Biocontrol tools encourage the use of natural mechanisms and interactions to the relations between species in a media.

In food processing, the concept of food preservation with protective flora is more then 40 years old concept. The purpose was to extend shelf life and to limit the risks of a microbiological contamination: a great alternative to chemical food preservatives. Now the applications fields are various and we can find biocontrol tools in cheese, bread, beverages, etc.

What does it mean in wine?

In wine, the use of SO_2 was an amazing improvement regarding product quality mainly because of its antifungal and antibacterial activity. But nowadays there is an increasing willingness for reducing chemicals in winemaking and SO_2 is obviously one of them. It remains an excellent tool to reduce spoilage population during the whole winemaking process, such as oxidative yeast flora, spoilage bacteria, and of course *Brettanomyces* which is still the number one enemy in wine. But recent studies (Curtin *et al*. 2012 ; Vigentini *et al*. 2013 ; Albertin *et al*. 2017) showed that there is a high genetic diversity in *Brettanomyces* and among them there is a significant number of *Brettanomyces bruxellensis* strains able to resist and survive to SO_2 (figure 1). AWRI (Curtin *et al*., 2012) demonstrate that 85% of the *Brettanomyces* population of their study was able to survive and grow at 0,6 mg/L of molecular SO_2. Albertin *et al*. (in 2016) identified 34% of *B. bruxellensis* strains among a collection of 33 strains as highly SO_2 resistant.

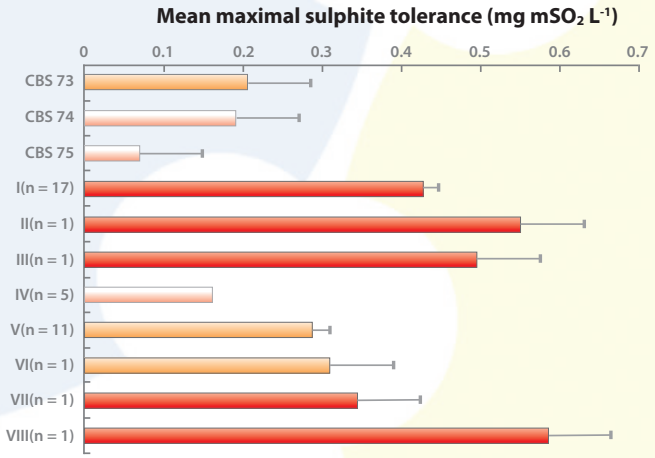

Figure 1. Sulfite tolerance of different *Brettanomyces bruxellensis* Australian isolates (from Curtin *et al*. 2012).

Can selected bacteria be used as biocontrol?

This kind of resistance would result in very high levels of final SO_2 in wines to reach the lethal level needed to eradicate such *Brettanomyces* population. Those results show the limits of SO_2 as a *Brettanomyces* control tool and highlight the need of alternative biocontrol tools to handle this risk. Yeast early inoculation (Gerbaux *et al*., 2004) is already known as a very efficient biocontrol tool against *Brettanomyces* growth. More recently it was found that some effective selected wine bacteria can also be considered as an efficient biocontrol tool to handle *Brettanomyces* contamination and volatile phenols levels in wine. These volatile compounds and mainly 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP) are responsible of off-flavors in wines. The proper inoculation of selected wine bacteria practice is also recognized by OIV (RESOLUTION OIV-OENO 462-2014) to reduce the growth of *Brettanomyces* during the winemaking process. It has been found that certain selected wine bacteria are considered as efficient biocontrol tools against spoilage micro-organisms to protect the wine quality.

THE RESULTS

BIOCONTROL WITH SELECTED WINE BACTERIA AGAINST GROWTH OF *BRETTANOMYCES*

Previous studies showed the clear impact of early inoculation of MLB on the reduction in final volatile phenols levels (Gerbaux *et al*. 2009 ; Pillet *et al*. 2011). It is known that the lag period between the end of alcoholic fermentation and the start of malolactic fermentation (MLF) is critical for spoilage microorganisms such as contaminant bacteria and *Brettanomyces*. In 2014, OIV recognized the co-inoculation of selected yeasts and selected lactic bacteria could help to reduce this lag phase and consequently limit the development of *Brettanomyces*. Recent research projects have studied if in addition to the reduction of this lag phase, some selected bacteria can have a direct inhibition on *Brettanomyces* growth. Research done in collaboration with IFV in Burgundy in 2015 and 2016 in Pinot showed the dynamics of the microorganism's population in wines inoculated just after alcoholic fermentation with *Brettanomyces* and then with various selected bacteria (1g/hL) at temperature between 16 and 18°C.

The results showed that there is a clear inhibition of *Brettanomyces* growth from selected bacteria for both vintages. In the figure 2 we can see the *Brettanomyces* population measurements in 2016 with 3 different selected bacteria compared to control where no bacteria were inoculated and in which MLF occurs spontaneously. The results were the same when *Brettanomyces* was at 10^3 cfu/mL.

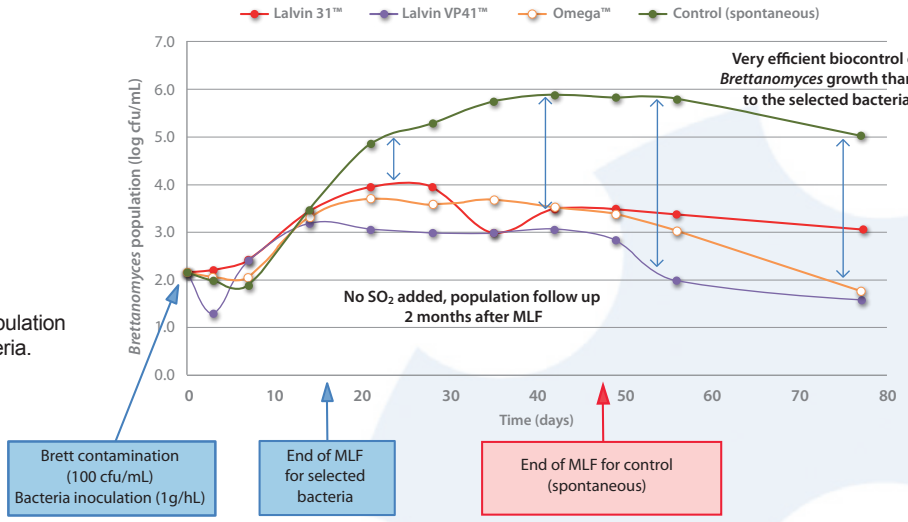

Figure 2. Biocontrol of *Brettanomyces* population with various selected wine bacteria.

The inoculation and the growth of those 3 selected bacteria limit significantly the development of *Brettanomyces* even if the *Brettanomyces* contamination was strong at the beginning. Final levels of *Brettanomyces* in presence of the selected bacteria was more or less the same level as initial (between 10^2 and 10^3 cfu/mL) whereas in the control with indigeneous bacteria (spontaneous MLF), final level of *Brettanomyces* is much higher (10^5) with a peak at 10^6 cfu/mL, i.e. a difference of more than 2 to 4 log.

Volatile phenols were measured at the end of the experiment in all the treatments (figure 3) and the wine in which MLF occurred spontaneously showed high levels of both 4-ethylphenol and 4-ethylgaïacol, above the perception threshold whereas the wines in which the selected bacteria were inoculated had very low levels of both phenols, close to zero.

THE RESULTS (cont'd)

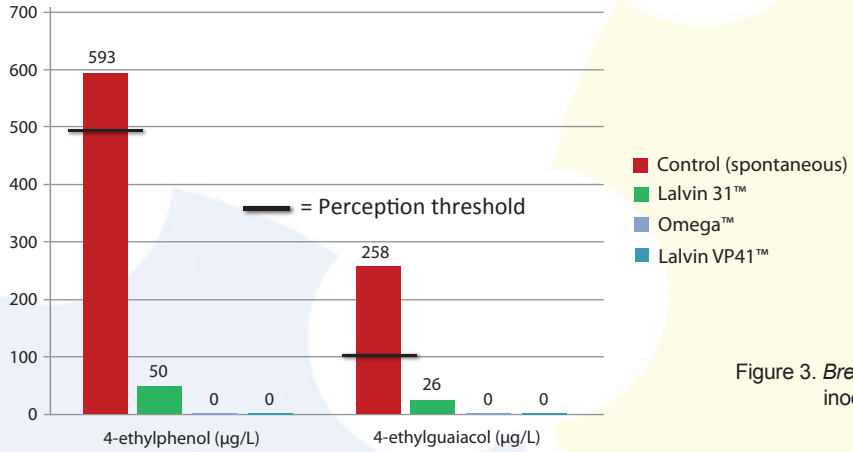

Figure 3. *Brettanomyces* biocontrol by selected bacteria inoculation. Impact on volatile phenols production

Those differences in volatile phenols are clearly linked with the differences in population and show once again the real benefit of a biocontrol of *Brettanomyces* thanks to the inoculation of some selected bacteria, also in the wine quality.

This work was confirmed by another study in 2016 in Pinot Noir. Four different selected bacteria were used in co-inoculation (24hrs after yeast inoculation) in a must initially contaminated with 1.7 x10^3 cfu/mL of *Brettanomyces* MLF finished in 20-40 days whereas the uninoculated control finished in more than 60 days.

Fig 4a and fig 4b illustrate the evolution of population of *Brettanomyces* and bacteria: in the case of co-inoculation with selected bacteria (fig 4a: results shown are an average of all the treatments with the selected bacteria) and in the case of the control - spontaneous (fig 4b). We can clearly see on the figure 4a that there is no growth of *Brettanomyces* population (even with this strong contamination) and this contaminant population decreases from the beginning to the end in opposition of the population of the selected bacteria growing at the same time. At the opposite in the figure 4b, *Brettanomyces* population maintains a high level until the 11th days (date of the rack off) and there is a regrowth due to the slow development of spontaneous bacteria population. Final 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 (more than one log ufc/mL of difference). Those results confirm the strong competition between our selected bacteria and a population of contaminant yeast such as *Brettanomyces*, especially co-inoculation thanks an easier growth of the selected bacteria and an excellent survivability of those bacteria.

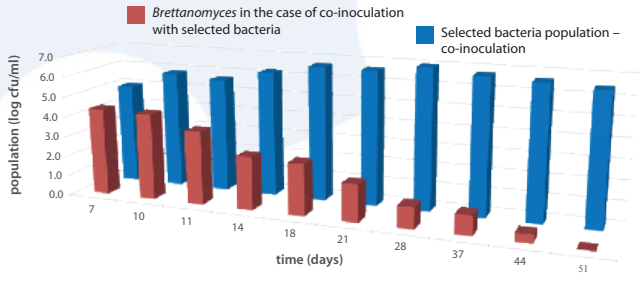

Figure 4a. *Brettanomyces* and bacteria population follow up. Co-inoculation with selected bacteria.

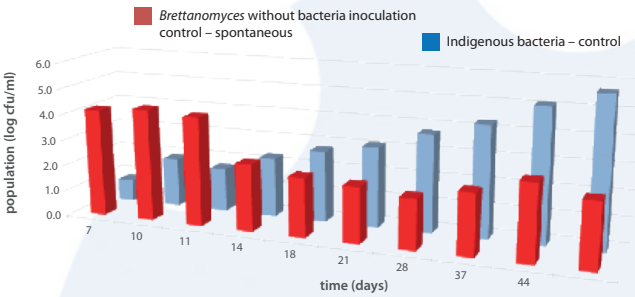

Figure 4b. *Brettanomyces* and bacteria population follow up. Control without bacteria inoculation.

BIOCONTROL OF CERTAIN SELECTED BACTERIA AGAINST RELEASE OF OFF-FLAVORS PRECURSORS

With *Brettanomyces*, 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP) are produced during the biotransformation of the hydroxycinnamic acids, p-coumaric acid and ferulic acid, which are precursors naturally present in grapes in the bound or free form. The transformation of these free precursors into 4-EG and 4-EP (figure 5) occurs in two steps: first with the cinnamate decarboxylase enzyme, followed by the vinylphenol reductase enzyme. But before *Brettanomyces* can use those precursors, they must be release from their bound form to the free form by the action of cinnamyl esterase.

THE RESULTS (cont'd)

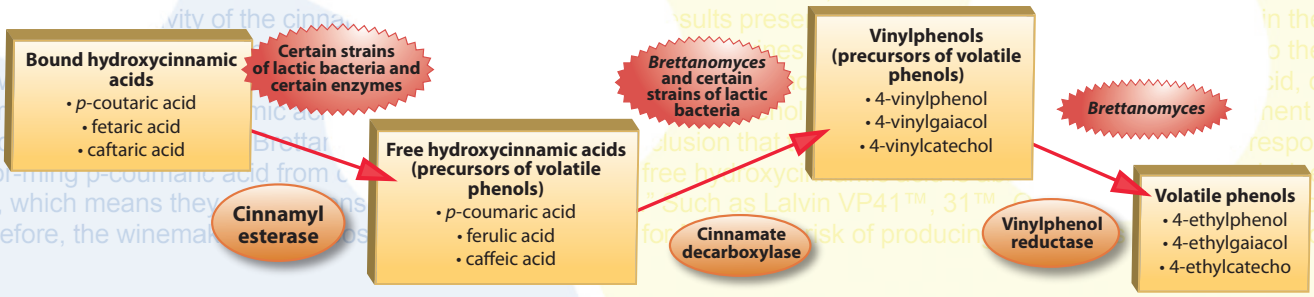

Figure 5. Steps in the production of ethyl phenols.

Certain lactic bacteria, including *Oenococcus oeni*, have this cinnamyl esterase enzymatic activity and could therefore increase the quantity of free precursors, made usable by *Brettanomyces* to produce volatile phenols (Burns and Osborne 2013). Their results showed that depending on the wine bacteria used for MLF, different concentraton of free precursors resulted.

This signifies that when using a wine bacteria, it must be cinnamyl esterase negative in order to avoid the production of the precursors of volatile phenols that would be used by *Brettanomyces*.

All our wine bacteria have been screened and we can confirm that they are all phenol negative as shown in Figure 6, from a study done by J.Osborne (OSU).

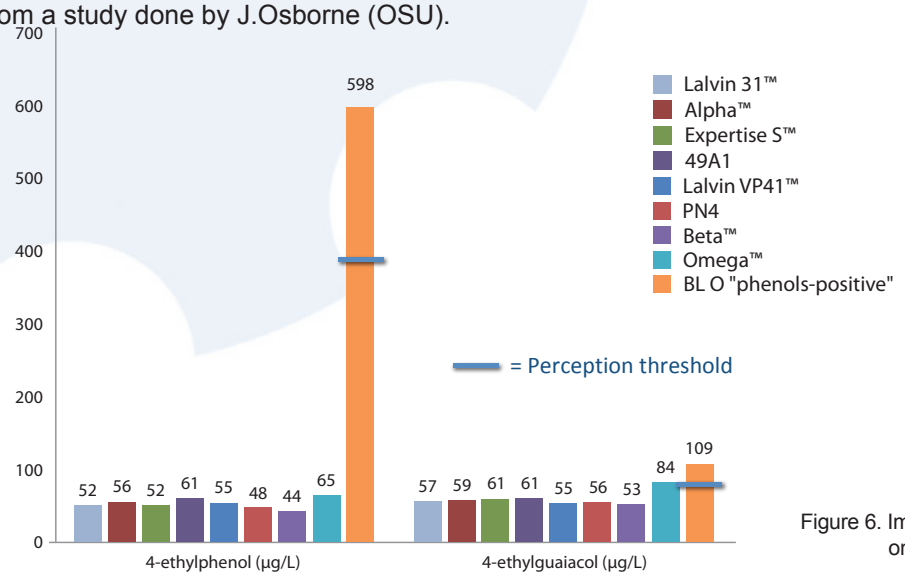

Figure 6. Impact on volatile phenols production depending on the selected bacteria inoculated .

IN SUMMARY

Biocontrol of contaminating microorganisms with certain selected wine bacteria is a secure and biological option for winema-kers. By carefully managing alcoholic fermentation with properly rehyrdrated and nourished yeast, and by using a selected wine bacteria in co-inoculation or in sequential inoculation, the population of *Brettanomyces*, and subsequently the production of phenolic off-odors is better controlled. When the level of contamination from *Brettanomyces* is high, it is better to co-inoculate since the biocontrol will start right at the beginning of fermentation.

Furthermore, our selected wine bacteria have been confirmed as phenol-negative, and can't supply to *Brettanomyces* the precursors to produce the off odors, 4-ethyl phenol and 4-ethyl guaiacol.

Biocontrol throught the protection of our selected wine bacteria, along with denying *Brettanoymces* its precursors are winning combination to respect the wine typicity by letting it fully be expressed and without the 'Brett' faults.

The Wine EXPERT

A WORD FROM OUR EXPERT

Vincent Gerbaux

Vincent graduated in 1982 from Ensbana (now AgroSup Dijon), Master degree in Biology applied to Nutrition and Food, and in 1983 from the national diploma of Enology. He studied wine lactic acid bacteria during his thesis at the University of Dijon (1983 to 1985). In 1985, he started working for IFV at Bordeaux then joined IFV Beaune three years later.

Vincent is in charge of the IFV project « Technoferm », gathering all the studies on alcoholic and malolactic fermentations. Collaborating with Lallemand for many years, he has selected and characterized original *Oenococcus oeni* strains, commercially available. He is also managing for 5 years studies on the selection of strains of *Saccharomyces* and Non-*Saccharomyces* for specific technological applications. In parallel, the team also works on spoilage microorganisms in wine, especially *Brettanomyces*.

The yeast *Brettanomyces* has always been associated with wine. A poem written during the First World War (Marc Leclerc, 1915) said "Hello house wine of the barracks, That tasted too little or taste of nothing. Except of the days you would tend, to stink of phenol or of manure....". But the involvement of *Brettanomyces* in the production of volatile phenols in wines, and the recognition as a problem, date only from the 1980s. The latest researches reduces the perception threshold of volatile phenols to about 200 µg/L, a value easily attained in *Brettanomyces* contamination. This yeast possesses a remarkable ability to adapt to unfavorable physicochemical conditions. *Brettanomyces* can be multiplied both during maceration, in the presence of sugars, and in bottles after several years of storage of a dry wine. Various studies show that cellars contain a wide bio-diversity of *Brettanomyces*.

The best strategy to control *Brettanomyces* involves good hygiene, the use of microbiological control and the control of fermentations. Control of malolactic fermentation is a key point in this struggle. Inoculating with selected wine bacteria provides different solutions for the control of MLF. Depending on the age of the site and the risk of the occurrence of volatile phenols, the MLF can be either very rapid when considered with a yeast / bacterial co-inoculation or slow with a late bacterial inoculation associated with a low temperature. These different options must allow completion of MLF before observing a pro-blematic growth of *Brettanomyces*. It is then possible to stabilize the wine with a conventional sulphiting or by using an alternative or complementary compound, chitosan. After MLF, growth of *Brettanomyces*, although still effective, is then less rapid and less important than if it had preceded the MLF. The induction of MLF by inoculation brings a massive population of wine bacteria that exerts a direct negative effect on the development of *Brettanomyces*.

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