Summary 48

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Title: **"Wine yeast inhibition by sulfur dioxide: A comparison of culture-dependent and independent methods"**

By: Luca Cocolin and David Mills

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These authors compare the microbial diversity that develops in fermentations without sulfur dioxide (SO2) addition versus fermentations with initial SO_2 addition. They also compare the diversity obtained using a plating system versus using DNA-based techniques.

• Non-*Saccharomyces* yeast species (*Candida, Hanseniaspora, Pichia, Torulaspora, Metschnikowia, Kluveromyces*) are known to grow in the early stages of fermentations. Later, as *Saccharomyces* becomes dominant, and ethanol levels increase, these wild populations decline. The use of SO_2 early in the fermentation is known to be very effective in knocking out the early native yeast populations. Whether we let the fermentation be carried out by native yeast or with a *Saccharomyces* inoculum is, as we know, a stylistic choice.

• Even though non-*Saccharomyces* populations fail to grow in an enriched medium after SO_2 additions, the authors suspected that they may actually persist later in the fermentations, but just be unable to grow. Some of the causes for this lack of growth could be that they had been damaged by the SO_2, or the SO2 made their environment temporarily too harsh for growth. To find out whether microorganisms that are not able to grow on a plate might actually still be viable in juice or wine, the authors compared the yeast species detected using 2 very different approaches: 1) one based on their ability to grow on a plate with an enriched medium; 2) the other based on the presence of each microorganism's genetic material (DNA and RNA).

• **Fermentations with and without SO_2.** The authors inoculated 2 Chardonnay juice containers with specific strains of *Saccharomyces*, *Candida*, and *Hanseniaspora* isolated from a commercial wine fermentation. They added 50 mg/l of SO_2 to one of the two containers. Then they allowed both to proceed through fermentation at 18°C. Samples were taken daily for DNA/RNA analysis. All fermentations were conducted in duplicate.

• **The plating method**. The authors spread wine samples on 3 types of media to study the presence of *Saccharomyces, Candida* and *Hanseniaspora*: 1) Wallerstein Laboratory Nutrient Agar (*WL*), which allows identification based on colony morphology, 2) *lysine medium*, which allows actual enumeration of colonies, and 3) *WL containing cycloheximide*, which enables specific enumeration of Hanseniaspora (both *Candida* and *Saccharomyces* are unable to grow in cycloheximide). After incubation (22°C), the plates were read by visual observation 5 days later.

• **The DNA/RNA method**. After isolating the DNA and the RNA from the microorganisms present in the fermentation, the authors used a specific piece of DNA, widely-used as a tool in taxonomic studies

(ribosomal RNA gene), as a template to obtain larger amounts of DNA or RNA belonging to each microorganism. Then they used a very powerful technique (denaturing gradient gel electrophoresis, or DGGE) to separate the products based on their sequence. This allowed the authors to identify which organisms were present based on their genetic makeup.

• **Plating results.** *(To help understand the results, we could think of a daily "class attendance list" of three students,* Saccharomyces, Hanseniaspora, *and* Candida, *to each of two classrooms, one with SO_2, one without).* As expected, the populations detected by the plating method were dramatically different depending on whether the fermentation had received SO_2 or not. In the fermentation without SO_2, the *Hanseniaspora,* and *Candida* reached high populations after only one day, whereas *Saccharomyces* did not do so until late into the fermentation. Then, *Hanseniaspora* and *Candida* started decreasing gradually, whereas *Saccharomyces* remained dominant until the end. In contrast, in the fermentation with SO_2, *Hanseniaspora,* and *Candida* were "wiped out" from the very beginning, and only *Saccharomyces* was able to recover after Day 3. The ratio of glucose to fructose was lower in the fermentation with SO_2 compared to the control, probably due to the absence of *Candida* -which is fructophilic- in the presence of SO_2.

• **DNA/RNA results**. The powerful detection method based on the genetic material depicted a completely different picture. In the fermentations without SO_2, two strong bands belonging to *Hanseniaspora* and *Candida* were detected from the very beginning, followed by the band for Saccharomyces soon after. This was true for both DNA and RNA bands. (Up to here, the results are similar to those observed with the plating method). But in contrast with the plating method, in the fermentations with SO_2, the DNA and RNA bands belonging to ***Hanseniaspora* and *Candida* remained visible well into the fermentation, even though the corresponding colonies had not grown on media plates**. The band corresponding to *Saccharomyces* started appearing on Day 6. This coincides with the time when the *Saccharomyces* populations detected with the plating method became important.

• The presence of strong bands of *Saccharomyces* DNA/RNA in the SO_2-ed populations in comparison with the weak bands belonging to *Hanseniaspora* and *Candida* introduced a bias in the resolution of the gels against those organisms present in smaller amounts. To avoid this interference, the authors went a step further and constructed probes specific to *Hanseniaspora* and Candida so they could detect the presence of these organisms independently from *Saccharomyces*. What they found was that *Hanseniaspora* and *Candida* could be detected using this technique for as long as 15 days after SO_2 addition. In other words, even though these two organisms were not visible by the plating method, their RNA persisted.

In summary, the authors showed that microorganisms unable to grow in a routine wine plating can long persist in a wine fermentation. What needs to be determined next is whether these microorganisms are metabolically active enough to be able to alter the finished product. If these yeasts were to recover or regrow, they could be a source of wine spoilage at a later stage in production or bottling. As the authors suggest, it may be necessary to specify from now on whether the microorganisms present in a wine are "alive", "injured", "viable but not culturable", or "dead as a doornail".

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