Techniques to detect *Brettanomyces* before it's too late

The early detection of *Brettanomyces bruxellensis* yeast or 'Brett' can allow winemakers to take action before the concentration of volatile phenols becomes detrimental to wine quality. In this article, AWRI Oenologist Ben Cordingley answers questions on different technologies for the detection of Brett cells in wine.

What are the main technologies used to test for Brett cells?

Most available Brett testing methods fall into two main categories:

- Culture-dependent methods where colonies of Brett cells from a wine sample are cultured/grown on agar plates in the laboratory
- Culture-independent methods where Brett cells are detected by other means. This includes molecular tests using polymerase chain reaction (PCR) to detect Brett-specific DNA sequences, as well as flow cytometry methods that automate the identification and counting of Brett cells in a wine sample.

Each testing method works differently and can generate different results for

the same sample due to the detection of different forms of Brett cells that can exist in a wine. A sample may contain a culturable population of viable Brett cells capable of growth. Several studies have reported that Brett can also exist in a dormant or viable but non-culturable (VBNC) state (Capozzi *et al.* 2016). In this theoretical state, live Brett cells may be metabolically active and capable of producing volatile phenols but temporarily unable to multiply (Nunes de lima *et al.* 2021). A sample may also contain dead Brett cells that contain detectable DNA, as well as free (extracellular) Brett DNA released during cellular lysis of dead cells.

How are Brett cells quantified by culturing?

Culturing of the microbes in a wine sample is a commonly used technique to quantify viable Brett cells. Volumes of wine are usually filtered through a sterile filter membrane which is transferred onto an agar plate and incubated. Brett colonies are observed and counted after seven or more days. Results are usually expressed as the number of colony-forming units (CFUs) per a given volume of wine. Specialised growth media can exclude the growth of other yeast types. Growth media containing the antibiotic cycloheximide will allow the growth of many non-*Saccharomyces* yeast species, meaning that Brett colonies must still be correctly identified. VBNC and dead Brett cells are not detected by culturing.

How do molecular tests using PCR detect Brett cells?

Polymerase chain reaction (PCR) is a molecular test that uses enzymes to make copies of Brett-specific DNA. Brett DNA is recognised by synthetic molecules called primers that allow a part of Brett DNA to be amplified over multiple heating and cooling cycles. The number of Brett cells present in the sample is proportional to the amount of DNA that results from amplification. Conventional PCR methods have a predetermined number of amplification steps followed by a semi-quantitative estimation of the resulting amount of Brett DNA produced in the reaction. Quantitative PCR methods count the number of amplification steps needed to reach a threshold amount of Brett DNA that is detectable by the instrument. The number of amplification cycles required to reach this threshold level is used to precisely determine the number of Brett DNA copies in the sample prior to any amplification.

Are dead or VBNC Brett cells detected by PCR methods?

Conventional PCR technologies will generally detect all intact Brett DNA from living and dead cells, and also free extracellular DNA. Some wines may contain high numbers of dead Brett cells that would still be included in a PCR-based cell count. The portion of dead cells present in a sample could depend on if there had been a treatment to kill Brett and how recently this treatment was applied. The time required for dead Brett cells to break down to the extent that their DNA is not detected by conventional PCR is unclear but may depend on wine conditions. Some newer PCR methods do not detect dead cells and only detect viable and VBNC Brett. These methods exclude DNA from dead Brett cells by making use of specialised dyes that are not able to enter viable cells but are able to enter dead cells. These dyes integrate within the DNA structure to prevent amplification by PCR (Navarro *et al.* 2020).

What other options for Brett testing exist?

More recently, flow cytometry-based methods have become available that directly count the individual Brett cells in a sample. These work by creating a thin stream of the sample that flows past several detectors that can identify Brett based on its physical properties. Flow cytometry techniques often use fluorescent dyes that selectively stain either living or dead Brett cells, so it is possible to distinguish between viable populations (potentially including VBNC Brett) and dead Brett cells. Rapid molecular tests using LAMP technology (loop-mediated isothermal amplification) have been developed for the detection of Brett (Hayashi *at al.* 2007). This technology works similarly to PCR to amplify specific DNA sequences. The main difference is that LAMP uses a complex set of multiple primers that identify and amplify a Brett-specific DNA sequence at a constant temperature. LAMP is a rapid testing method that gives qualitative results (positive or negative) rather than a quantitative result expressed as the number of cells per mL of wine.

Is there a threshold Brett cell density that is a concern for winemakers?

Brett cells can multiply from very low densities to result in a wine containing high levels of volatile phenols. Detection of any number of Brett cells should therefore prompt winemaking interventions to inactivate or remove Brett if a 'Brett character' is to be avoided. Wine conditions and the specific Brett strain can influence the production of volatile phenols, meaning that the Brett cell density is not a direct indicator of the level of potential 'Brett character'.

For further information about *Brettanomyces bruxellensis* detection or other technical winemaking or viticulture questions, contact the AWRI helpdesk on (08) 8313 6600 or helpdesk@awri.com.au

References

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