

Molecular-biological wine analysis with real-time PCR Yeasts and Bacteria

- *Brettanomyces* / Yeasts
- Bacteria
- Bacteria forming Biogenic Amines
- Multiplex Screening



Yeasts analysis for wine becoming increasingly important

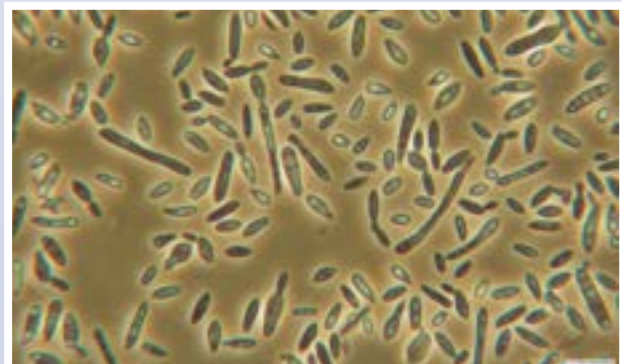
Scientific methods are now applied in modern wine production. Nowadays, the wine industry tries to reduce the dependence of soil, climate and weather conditions in the wine production. Wine-making starts with grapes and production of grape juice, followed by maceration, fermentation, aging, clarification and stabilization before ending with the bottled wine. Many parameters must be or can be tested during wine production in order to allow decision-making at different stages.

Usually, cellar technically controlled fermentation runs with the addition of selected yeasts from the *Saccharomyces* strains and possibly the use of *Oenococcus oeni* (*Leuconostoc oenos*) to control the optional malolactic secondary fermentation. A spontaneous fermentation with yeasts present in the environment can be controlled worse. But even with the controlled fermentation, wild yeasts and specialized bacteria can cause a sensory change and adverse effects on wine.

The analyses of the yeast *Brettanomyces* (*Dekkera bruxellensis*) is becoming increasingly interesting. It is the most important wild yeast in wine forming phenolic metabolic products 4-EP (4-ethylphenol) and 4-EG (4-Etylgujacol). Mainly, it causes blemished smells described as leather, sweat and/or horse. The *Brettanomyces*, however, can also be specifically used in low concentrations to achieve a stronger sensory effect.

An early quantitative identification of this yeast is of great interest. The traditional microbiology needs an incubation time of about 10 days and moreover

the differentiation of *Dekkera bruxellensis* requires a lot of experience to avoid any misinterpretations. With the help of a DNA standard (DBST 0100) of 200,000 cfu and derived DNA, a simple quantification in FAM channel can be done even with dark red wine. In ROX or HEX channel, an internal amplification control is performed for each sample. Optionally, other yeasts are of interest such as *Pichia* strains.



Mikroskopische picture of *Dekkera bruxellensis* (LVWO Weinsberg)

Desirable or undesirable bacteria

The malolactic fermentation is supported by *Lactobacillus*. During this process the bacterium *Oenococcus oeni* can be added as highly specialized bacteria. It effects a transformation of malic acid into milder lactic acid. However, depending on the

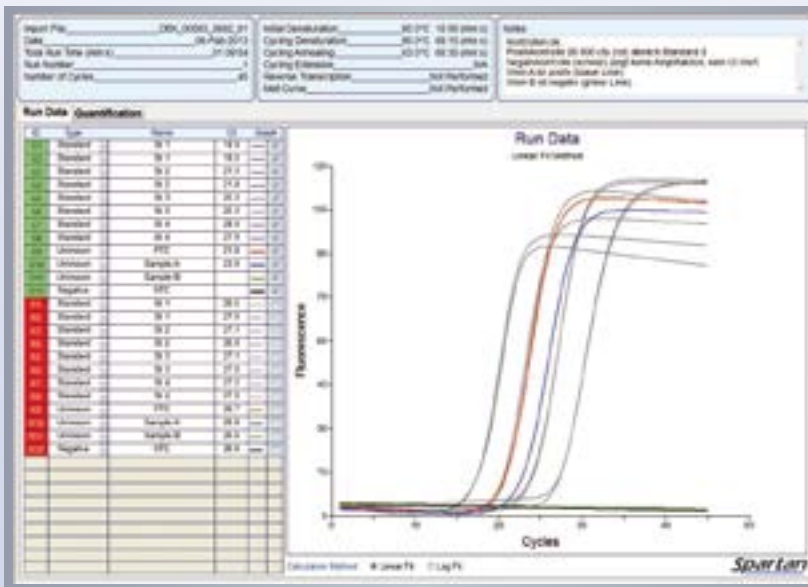
desired type of wine, the presence of *Oenococcus oeni* may also be undesirable.

Undesirable bacteria include these ones forming acetic acid.

The new 4x multiplex kit (First-Wine PCR Kit TaqMan™ Screening-TPWS 0050) detects

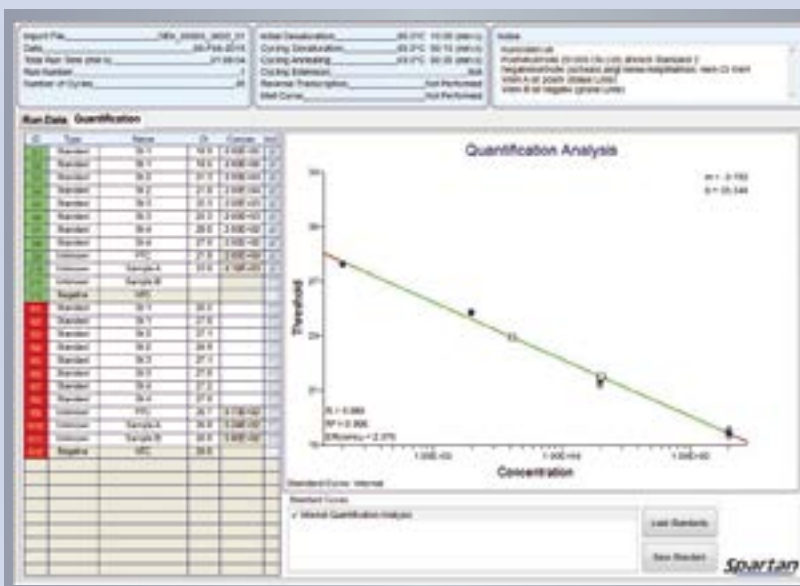
- **Lactobacillus, Pediococcus, Oenococcus oeni** (FAM)
- **Acetic acid forming bacteria** (ROX)
- **Yeasts** (Cy5)
- **Internal Amplification Control** (JOE)

on suitable equipment. This can all be analyzed in one measurement and enables a fast overview of the microbiological status..

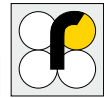


Picture 1 shows a typical PCR result. The exponential curves show the amplification of specific DNA. A flat straight line represents a negative sample.

Here, the analysis for *Dekkera bruxellensis* shows qualitatively a valid measurement due to its controls. One of two different wine samples shows a positive result (blue line) and one a negative (green line). The internal amplification control has similar Ct-values.



Picture 2: The quantification shows a positive control PTC (square) calculated with $2,05 \times 10^5$ cfu (equates to standard 2 with 2×10^5 cfu). A quantification with an external standard curve is possible. The positive wine sample (square) is automatically calculated with $4,14 \times 10^3$ cfu /ml wine.



GEN-IAL® Products

Product	Description	No. of Tests/Amount	Art. No.
GEN-IAL®			
DNA preparation			
Simplex® Easy Wine kit	DNA preparation of wine samples	100 reactions	SEW 0100
Simplex® Easy DNA kit	DNA preparation of beverage samples	100 reactions	SE 0100
GEN-IAL® Species			
Qualitative real-time PCR			
Dekkera bruxellensis Standard	DNA standard for Dekkera bruxellensis quantification	200.000 cfu	DBST 0100
Dekkera bruxellensis TaqMan™ FH	Specific DNA detection of yeasts (FAM HEX)	50 reactions	TPYDB 0050 FH
Dekkera bruxellensis TaqMan™ FR	Specific DNA detection of yeasts (FAM ROX)	50 reactions	TPYDB 0050 FR
Dekkera bruxellensis TaqMan™ Spartan DX-12	Specific DNA detection of bacteria	50 reactions	TPYDB 0050 SP
New! Dekkera bruxellensis TaqMan™ MyGo Pro	Specific DNA detection of yeasts (coated strips)	48 reactions	TPYDB 0048 MG
Acetic acid bacteria TaqMan™	Specific DNA Nachweis von Bakterien	50 reactions	TPA 0050
Pichia anomala TaqMan™	Specific DNA detection of yeasts	50 reactions	TPYPA 0050
Saccharomyces diastaticus TaqMan™	Specific DNA detection of yeasts	50 reactions	TPYSD 0050
Pichia membranaefaciens TaqMan™	Specific DNA detection of yeasts	50 reactions	TPYPM 0050
First-Wine Screening Biogene Amine	Specific DNA detection of biogenic Amines forming bacteria	50 reactions	TPOE 0050
First-Oenococcus oeni PCR-Kit	Specific DNA detection of bacteria	50 reactions	BAM 0050
GEN-IAL®			
Multiplex Screening			
First-Wine PCR Screening TaqMan™	DNA screening and differentiation of wine spoilage bacteria and yeasts: <i>Lactobacillus</i> ; <i>Pediococcus</i> ; <i>Oenococcus oeni</i> ; acetic acid bacteria; yeast	50 reactions	TPWS 0050
First-Wine PCR Screening TaqMan™	DNA screening and differentiation of wine spoilage bacteria: <i>Lactobacillus</i> , <i>Pediococcus</i> , <i>Oenococcus oeni</i> , acetic acid bacteria	50 reactions	TPWSOH 0050

Further parameters for beverage analysis on request.



GEN-IAL® First Yeast Dekkera bruxellensis
Art.No. TPYDB 0050 FR



Simplex® Easy Wine Kit
Art.No. SEW 0100

Real-time PCR

- Quick results (approx. 2 hours)
- Highly specific
- Easy handling
- Cost reduction due to time savings
- Even performable in small wine laboratories
- Little effort