FACT SHEET

BRETTANOMYCES BRUXELLENSIS

***Brettanomyces bruxellensis* (*B. bruxellensis*)** is a spoilage yeast commonly found in wineries, in part due to its tolerance for high alcohol, low pH and low oxygen levels. *B. bruxellensis* has the potential to cause off flavours in wines, through production of the volatile phenolic compounds 4-ethylphenol (4-EP), 4-ethylguaiacol (4-EG).

Where does *B. bruxellensis* come from?

Vineyard, cellar, cellar equipment. *B. bruxellensis* particularly like growing in oak barrels. They grow very slowly in comparison to other yeast. Careful control and continued monitoring of SO_2, pH and residual sugar can reduce the risk of infection.

How can Winechek help?

In addition to the chemical monitoring of your SO_2, pH and residual sugar, Winechek has developed a rapid, quantifiable method to detect and quantify *B. bruxellensis* in juice, musts and finished wine. Using quantitative real time polymerase chain reaction (qPCR) DNA from both dead and viable cells in very small numbers, a valuable tool for your wine making needs. Using qPCR to identify and quantify *B. bruxellensis*, any changes in the level of *B. bruxellensis* DNA can serve as a quick health check for your wine as it matures.

Winechek has additionally devised a technique for measuring the levels of 4EP and 4EG within wine samples, employing a GC-MS approach. This methodology ensures precise detection and quantification of these volatile compounds, often linked to sensory descriptors such as "band-aid" or "wet leather." The proportion of 4EP to 4EG differs across various wines, with a 3.5:1 ratio for Pinot, 8:1 for Merlot, 9:1 for Shiraz and a 10:1 ratio for Cabernet Sauvignon.

B. bruxellensis testing methods

Culturable cells	qPCR	4EP/4EG
(PLATING) This tells us how many cells can grow in a lab environment. An inexpensive monthly follow up during wine maturation.	Measures viable and non-viable *B. bruxellensis* DNA. A fast, highly specific test for the detection and the quantification of Brett.	4-Ethylphenol (4EP) and 4-Ethylguaiacol (4EG). Chemical taint produced by 'Brett' once it reaches critical population.

Test results – what do they mean?

Quantity of *B. bruxellensis* detected →	Cells not detected	<10 cells/ml Low risk	<50 cells/ml Medium risk	50-100 cells/ml High risk	>100 cells/ml Very high risk
Recommended actions →	Check G/F, pH and SO_2 Reanalyse in 3 months	Check G/F, pH and SO_2 Reanalyse in 3 months	Check G/F, pH and SO_2 Check viability with plating OENOBRETT®	Check G/F, pH and SO_2 Check 4-EP/4-EG Check viability with plating Measure 4-EP/EG Treatment with OENOBRETT®	

Spoilage risk increasing

Frequently asked questions

If my wine comes up positive for *B. bruxellensis* via PCR, but negative for plating, what does that mean?

qPCR measures DNA which is present in both live and dead *B. bruxellensis* cells. SO_2 additions may cause *B. bruxellensis* cells not to show up positive via plating. qPCR can pick up cells that are not necessarily culturable via plating which are sometimes called Viable but non-culturable (VBNC)

Picking up VBNC by qPCR may give you a positive result that you would not have otherwise gotten with plating. If *B. bruxellensis* is not picked up due to a recent SO_2 addition, it may falsely lead to the conclusion that *B. bruxellensis* is not present. It is also possible that the cells are no longer alive, and only dead cells have been detected, but they may have still been alive long enough to produce some 4EP/4EG. This may also be the case when chitosan-based products such as OENOBRETT® are used, as the cell membrane is compromised creating a dead cell, whereby the DNA is still able to be detected via qPCR.

Talking about your specific results with a Winechek laboratory manager will provide insight into where the problem lies. Ongoing routine checks are recommended to detect any growth before the cells start producing ethyl phenols.

What if my wine comes up positive via PCR and plating, but there is no 4EP/4EG being produced?

B. bruxellensis cells need to reach a critical population before it can start producing off-flavours (4EP/4EG). This population level may be different for each wine depending on the chemical factors and other microorganisms present. It is likely that if there is a population in the wine that it will start producing 4EP/4EG over time.

My wines are at 50 ppm free SO_2– why am I getting a viable result via plating?

B. bruxellensis are highly SO_2 tolerant and can survive significant levels of SO_2. The amount of available SO_2 will be dependent on pH. Any residual sugar available can stimulate their growth. Each wine matrix is different and will have factors working with and against *B. bruxellensis* growth. Brand new barrels contain carbohydrates which can also be metabolised by Brett, further supporting population growth.

Will *B. bruxellensis* keep growing after it goes to bottle?

B. bruxellensis will keep growing in bottle if the environment allows. They are able to grow without any air (anaerobically) as well as with air (aerobically), so if the product is not sterile filtered to bottle via membrane filtration, there is a risk that a single cell could turn into a large population over a number of years and produce significant levels of 4EP/4EG .

What is the sensory threshold of 4EP and 4EG?

The sensory threshold of 4EP 430 ug/ is 430 and this value is 33ug/L for 4EG.

References

Agnolucci, M., Rea, F., Sbrana, C., Cristani, C., Fracassetti, D., Tirelli, A. and Nuti, M., 2010. Sulphur dioxide affects culturability and volatile phenol production by Brettanomyces/Dekkera bruxellensis. International journal of food microbiology, 143(1-2), pp.76-80.

Serpaggi, V., Remize, F., Recorbet, G., Gaudot-Dumas, E., Sequeira-Le Grand, A. and Alexandre, H., 2012. Characterization of the "viable but nonculturable" (VBNC) state in the wine spoilage yeast Brettanomyces. Food microbiology, 30(2), pp.438-447.

Willenburg, E. and Divol, B., 2012. Quantitative PCR: an appropriate tool to detect viable but not culturable *Brettanomyces bruxellensis* in wine. International journal of food microbiology, 160(2), pp.131-136.

Avramova, M., Vallet-Courbin, A., Maupeu, J., Masneuf-Pomarède, I. and Albertin, W., 2018. Molecular diagnosis of Brettanomyces bruxellensis' sulfur dioxide sensitivity through genotype specific method. Frontiers in Microbiology, 9, p.1260.