

Brettanomyces bruxellensis: the current state of play

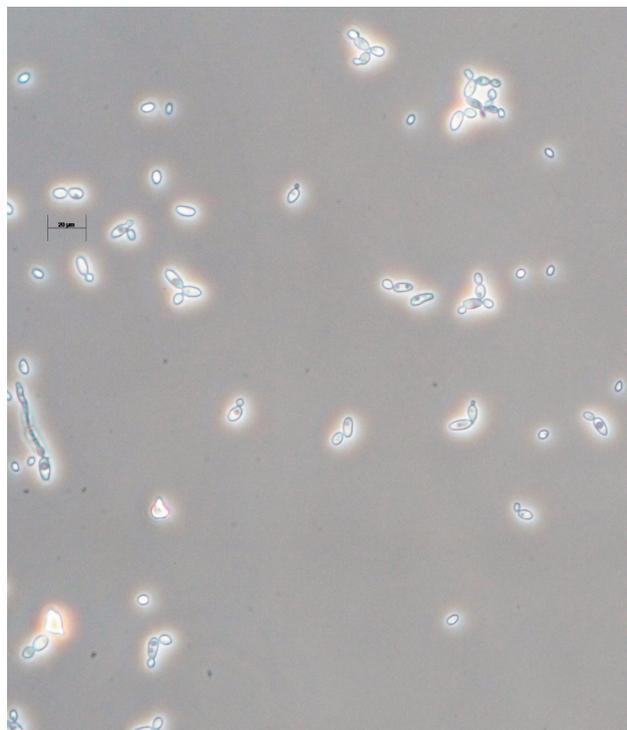
>>> *B. bruxellensis* appeared in inventories of must and wine yeasts as from the 1950s, and was quickly blamed for wine spoilage. Much later, the volatile phenols produced by these yeasts were identified as the cause of sensory deterioration. Their presence is an increasingly important issue, especially during barrel aging. Sulfite addition is the ideal means of eradication, but sometimes it is not enough. Especially well adapted to wine, *B. bruxellensis* is now of great concern to winemakers and microbiologists. <<<

In 1960, Peynaud and Domercq attributed wine spoilage to *Brettanomyces*, and in 1986 Heresztyn demonstrated that the olfactory fault was due mainly to the accumulation of volatile phenols produced from cinnamic acids. Studies of *B. bruxellensis* were launched and have not stopped since. Molecular methods and genetic analysis have been used on the one hand, and chemical and sensory analysis on the other. At the same time, winemakers and producers have been reporting field observations to scientists.

■ What is known

In the aging cellar and later: the most critical period is during barrel aging. Spoilage often occurs in the first summer after the harvest, when the increase in temperature leads to growth of *B. bruxellensis* populations. A population of 10^2 - 10^3 CFU/mL is a sign of likely spoilage if nothing is done to stop proliferation. Racking, simply by virtue of sedimentation, can eliminate all or part of the population. Work and observations in the cellar show that adjustment of SO₂ to 0.6 mg/L (molecular) is necessary in case of contamination¹. After bottling, the yeasts do not die immediately and the accumulation of volatile phenols can continue. A small surviving population can multiply as soon as conditions are favorable.

Scientific knowledge of the species: the diversity of *B. bruxellensis* strains is known, and very recently genetic studies have established the causes of the differences. DNA-based methods have successively been able to specifically detect *B. bruxellensis* by a simple PCR (polymerase chain reaction), to quantify it (even though commercial detection kits are not always easy to use and lack robustness²), and in most cases to distinguish one strain from another and sequence whole genomes. Genome analysis, combined with phenotypic data, provides answers to oenological questions, but also a basis for understanding how these yeasts work. The role of polyploidy in the evolution of the species has been highlighted.



B. bruxellensis ©Microflora

Physiology: *B. bruxellensis* has adapted in an exemplary way to be able to multiply and survive in the oenological environment on grapes, in fermenting must, in wine and in the cellar. Its population (in number and in strains) grows during the vinification process and aging. It is selected as the alcoholic fermentation progresses³. It requires little in the way of nitrogen nutrients and sugars to ensure its growth and survival. The pH has little effect, and ethanol is tolerated up to 14 % and more. A small quantity of dissolved oxygen is favorable, even at the end of alcoholic fermentation.

Production of ethyl phenols (EPs) and other undesirable metabolites: the *p*-coumaric and ferulic acids in wine are transformed into vinyl then ethyl phenols. *B. bruxellensis* has the two enzymes needed, decarboxylase and reductase. The substrates are free acids; they are also released from more complex molecules, *p*-coumaroyl and feruloyl glucose and *p*-coumaroyl and feruloyl tartrate⁴. While *B. bruxellensis* seems to be able to hydrolyze only the glucose esters, *O. oeni* hydrolyzes the tartaric acid esters. Peynaud attributed "mousey taste" to *B. bruxellensis*; indeed, the characteristic 2-acetyl-tetrahydropyridine is produced, apparently, by all strains, but less frequently than EPs, as well as acetic acid and isovaleric acid⁵.

■ What is said

About their origin: *B. bruxellensis* yeast is mainly present in the cellar on winemaking equipment and in

particular in the wood of barrels that have already been used³. This observation is accurate, but these yeasts also exist on the grape berry³. They often go unnoticed there because, as for most epiphytic flora, the optimal conditions for culture and isolation are unknown. Observations confirm their presence in greater number in the more abundant microflora of rot-affected grapes. The cellar and barrels are contaminated by contaminated wines, made from grapes naturally colonized by *Brettanomyces*³.

About detection of the risk during aging

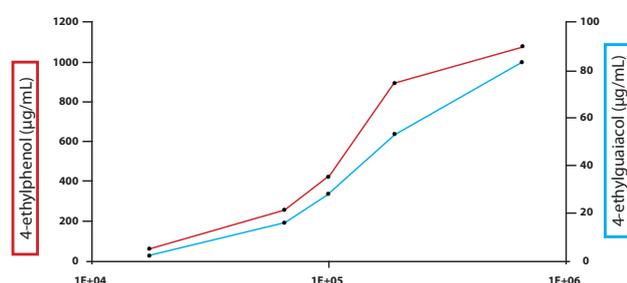
Measurement of EP concentrations is often used to detect the risk of spoilage. If their concentration is high, or just above the critical threshold (200-300 µg/L and sometimes even less depending on the matrices and tasters), this does not mean that the *B. bruxellensis* population is high at that particular time. It may also be in decline. Conversely, a low EP concentration does not mean an absence of risk. *B. bruxellensis* can develop later. Microbiological monitoring is an essential complement to phenol monitoring.

About their activity: contrary to what is sometimes said, the current state of knowledge shows that all the strains isolated from wine have cinnamate decarboxylase and vinyl reductase enzyme activity; but specific activities vary. The accumulation of EPs depends above all on the concentration of the viable population (Figure 1), the availability of substrates and probably other factors.

Prevention

The "Code of good vitivicultural practices in order to avoid or limit contamination by *Brettanomyces*" (OIV-OENO 462-2014) brings together the precautions to be taken during the pre-fermentation, fermentation and aging phases. Not surprisingly, the hygiene of the cellar and all its equipment is at the heart of the recommendations. The same is true of racking and fining, as *B. bruxellensis* sediments easily. Elimination of persistent populations requires sulfite addition (adjusting the dose to the population⁶) or physical treatments: membrane filtration or heat treatment. Any treatment must be accompanied by a cell count before and after application. Other methods have been recommended following the trend to reduce sulfite levels, including "bioprotection", chitosan, DMDC; the results are contradictory or questionable. Physical procedures (pulsed fields, high pressure) have been slow to prove their worth. Whatever the means, any opportunity for subsequent contamination must be excluded.

Figure 1. Change in the concentration of volatile phenols during barrel aging as a function of the cumulative population of *B. bruxellensis* (CFU x 1/mL)³.



Prevention requires risk prediction, which remains difficult because of the variability of the environmental parameters and of the strains. The *B. bruxellensis* cell count is essential. It should be performed throughout the winemaking process, and more frequently at crucial periods (sluggish fermentations, aging) because only this monitoring can give information on the capacity of the population to multiply. New methods make it possible to detect SO₂-tolerant strains⁷, providing additional information for choosing the right control method.

What should be known and understood

Why this surge in cases of spoilage, why are some cellars often affected and others never? The species has been domesticated, probably aided by its polyploidy, by the pressure of the oenological environment. Some strains are more tolerant than others to certain constraints, doubtless not only to SO₂ as has already been demonstrated. One question arises: will the selective advantage of triploid strains lead to domination by these strains? Will other environmental factors come into play to counter it? All this is difficult to predict. But predicting the impact of new winemaking practices is much easier. All the elements favorable to microbiological spoilage in general and *B. bruxellensis* in particular are brought together. In the first place, the reduction or absence of sulfite addition, difficult to replace without risk. Then everything that is used to enrich the wine with carbon and nitrogen nutrients, and EP precursors: over-ripening of grapes and pre/post-fermentation practices that extract the maximum amount of material from the grape. Unequivocal studies are essential to evaluate the impact of nitrogen nutrient additions in all forms (chemical, yeast derivatives, yeasts). The subject is difficult, because *B. bruxellensis* is in a system, and the interactions with other microorganisms are not limited to the inhibition that is supposed or hoped for. ■

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1 Du Toit, W.J., Pretorius, I.S. & Lonvaud Funel, A. (2005). The effect of sulphur dioxide and oxygen on the viability and culturability of a strain of *Acetobacter pasteurianus* and a strain of *Brettanomyces bruxellensis* isolated from wine. *Journal of Applied Microbiology*, 98, 862–871.

2 Longin, C., Julliat, F., Serpaggi, V., Maupeu, J., Bourbon, G., Rousseaux, S., Guilloux-Benatier, M., & Alexandre, H. (2016). Evaluation of three *Brettanomyces*; qPCR commercial kits: results from an interlaboratory study. *OENO One*, 50(4). doi:10.20870/oeno-one.2016.50.4.1274

3 Renouf, V., Lonvaud-Funel, A. & Coulon, J. (2007). The origin of *Brettanomyces bruxellensis* in wines: a review. *OENO One*, 41(3), 161–173. doi:10.20870/oeno-one.2007.41.3.846

4 Hixson J.L., Hayasaka Y., Curtin C.D., Sefton M.A. & Taylor D.K. (2016). Hydroxycinnamoyl Glucose and Tartrate Esters and Their Role in the Formation of Ethylphenols in Wine. *J Agric Food Chem.*; 64 :9401–11.

5 Romano, A., Perello, M.C., de Revel, G., Lonvaud-Funel, A. (2008). Growth and volatile compound production by *Brettanomyces/Dekkera bruxellensis* in red wine. *J Appl Microbiol.*, 104:1577–85.

6 Longin, C., Degueurce, C., Julliat, F., Guilloux-Benatier, M., Rousseaux, S. & Hervé A. (2016). Efficiency of population-dependent sulfite against *Brettanomyces bruxellensis* in red wine, *Food Research International*, Volume 89, Part 1, Pages 620-630, ISSN 0963-9969, doi:10.1016/j.foodres.2016.09.019

7 Avramova M., Vallet-Courbin A., Maupeu J., Masneuf-Pomarède I. & Albertin W. (2018). Molecular Diagnosis of *Brettanomyces bruxellensis* Sulfur Dioxide Sensitivity Through Genotype Specific Method. *Front Microbiol.*, 9:1260.