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Yeast and bacteria analysis of grape, wine and cellar equipments by PCR-DGGE

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These authors inventory the yeast and bacteria species present at every step of the process from the grape to the bottle.

• Several post-fermentation microorganisms are detrimental for wine quality, such as the yeast *Brettanomyces bruxellensis*, which produces volatile phenols, or some lactic acid bacteria, which can produce polysaccharides, biogenic amines, or ethyl carbamate. For this reason, a microbial survey, both before and after completion of fermentation, is important in preventing spoilage.

• These authors studied the microorganism population present at every step of the winemaking process in 3 estates located in 3 Bordeaux appellations: Libournais, Graves, and Medoc. For each estate, they started inventorying the microorganisms present at the vineyard level, continued with those present in the water resulting from cleaning the cellar equipment, and ended by analyzing the microorganism present in 2 wine bottles, a recent vintage and an older vintage.

• The authors chose to bypass the tedious culturing method and used PCR-DGGE instead. This technique stands for Polymerase chain reaction- Denaturing gradient gel electrophoresis. In the first part of the technique (PCR), a specific portion of the total DNA present in the samples is amplified, by the use of commercial primers available for identification purposes. In the second phase of the technique (DGGE), the resulting amplified DNA fragments –called an *amplicon*-, all of the same size and differing only in their nucleotide composition, are separated by an extremely sensitive gel electrophoresis set-up. The resulting sequences are then compared with sequences available in databases to reveal the identity of the different microorganisms present.

• **Yeast and bacteria present on the berry surface**. The community of microorganisms the authors found on grapes was very large and diverse. It could be divided into 3 groups: 1) species **without fermentation ability** (such as *Auresbasidium* and *Burkholderia*, never before seen in wine); 2) species **with some fermentation ability** (*Lactobacillus, Pichia, Candida, Metschnikowia*, which could act during the first stages of winemaking); and 3) species that are the **main fermentation agents** (*Saccharomyces cerevisiae* and *Oenococcus oeni*). The proportions of these 3 groups changed during grape ripening. Group 1 was dominant before veraison, whereas at harvest, species of Group 2 were dominant.

• **Yeast and bacteria present during winemaking.** At the beginning of fermentations, the main species detected in this study corresponded to Group 2, which all have low alcohol tolerances. The authors noticed how the concentrations of these species could be influenced by winemaking practices in the different chateaux (such as SO2 addition, cold maceration, oxygenation, and the use of a commercial yeast starter). After that, the predominant species became those of Group 3, that is, *S. cerevisiae* during alcoholic fermentation, and *O. oeni* during malolactic fermentation. Finally, at the very end of the fermentations, and after SO2 addition, it was *Brettanomyces bruxellensis* and *Pediococcus parvulus* which dominated. This is likely due to their high ethanol tolerance and poor nutrient requirements.

• At this point, the authors point out a shortcoming of the PCR-DGGE technique. Because the best adapted species at each stage constitute an overwhelming majority of the total DNA analyzed, their magnitude makes the minor species very difficult to detect. (This effect happens when the ratios between species exceed 1,000-fold). So the authors warn us: failure to detect some species by direct DGGE analysis does not necessarily mean that the species is absent, but that the species is much less numerous than others. Going back to our situation, this means that *B. bxuxellensis* and *P. parvulus* are likely present throughout fermentation, just unable to grow enough to be detected with PCR DGGE until *S. cerevisiae* and *O. oeni* succumb.

• **Yeast and bacteria present in barrels and tanks.** Analysis of barrels revealed species common in wine (*O. oeni, S. cerevisiae*), but also wood-specific yeasts, such as species of the genus *Cryptococcus*. As for the analysis of stainless steel tanks, species of wine origin could be detected *(O. oeni, P. parvulus*), but species of grape origin as well, such as *Burkholderia*. This means that species that originate on grapes could survive in the tanks despite the fact that they were unable to grow in the wine. To avoid these sources of contamination, the authors recommend thorough sanitation of this equipment.

• **Yeast and bacteria present in bottled wine.** *S. cerevisiae* was detected only in bottles of recent vintages. This was also true for species of Group 2 (*Lactobacillus, Pichia, Candida*). *Zygosaccharomyces*, a species known to be able to referment sweet wines**,** was detected only in bottles of older vintages. Finally, ***B. bruxellensis*** **was the predominant yeast in every bottle, recent or old**.

In conclusion, using a direct DNA identification technique, the authors were able to show that the microbial diversity decreased importantly from grape to bottle. Only the most resistant species were able to survive ethanol, SO2, low oxygen, and low pH. These most resilient species were *B. bruxellensis* for the yeast, and *P. parvulus* for the bacteria. The authors believe that PCR-DGGE is an adequate tool for monitoring fermentations at the commercial level, as it will allow the user to react to spoilage organisms quickly, before the wine is bottled. It would also allow a direct comparison of the microflora present in different vineyards, as well as to establish a connection between the different spoilage organisms and particular wine defects.

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