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Title: **"Use of WL medium to profile native flora fermentations"**

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To evaluate microbial diversity, these authors studied the type of microorganisms present in the vineyard, winery, and barrel fermentations of a commercial winery by looking at their colony morphology.

• Many yeast species can be present during a native fermentation, but three genera are dominant: *Hanseniaspora, Metschnikowia*, and *Saccharomyces*. The end-products of the first two may or may not be desirable, depending on wine style. This justifies the importance of being able to identify the relative amounts of the different organisms present.

• Other researchers had shown that a medium designed for the brewing industry (called Wallerstein Laboratory Nutrient Agar, or WL for short) could also be useful for the wine industry to identify and quantify wine microorganisms. The beauty of this medium is that colonies have different colors and shapes depending on which yeast is growing: *Hanseniaspora* is bright green, *Metschnikowia* is brown/red, and *Saccharomyces* is pale green-to-cream color.

• The authors used WL media to plate 1998 Chardonnay wines from the collaborating winery (Luna Vineyards and Winery, Napa, CA). Must was transferred from the winery tank to glass carboys (5 gal), and fermented in triplicate at either 13°C or at 18°C. Fermentation samples for analysis were plated daily. Juice from the vineyard and from winery equipment, as well as wine from both old- and new-barrel fermentations, were also plated. Once the colonies were identified based on their morphology, their DNA was isolated and the fragment of interest sequenced to confirm that the microorganisms had been correctly identified.

• **Effect of juice source**. The wild yeasts *Hanseniaspora* and *Metschnikowia* were present at all sampling sites: vineyard, winery equipment, barrels, and small-scale fermentations. In contrast, *Saccharomyces* was occasionally spotted in vineyard and winery equipment, but was found predominantly at the later stages of fermentation. The authors also spotted colonies that did not fit any of these 3 main genera. After studying their DNA and comparing it with available sequence databases, they were able to identify one of the colonies (found in samples from the vineyard and from 18°C fermentations) as *Candida*, and the two other colonies (found in samples from the 13°C fermentations) as *Pichia* and *Issatchenkia*.

• **Effect of fermentation temperature**. The authors studied the effect of fermentation temperature on the distribution of colonies. They found that *Hanseniaspora* was present throughout the fermentation at both temperatures. *Metschnikowia* declined quickly at the higher temperature (18°C). *Saccharomyces* was initially undetectable, and colonies did not start appearing until day 2 (18°C) or day 3 (13°C) into

the fermentation. The presence of *Candida* could be found only in the 18°C fermentations, whereas *Pichia* and *Issatchenkia* were restricted to the 13°C fermentations.

• **Effect of old versus new barrels**. There were no significant differences between the microorganism populations of wine fermented in old versus new barrels. The only difference the authors observed was that *Saccharomyces* populations increased faster in the old barrels, but final populations were the same.

• The authors were surprised about the large variation in colony morphology found within the genus *Metschnikowia*. So they studied this genus in great detail, comparing their DNA sequences and proposing a phylogenetic relationship among the different species, something not so relevant for us. Since colonies initially showing different morphologies were later identified as the same species, the authors concluded that, unlike *Hanseniaspora* and *Saccharomyces*, a more systematic analysis than colony observation is needed to correctly identify the different species within *Metschnikowia*.

In summary, the study demonstrates the utility of the WL medium plating as a tool to monitor yeast population diversity during fermentations. The colony morphologies were sufficiently unique that their characteristics could be used to identify the corresponding yeast's genus and species (the exception being *Metschnikowia* which can only be identified by genus). Because of the dramatic difference in the appearance of their colonies, even yeasts present in low numbers (*Pichia, Candida, Issatchenkia)* could also be identified. Plating is already a common practice in many large wineries as a post-bottling quality assurance.

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