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SMARTLINK

An exclusive SARCO laboratory offer.

TYP*Brett* : detection and quantification of *Brettanomyces bruxellensis* strains resistant to sulfites, for a reasoned strategy of prevention of the alteration of wines.

Despite clear progress in preventive oenology, *Brettanomyces bruxellensis* remains the leading cause of microbial contamination of wines. Some issues come in a very pernicious way, notably at the end of the itinerary, whereas the levels of free SO_2 seems perfectly adapted to limit the growth of *Brettanomyces bruxellensis*.

Isabelle Masneuf and Warren Albertin's research teams from ISVV of Bordeaux have recently showed that the resistance to sulphites of *Brettanomyces* depends on their genetic patrimony. During these studies, several hundred strains were characterized. Isolates were discriminated by genetic analysis of microsatellites (which are repeated areas of the genome), which allowed to distinguish groups of diploid and triploid strains (three lots of chromosomes instead of two).

Several representatives of each group were analyzed to determine if they were resistant or sensitive to sulphites. 40% of the tested strains were found to be resistant to sulphites at the usual concentrations of 0,4 mg/L of molecular SO_2. This explains why some deviations occur despite free SO_2 contents quite consistent with the usual recommendations. All the strains which are resistant to sulphites have the particularity of having a specific triploidy. This triploidy allowed the development of a molecular test whose purpose is to detect and count these strains, the **TYP*Brett*** test.

The SARCO laboratory has all the analytical tools available for the analysis of *Brettanomyces bruxellensis*. Our goal is not to promote one technique over another but to help our partners to have the most useful tool, depending on the situation. From the first communications of Isabelle Masneuf and Warren Albertin, we have been very interested in their work. In the last several months, the use of the **TYP*Brett*** test has allowed us to characterize the sulphite resistance of strains of *Brettanomyces bruxellensis* isolated on grapes and in wines analyzed in the laboratory, but also during hygiene audits in cellars. This has already provided many interesting indications:

- The vast majority of *Brettanomyces bruxellensis* strains present in the vineyard are sensitive to sulphites.
- Strains resistant to sulphites are often the most ferocious in terms of production of volatile phenols.
- The share of resistant strains in the musts and in the wines varies according to physicochemical parameters but also according to the oeno-technical practices implemented (sulphiting of course but not just it ...). This distribution also varies according to the production sites.
- Isolated strains from the atmosphere, on the surface of containers or small material are in the vast majority triploid strains resistant to SO_2.

These data provide precious information to better understand *Brettanomyces* colonization pathways. Some *Brettanomyces* strains are present on grapes, but they are different from the strains present in cellar, which developed mechanisms of resistance to this hostile environment, including resistance to SO_2. Recently, microbiologists working on yeasts in drinks (wines, beers ...) drew a very interesting phylogenetic tree. At the top of this tree, we find *Saccharomyces cerevisiae*, which is considered as the most «primitive» yeast. In other words, it is the one that has evolved the least. At the bottom of this tree is *Brettanomyces*. It is the proof that this species can develop remarkable tools allowing it to have adaptive advantages according to the conditions, and notably the sulphite resistance in the wines.

During the 2018 vinifications, we monitored the distribution of the sensitive and resistant strains in different wineries. The results in the table below show that, indeed, more the conditions are restrictive (languishing alcoholic fermentation for example), more the resistant strains gain the upper hand quickly. Nowadays, we are convinced that limiting the takeover of resistant strains is a major challenge for a reasoned and preventive fight against wine faults caused by volatile phenols.

	SO_2 sensitive diploid *Brettanomyces* strains	SO_2 resistant triploid *Brettanomyces* strains
Analysis performed on 2018 grapes.	100%	Ø
At the end of AF in wines without fermentation issues.	85%	15%
At the end of AF in wines with fermentation issues.	25%	75%
In 2018 wines that already had more than 30 µg/L of volatile phenols in December 2018.	5%	95%
Strains isolated during cellars audit after vinifications.	5%	95%

Some partners are already managing their microbiological monitoring based on this distinction between sensitive and resistant strains. This avoids the unnecessary use (if the main strains present are resistant) or counterproductive (allow resistant strains to use their adaptive advantage to become majority) to sulphites. It is likely that high-dose of sulphites will promote adaptation and resistance phenomenon. Indeed, a group of individuals called «super-resistant» has already been isolated from properties whose use of sulphites occurs at concentrations higher than those usually recommended.

Several TYP*Brett* applications are possible

- As part of a regular monitoring of volatile phenol levels*, during an increase of concentrations, **TYP*Brett*** allows to determine whether the use of SO_2 is suitable to limit contamination or if it is more effective to resort to other actions (chitosan, filtration, heat treatments ...).
- During the two first notable additions of SO_2 in the winemaking route, at the vatting and at the end of the MLF, **TYP*Brett*** allows a precise adjustment of the SO_2 dose.
- As part of the hygiene audits of the wineries, **TYP*Brett*** validates the disinfection processes after barrel cleaningsulphur burning and small equipment (rinsing with sulphite water).
- At the end of the itinerary, **TYP*Brett*** can avoid the overdosing of sulphites, the risk being to use them unnecessarily and to induce a modification of the REDOX balance ("closing" of the wines at bottling).

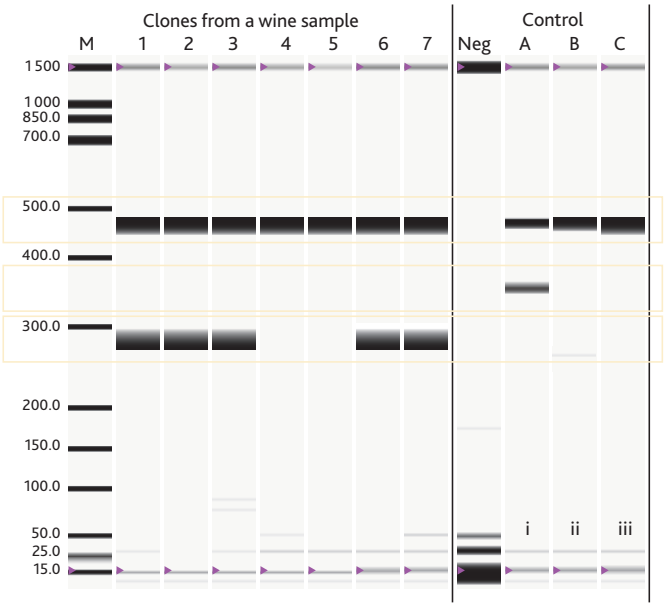
The analytical approach is as follows

1. An enumeration of the total population of *Brettanomyces* in the sample.

2. A **TYP*Brett*** test performed on a number of clones representative of the enumerated population.

The result provided by the **TYP*Brett*** expertise is the total population of *Brettanomyces* and the % of SO_2 resistant strains.

The figure below illustrates this approach through a concrete case:



***TYP\Brett**, it is the enumeration of the total population of Brettanomyces bruxellensis, the percentage of resistant strains, the measurement of free and total SO_2 and the calculation of active SO_2.*

Interpretation

The enumeration of the total population of *Brettanomyces* indicates a total population of $5{,}10^1$ CFU/mL.
Seven colonies are analyzed by **TYP*Brett***, 5 of which are triploid strains SO_2-resistant and 2 are diploid strains SO_2-sensitive.

Conclusion

The analyzed wine shows a notable population of *Brettanomyces bruxellensis* including 70% of SO_2 resistant strains. In order to reduce the population and to limit the risk of contamination, it is better to opt for another treatment than free SO_2 readjustment.

Legend

M : Molecular weight marker
1 to 7 : Genetic profiles of isolated colonies of a wine
Neg : Negative control
A : SO_2 sensitive triploid reference strain
B : SO_2 sensitive diploid reference strain
C : SO_2 resistant* triploid reference strain

*ability to grow even if the active SO_2 in the wine is ≥ 0.4 mg/L.

In addition to quantifying the total population of *Brettanomyces bruxellensis*, **TYP*Brett*** is to this day the only microbiological analysis test that combines the specificity of genetic typing with practical information about the action to carry on to control the development of cells and thus the deterioration of wines.

**Even if these dosages have become more and more «routine», it is essential to pay close attention to the precision and the sensitivity of the dosages carried out. SARCO laboratory is accredited COFRAC on the determination of 4-ethylphenol and 4-ethylguaiacol.*

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