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Title: **"Direct identification of the indigeneous yeasts in commercial wine fermentations"**

By: Luca Cocolin, Ashley Heisey, and David Mills

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These authors develop a DNA-based method to characterize the yeast diversity in wine fermentations, bypassing the need for plating.

• Wine fermentations are carried out by a succession of microorganisms involving both yeast and bacteria. The quality of the final product will depend not only on which microorganisms performed the fermentation, but as well in which order. This is because the presence of one organism often affects the levels of the next –either enhancing or inhibiting- and, therefore, it has an impact on the overall populations that carry out the fermentation.

• In the past, the microbial composition of fermentations was studied by plating out a sample of wine in an enriched medium that was "cozy" for a wide range of microorganisms to grow in. Then researchers would attempt to identify the microorganisms present by performing a series of physiological tests. Besides being slow, this method failed to characterize organisms that would not grow on the plate.

• The method these authors developed characterizes the organisms directly based on the presence of their DNA. To do that, they focused on a specific piece of DNA (ribosomal RNA genes) that is highly conserved among organisms and, for that reason, widely accepted for classification purposes. The method comes with the un-appealing name of PCR-DGGE (PCR stands for "polymerase chain reaction", the part that allows amplification of the DNA; DGGE stands for "denaturing gradient gel electrophoresis", the part that allows separation of the amplified DNA).

• Briefly, the method consists on, first, isolating the DNA of all the organisms present in the fermentation (this is easier said than done, as there are a lot of grape polyphenols and polysaccharides in the way!). Then the authors amplify the piece they are interested in by using available sequences for those specific genes as templates (called primers). The next step is to separate all the pieces present in the amplified DNA based on their sequence. This results in a series of bands that look like a barcode. The final step is to isolate each individual band and sequence it, to confirm the microorganism's identity. This is not unlike what a CSI lab does to identify a criminal.

• **Fermentations.** The authors selected to study botrytized wines because of their high microbial load. The collaborating winery (Dolce Winery, Oakville, CA) uses a blend of Semillon and Sauvignon blanc that they sprayed with a strain of *Botrytis cinerea* two weeks before harvest. After thorough clarification of the juice, fermentations were carried out by indigenous yeast in four oak barrels at ambient

temperature (ranging from 15 to 20°C). Wine fermentation samples were taken daily. Samples were also taken from the press pan and the settling tank.

• **Results.** 1) The **press pan** analysis revealed the presence of 2 molds (both *B. cinerea*), and 4 yeasts species (two species of Candida, one of Pichia, and one of Metschnikowia). 2) The **settling tank** analysis revealed a clearly different profile: the Botrytis species had disappeared, whereas the wild yeast had persisted. As the authors point out, this is probably due to the inability of Botrytis to switch from aerobic to the more anaerobic conditions of the settling tank. 3) **Eight days** into the fermentation, a new species appeared, which was identified as *Saccharomyces cerevisiae* (a second band did not match the GeneBank database for any species, and so, could not be identified). Interestingly, even though most of the wild species had disappeared by day 8, the two Candida species persisted throughout the fermentation. As the authors note, this is likely to affect both the sensory and the stability of the final wine.

What is the application for the winemaker? With many winemakers wanting to decrease their use of SO2, there is an increased interest in knowing the yeast and bacterial ecology present in a wine. The use of Saccharomyces (starter inoculation) versus non-Saccharomyces yeast to conduct a fermentation is a clear stylistic choice that comes with its pros and cons. Here the authors developed a method of microbial characterization that takes one day to run and uses as little as 1 ml of must. In fact, Dr. Mills was the first one to demonstrate the use of this DNA technology to characterize wine fermentations, a technique that commercial labs have adopted since. So if you need to find out what is growing in your wine, there are labs available that offer this service.

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