

Wine, Cheese, and the Microbiomes That Influence Their Flavor

Researchers use the MiSeq[®] System to identify the microbiome profiles of wine and cheese, and the facilities in which they are made.

Introduction

Most microbiology studies focus on how to identify and remove microbes from our food—and for good reason. Pathogens like *Salmonella enterica, Listeria monocytogenes*, and *Escherichia coli* sicken countless people every year. But University of California, Davis (UC Davis) food scientist David Mills, PhD, is quick to point out that microbes are actually the true chefs behind some of our favorite foods. Lactic acid bacteria metabolize the lactose in milk, helping convert it into the savory cheeses that we enjoy such as cheddar, brie, or pecorino romano. And the yeast *Saccharomyces cerevisiae* performs its magic on grapes, transforming their juice into a seemingly infinite variety of wines for every taste and occasion.

Dr. Mills studies the microbiomes of cheese and wine, and the facilities that produce them, to understand how microbes work their magic. It's a question that scientists have only recently been able to tackle, thanks to next-generation sequencing (NGS) instruments, like the MiSeq System.

He hopes to discover how cheeses and wines cultivated in different regions develop their own distinctive flavors by observing and cataloging their unique constellation of microbes. "The concept of regionality in food might be traceable through the microbes that are associated with it," Dr. Mills said. "The microbiota profile at the front end, before the grapes are fermented, could correlate with the profile of the chemical constituents of the finished wine. A very exciting aspect of our research is that we might be able to predict flavor outcomes by studying these microbes."



David Mills, PhD, is a Professor in the Departments of Food Science and Technology, and Viticulture and Enology at the UC Davis.

A Different Wine and Cheese Pairing

Dr. Mills began his career in biochemistry and biotechnology. As a PhD student at the University of Minnesota, he switched to the microbiology program after becoming interested in the work of Larry McKay, PhD, a microbiologist who studies the bacteria that make cheese. This appealed to Dr. Mills' interest in translational science. "While I enjoy basic research, what drives me is studying its application in processes and the environment," Dr. Mills stated.

He decided to focus on the genetics of *Lactococcus lactis*, one of the lactic acid bacteria needed to make cheddar cheese. After a short postdoc at North Carolina State University, Dr. Mills was hired by the renowned Department of Viticulture and Enology at UC Davis. Although wine's main fermenter is *S. cerevisiae*, lactic acid bacteria also perform some of the fermentation. It was a wine and cheese pairing of a different sort, and Dr. Mills loved it.

Sampling Cheesemaking Facility Microbiomes

Understanding the microbial ecology of any food involves more than a focused study of the hundreds or thousands of different microbes found in the food itself. It also means studying the microbes found in the facility where the food is prepared and packaged.

"We're very interested in understanding the microbial landscape of the whole food production facility, such as the seasonality of microbial movement inside the facility," Dr. Mills said. "For example, how activities inside the facility lead to microbial transfer back and forth.



Photo courtesy of UC Davis Communications

UC Davis viticulture and enology student Javier Avina prepares a fermenter containing crushed grapes for primary fermentation.

Often, I'm studying facilities where microbes are purposely introduced for making cheese or wine. The question is, do these microbial colonies grow to populate the entire place? Where else do they end up? In instances where sanitation is performed correctly, we want to understand how other microbes present in the facility take over."

No matter the type of facility being studied, identifying these microbes means taking samples—lots of them. Dr. Mills and colleagues use cotton swabs to take thousands of samples from buildings and the foods they produce.

After chemically isolating and extracting the DNA, Dr. Mills originally employed "very rudimentary tools" to identify the different microbes by hand, using denaturing gradient gel electrophoresis. "Old school methods had very low throughput," Dr. Mills said. "We could only analyze 20 samples at a time and the resolution was terrible. Graduate students hated it because it was a tough method and very timeintensive. We could get an indication that there were a bunch of microbes present, but we weren't sure what they were."

Role of House Microbiota in Cheese and Winemaking

While the interest in decoding the microbes that make wine, cheese, and beer has always been high, the research stalled in the absence of efficient tools. This changed several years ago, when the sequencing core at UC Davis invested in NGS and purchased several MiSeq Systems. Taking advantage of this advanced technology, Dr. Mills began identifying microbes from large numbers of samples. Together with graduate student Nickolas Bokulich, he began to look more closely at the microbes in a California artisanal creamery to see if a facility-specific or "house microbiota" could be identified. They sampled microbes from the surfaces of the washed rind cheeses (a class of cheese that includes Limburger and other pungent varieties), and examined some of the surfaces in the facility itself.¹

"We could see that the microorganisms on the surface of a cheese were the same ones present on the surface of some parts of the facility," Dr. Mills stated. "We're still unsure which direction the microbes colonized: from the cheese to the facility surfaces, from the surfaces to the cheese, or in both directions over time."

Regardless of the colonization dynamics, the results supported the idea that a house microbiota helps to inoculate a cheese or wine and cause it to take on a specific flavor. Vintners in the Bordeaux region of France have said for years that the winery itself inoculates the wine. It's a concept called *terroir*, the idea that the place where a wine or food is grown or made is ultimately expressed in its final taste.

"People will taste a wine and say 'that's a Bordeaux," Dr. Mills said. "A good sommelier can even tell you the vintage and region of origin. Yet, we don't know what causes that regionality. How do we connect the sense of place to actual sensory metabolites in the wine, so we can understand why a Napa Valley cabernet delivers a specific flavor that can't be found in a cabernet from the California Central Valley?"

No one knows exactly what creates *terroir* in a food, but Dr. Mills' work is showing that microbes likely play a role. His sequencing analysis studies begin with extracting DNA from samples and amplifying portions of bacterial and fungal ribosomal genes with different sets of barcoded primers, some of which were developed in his lab. The library sets are sent to the UC Davis Genome Center, where the MiSeq System is used to sequence 500 samples in a single run. The amplicon sequences tell the researchers which microbial species are present in a given sample, allowing them to take a census of microbes across a facility and over time.

Identifying Winery Microbiomes

Back at the UC Davis winery, Dr. Mills and Bokulich sampled the whole facility about 150 times using cotton swabs at several points throughout the winemaking season. They started in August, before the grapes were harvested and brought inside. They returned in the middle of the season, as the grapes were brought into the winery to be crushed. And they made a final visit in mid-December, when the facility was being cleaned and the winemaking equipment stored for the season. As they predicted, they saw dramatic shifts in the microbes over that time. They also found some things they didn't expect to see. For example, *S. cerevisiae* arrived on the doorstep of the winery long before a single grape came in, perhaps brought in by workers walking back and forth from the vineyard to the winery.²

Dr. Mills and his colleagues expanded their study to wineries throughout California, looking at the microbes that grew on grapes and in the facilities over 1 to 2 seasons. The goal was to see if microbial populations were unique for different wines from different regions, and to study how these microbes moved through the system from harvesting to the final product. They also sought to document the role of microbes in producing a specific wine and its flavor.

Clearly, the flavor molecules in the grapes themselves strongly influence the final flavor of the wine, and certain regions produce grapes that yield better wines than others. "Whether microbes play a 1% or an 80% part of the flavor-generation process, I don't know," Dr. Mills stated. "The Holy Grail in wine is to understand where a good wine flavor comes from, and we know that there is an amalgam of microbes needed to produce the final product. Many of the most-desired wines have much more complex flavors that are driven by more than just *S. cerevisiae*. We're attempting to understand the constellation of microbes that delivers that actual flavor."

The research showed that "there are different sets of microbes entering the wineries from different regions on the same grapes," Dr. Mills said. "We know some of those microbes influence the flavor of wine, providing the potential connection between something in the vineyard to a flavor molecule in the wine."

Dr. Mills and his team studied *must*, the freshly pressed juice created from crushed grapes before they are fermented.³ The data revealed that the microbes entering a winery on *grape must* are region-specific. For example, the microbes on *chardonnay must* from the California Central Coast are different from those on *chardonnay must* from Napa Valley or Sonoma County.

"If you take the same vine type and grow it in Sonoma and in Napa, I wouldn't expect to see that much difference in the microbial landscape," Dr. Mills stated. "Plants, like humans, have typical microbes associated with them. So I was a bit surprised at how robustly we could differentiate the regions based on the microbial communities we found."

Different populations of microbes that enter into fermentation could cause flavor differences in the final product, a hypothesis that Dr. Mills and other scientists are just beginning to test.

NGS Enables Efficient, High-Quality Microbiome Research

None of this work would have been possible without NGS systems, such as the MiSeq System, and bioinformatics programs that help researchers make sense of the data. "The irony of the situation is that collecting the samples is now the biggest problem I have in performing these studies," Dr. Mills said. "Processing the samples and running them on a sequencer is easier than going to a facility and spending 8 hours obtaining 300 swabs, all documented so we know exactly where the sample was taken. In one of the papers we're submitting soon, the winery had to collect about 2000 fermentation samples for us. That's a large number of samples and they all have to be labeled correctly and stored somewhere. It's a lot of work."

For Dr. Mills' research, the MiSeq System provides an optimum combination of speed and cost. "I like the MiSeq System because it's a scaled down version of a high-throughput sequencer," Dr. Mills stated. "It turns around samples quickly and I know that the quality is good. While I'd love to see a larger read, I'm happy with the reads that I get."

His work has implications beyond identifying the microscopic creatures that help to make some of our favorite foods and wine. It might also help us understand how microbes move through our world.

"Our goal is to develop a microbial map of a whole facility," Dr. Mills added. "One of the beauties of using NGS tools is that we can process a large number of samples. Our MiSeq runs are often 500 samples or more. In the old days, plating 500 samples would not have been feasible. We could never have performed the kind of highthroughput studies that we can today. With NGS data, we are opening up a completely new window, enabling us to view the microbial landscape of food facilities."

References

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Learn more about the Illumina system mentioned in this article and the art of cheesemaking:

- MiSeq System, www.illumina.com/systems/miseq.html
- Illumina SciMon Video: Cheesemaking: Ancient Art or the Simplicity of Science, www.youtube.com/watch?v=M0QUzpRCvP4&feature= youtu.be&list=PLKRu7cmBQlahAeoVCcb-pM1oVCKFkggFh



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