



“Real-time PCR assay for detection and enumeration of *Hanseniaspora* species from wine and juice”

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These authors developed an assay to detect and measure populations of the yeast *Hanseniaspora/Kloeckera*, the dominant yeast in grapes and initial phases of alcoholic fermentation before *Saccharomyces cerevisiae* dominates.

- *Hanseniaspora* can be a positive contributor to a wine’s final complexity through the production of esters, glycerol, and acetoin. However, it has also been associated with negative ester taints and with high acetic acid. Additionally, its growth in large quantities (such as on damaged grapes) can deplete a juice of nutrients, contributing to stuck or sluggish fermentations.
- While traditional methods of identifying yeast in wine have relied on cultures using selective media, advances in molecular tools have dramatically enhanced our ability to identify and quantify yeasts. There are 2 main advantages to the direct characterization of yeast through DNA as opposed to through plating: 1) many grape and wine microbes might not respond to enrichment plating because of injury, lack of the proper nutrient, or simply because they are “alive but non-culturable”, and 2) direct molecular analysis takes less time and is high throughput, making it much more efficient.
- One such molecular method is called real-time PCR, also known as quantitative PCR (Q-PCR). (PCR stands for polymerase chain reaction, a technique that does to DNA what a photocopier does to paper: copy, copy, copy). Briefly, the DNA from the unknown microorganisms is isolated, then the PCR multiplies this DNA in a very controlled fashion, and, finally, the analysis of the resulting products allows not only the identification of the organism present, but an estimation of its population size as well (the more initial cells, the more DNA, the more final amount of copies).
- Obviously, the key to the technique is to design a piece of DNA (called a *primer*) that is able to specifically attach to the DNA of our target organism (in this case, *Hanseniaspora*). This allows for a given fragment of the *Hanseniaspora* DNA to be replicated, leaving the DNA from other organisms untouched. To this date, specific primers like this have been used to develop assays for a number of microorganisms of interest (*Oenococcus*, *Acetobacter*, *Leuconostoc*, *Brettanomyces*, *Saccharomyces*, *Zygosaccharomyces*). The goal of these authors was to develop the equivalent assay for *Hanseniaspora*.
- **Specificity of the assay.** The authors first constructed two primers based on a DNA region previously used to distinguish between yeast strains. When they tested these primers using PCR, only the DNA of *H. uvarum*, *H. guilliermondii*, and *H. valbyensis* was replicated. This meant the primers were specific for *Hanseniaspora* species, and could be used to generate a QPCR-based assay.

• **Reproducibility of the assay.** To test the reproducibility the assay, the authors made serial dilutions of a *H. uvarum* culture. These various dilutions were then plated on selective media, as well as tested with the QPCR assay. In all cases, the colony-forming units and the QPCR results were very highly correlated.

• **Detection limits of the assay.** To test the power of the QPCR assay in a variety of situations, a culture of *H. uvarum* was serially diluted in a rich media, in a sterile juice, and in a juice that had been inoculated with *Saccharomyces cerevisiae*. In all cases, the assay was able to detect *Hanseniaspora* in concentrations as minute as 10 cells per milliliter. The authors were also able to see that the relationship between the plating and the QPCR results was linear over four orders of magnitude (from 100 cells/ml to 100,000 cells/ml). For tiny amounts (<100 cells), the assay did not work so well.

• **Ability to measure *Hanseniaspora* in a commercial juice.** The authors obtained juice samples representing several grape varieties from a local winery. Then, various dilutions of these samples were plated onto a medium that allowed for visual identification of *Hanseniaspora* colonies (which show as green and flat). At the same time, DNA was extracted from the different dilutions and quantified through QPCR. The researchers found an excellent correlation between the *Hanseniaspora* populations estimated by QPCR and the populations determined by plating. Thus, the ultimate test was passed!

In summary, Dr. Mills and his team were able to develop an assay for the enumeration of *Hanseniaspora* sp. in must and wine that can detect as few as 10 cfu/ml, is linear over four orders of magnitude, and is not influenced by high concentrations of contaminating *S. cerevisiae*. High levels of *Hanseniaspora* have been associated in some instances with heavily damaged grapes, and with sluggish fermentations. This assay now provides a rapid method to evaluate grape/must microbial quality, and to predict potential fermentation problems.

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