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Microbial War & Peace in Cheese Rind Microbiomes [enriched transcript]

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Editors note: This publication contains the video of the talk from the Fermentology webinar series, as well as a lightly edited transcript of the lecture. The transcript has been enriched with media, annotations, and links to other material by the digital publication team in order to amplify and extend the content for a reading experience.

Abstract

In this talk, Benjamin Wolfe will explore how microbes compete and cooperate in cheese rind microbiomes. From fungal highways on wheels of Saint Nectaire to antibiotic producing fungi in cheddar, we'll learn about the ecology and chemistry of microbial interactions in some of your favorite stinky cheeses. We will also learn how cheesemakers can use this knowledge of microbial interactions to improve the safety and quality of their products.

Benjamin Wolfe is the Aptman Family Assistant Professor of microbiology at Tufts University. The Wolfe Lab at Tufts uses fermented foods as model systems to identify the processes that shape the diversity of microbiomes. In addition to research focused on the basic biology of microbes, the Wolfe lab has worked with chefs and food producers, including David Chang's Momofuku Culinary Lab and Jasper Hill Farms, to understand the roles of microbes in creating the diversity of flavors in fermented foods.

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Microbial War and Peace in Cheese Rind Microbiomes | Fermentology mini-seminars

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Introduction

What I'm going to be talking about today is the *microbiome*. And of course, this is a term that many of you are hearing these days, particularly now during a pandemic.

The microbiome is simply the collection of microbes living in a particular environment. Everything really at the end of the day has a microbiome. Human bodies have a microbiome, soil has a microbiome, my cat has a microbiome because she is an organism — and on her, she has a bunch of microbes living on her surface of her fur, living inside of her belly, living on her paws.

Microbiomes contain many types of microbes. It's often bacteria that we're thinking about, but there are also fungi, like yeast and molds, as well as viruses. And microbiomes are *everywhere*.

The Challenges in Studying Microbiomes

The reason I'm talking about microbiomes today is there's some really big challenges in terms of studying microbiomes. in terms of understanding what this collection of microbes really is, what is this community? Some of the really big questions that are still relatively open are: **What generates microbiome diversity? Why do we see different types of communities of microbes in different habitats?**

For example, up in your nose you have various Actinobacteria. Between your toes, you have a very different community of bacteria, a very different type of microbial community present. And so one really big question is: *Why*? What's creating that diversity? This is still a relatively unresolved question in the science of microbiomes.

Another really big challenge for microbiologists who are studying microbiomes is trying to understand the parts in a microbiome. Those would be the microbial species, the yeast, the fungi, the bacteria, the molds, the viruses, all these different types of microbes. **What are the parts? And also how do those parts fit together?**

What I like to think about is an assembly line like when you're building a car. There are particular ways that the wheel has to interact with the axle, which has to interact with the steering wheel for example. So what we're trying to do in the world of the microbiome in studying microbiomes is understanding:

1. Are there particular parts in a microbiome that you need to accomplish a function?
2. How do those parts fit together?
3. How do those microbes and those cells interact with each other and communicate in some ways with each other?

Another really big challenge in studying microbiomes is that so much of what we know about microbes comes from a history of microbiology where we studied microbes all by themselves in a Petri dish. So much of the science of Microbiology (and it's been amazing, fantastic science!) comes from monoculture, studying just a single organism living all by itself. But as I've been explaining, microbes live in these very complicated systems where there's many different neighboring species that could potentially help them, hurt them, or maybe not interact with them at all.

Fermented Foods instead of Lab Rats

What we're trying to do when we're studying microbiomes is understand those microbial interactions. What we do in my lab and the way we address these really big questions about diversity and interactions in microbiomes is that we use fermented foods almost like a lab rat. The reason we do this is that most microbiomes are very complex. They have hundreds or thousands of species in them and they're being worked on by many scientists but they're relatively challenging puzzles to put together and take apart.

The great thing about fermented foods is the relatively simple system. For hundreds of years, people have been taking microbes and mixing them with raw materials, like cabbage if you're making a fermented vegetable or tea if you're making kombucha. And with things like salt, heat, and time, we've been able to come out on the other side of this with these microbial communities that live in fermented foods.

From a scientific perspective, this is really awesome because:

- These are being made all over the world — they're almost like little Petri dishes that people are putting out for us to study, like little islands of biodiversity.
- They're reproducible in the sense that they generally make the same thing over and over again, which is such a fascinating ecological problem to solve.
- They're also easy to grow, so most of the microbes in fermented foods we can coax them to grow in a Petri dish in the lab. That's not necessarily true in a lot of other systems like your gut or in soil, where many microbes are really hard to grow in the lab.
- And there is tractable complexity — they're relatively simple systems. There's usually one, two, three, maybe up to 20 different species of microbes living in a fermented food, as opposed to your gut where there may be thousands of species.

What we can do is use fermented foods as a simple lab rat to study and understand microbiomes, and then take it back out into the world and maybe learn general principles in more complex systems.

Surface-Ripened Cheeses

Surface-ripened cheeses are really where a lot of the research in my lab has been focused. When I say surface-ripened cheese, what I'm talking about is not that plastic wrap cheddar that you see in the supermarket. What we're talking about here are cheeses that are furry, and wrinkly, and spotted, and sort of funky sometimes.

These are cheeses where you grow a microbial community: you grow a microbiome on the surface of the cheese and over time that microbial community decomposes that cheese, that curd, that solidified milk. From the outside, it's slowly rotting that cheese and kicking off all these delicious flavors that we enjoy as consumers of cheese. You get buttery flavors, fruity flavors, and a lot of funky cabbage flavor sometimes as well.

How Cheese is Made

Here is just a quick review of how cheese is made to understand where these rinds come from.

1. You have to get milk, of course.
2. You make curds so you actually solidify that milk through a whole other fermentation process that I'm not going to address here.
3. You drain those curds to make a shape, some kind of circle or square.
4. You add salt.
5. And then what we're most excited about in terms of today's talk is understanding affinage: the aging step.

We're really excited about understanding how the microbial community grows on the surface as the cheese is aged over time. Again, that's the rind. These rinds, these microbial communities on the surface of the cheese develop in aging facilities all over the world.

One of my favorite places to go is Jasper Hill Farms up in Vermont, which is a really high-tech example of cheese aging — whereas we can see low-tech aging facility in France, where a particular cheesemaker is making a cheese in a very traditional way.

You can look at different stages of cheese rind development, which would include fresh cheese, middle-aged cheese, and cheese about ready to be shipped out to the consumer. Overtime, pigmentation develops on the surface of the cheese and that's the rind that's growing on the cheese.

Microbial Cheese Rinds

Now, there are three different types of microbial cheese rinds.

The first would be a bloomy rind cheese. These are called bloomy rind cheeses because they bloom with a white fungus during the aging process. These tend to be heavily inoculated with starter cultures that the cheesemaker can buy and add to the cheese.

The second type of cheese are washed-rind cheeses. These are those really stinky, funky things like Taleggio and Limburger. They grow usually orange or sometimes red or pink rinds on their surface that are stinky and sticky. And they're washed with a brine solution during the aging process to promote the formation of this particular rind.

And third, we have a natural rind cheese. These are cheeses where we don't really do a whole lot. We just let a microbial community establish. We don't inoculate it very much. We don't manipulate it very much, kind of like the old-growth forests of cheese rinds.

Just by looking at the shapes and the colonies that are growing, you are able to see how we get very different types of microbial communities in these three different types of rinds.

The Science of the Cheese Rind

One of the things we've been doing is just sequencing the microbiome of these cheese minds. We first go out and ask: What are the patterns of diversity across cheese rinds? This is to understand just who's out there, what microbes are out there. The way we do that is sort of like microbial CSI when they go out and do a crime scene investigation but in this case, we're looking at what microbes are there, not what criminal was at a particular site. What we do is we extract DNA from the cheese rind: we go in and find particular DNA regions that are useful for identifying microbes, we sequence that DNA, and we match it to databases. So: extract, amplify, sequence, and match.

Across difference cheeses, we see a lot of variation in the overall composition of the cheese rind microbial communities. There's a lot of diversity out there. But what I really want to get to today is thinking about how those communities come together. How are they built in this idea of microbial war and peace that I brought up in the title?

Microbial community assembly refers to the microbes coming together to this particular habitat, in this case, its cheese, and growing together to form a community.

What we've been able to see in a cheese that we studied a long period of time is a succession (this is something you hear a lot about in ecology) — where in the early stages of the rind development, we get a particular type of microbe, these bacteria called staphylococcus. These are not staph aureus. These are other staph species, then we get some yeast coming in. And then, later on, we get Actinobacteria and filamentous fungi or mold. We are able to see this clear temporal progression as the cheese rind develops over time.

And so what we've been trying to do is use this cheese rind system to understand that process, the process of assembly, and explore what's actually controlling that. If we can understand that, it goes back to the questions I introduced at the very beginning, then we can understand what drives diversity more generally in microbiomes. We could understand maybe some of the variation we see in the human microbiome.

The first thing we think about is the process of dispersal: **What are the parts available to build a microbiome?** What I mean there is that there's a certain set of microbial species in a particular place and only some of those (in this case, it's the orange, blue, and green bacteria) get to the cheese rind. They actually have to get there physically and move there. That's step one.

The second way we think about cheese rinds building, coming together in this assembly process, is: **How do they fit together?** How do those microbial parts, the microbial species, interact?

And then third: **How do microbiomes change over time**? This is the idea that once a community actually comes together and is established, if it's continuously being grown in an environment (as you see in kombucha, sourdough, and sometimes in cheese), those microbes could evolve, mutations can pop up in their genomes, and you can actually see new genome genetic types, but you could also see new phenotypes.

Microbial Interactions: A Short Story

We've been thinking about microbial interactions in cheese rinds and how fungi interact with bacteria in their environment. And so now I'll be sharing with you a very short story about how bacteria can use molds, the filamentous fungi in cheese, as a superhighway because I think it most clearly illustrates the importance of the interactions in this particular system.

So some bacteria, including those that can live on cheeses, are motile. These would be bacteria that have little tails called flagella — and they use these tails to swim around in liquid environments or sometimes they can even use them to spread across the surface of a thing like a cheese.

One of the things we got really interested in with the cheese rind system is: **How do these bacteria that can swim around and that can actually actively move themselves on surfaces? How might that motility work if there are other microbes present?** And what we happened to do is stumble across something really awesome, really cool.

We noticed this weird morphotype in a particular cheese when we plated it out. When we zoomed in really closely what we noticed is that there were fungal hyphae, the little filaments that make up a mold — and around those filaments, we noticed that there are these bacteria.

What we found is that these bacteria were actually swimming along the fungal hyphae. They were actually quickly moving around the tips of the fungus, almost like a swarm of bees moving around a tree. The bacterium here is called Serratia and the fungus is called Mucor.

What we noticed is if you put the bacteria in the middle of a Petri dish and then you add the fungus, this filamentous fungus and the mold, the fungus acts like a superhighway. It actually creates this filamentous network and then the bacteria are able to hop on that network and swim around it and move around it. When we spike in the fungus, when we add that filamentous fungus network, this results in dispersal facilitation. The fungus is helping the bacteria move across the surface. And while this is in a Petri dish, we've actually shown the same thing can happen on the surface of a cheese-like medium.

Additionally, what we noticed is that not all bacteria use fungal superhighways. Some bacteria like proteobacteria can move very far and are able to spread really far on the fungal hyphae. They're using the highway. Versus other bacteria, they don't have a pass to use the fungal superhighway if you will, and they don't move on those fungal networks.

What was interesting to us is that if you add in the fungal networks in a community when you have many species living together, you might expect that you can then promote the growth of these bacteria. The ones that can use the superhighway will be able to get around the cheese more quickly and become the dominant species.

To test this we did an experiment where you mix together four different bacteria, including one called Serratia that uses the fungal superhighway, and then three bacteria that don't use the fungal superhighway. We mixed them together equally, added them to our lab cheese, and then after a couple of weeks of aging — we found that the Serratia takes over pretty nice in the community, but it's not dominant, the other things are still around. If you give the superhighway to this bacterial community it does indeed favor the Serratia: it allows it to get everywhere across the surface of the cheese and really take over the community. This is really fascinating because this is a mechanism of interactions between microbes, facilitation whereby that facilitation can change the entire community composition.

We went on to do some genetic experiments where we were trying to figure out what genes are controlling this cool phenomenon. And it turns out that it's flagella. You need to have functional flagella, you need to have those little bacterial tails to be able to swim along the fungal hyphae and migrate throughout the cheese on these fungal superhighways.

Teamwork in Cheesework

This is just one example of how fungi and bacteria work together on a wheel of aging cheese and allow the bacteria to grow much better. And in general, we think it's a cool example of how pairwise interactions, how microbes coming together, can actually change the entire community.

One thing that is important to think about is that there are some pathogens, like Listeria, which can be problems in cheese making and maybe these fungal superhighways, if you have a pathogen that's motile with these flagella, maybe they can use them to get around. That could actually be a problem.

We've also been really exploring microbial interactions beyond just dispersal facilitation. We have a really cool example of a Penicillium mold, a relative of the mold that brought us penicillin, that kills rind bacteria through antibiotics — completely wipes them out. That's some ongoing work with Nancy Keller.

Another set of experiments in the lab are looking at how bacteria respond to the aromas of cheese. Those aromas are breakdown products of the cheese, the microbes breaking down that cheese curd. And what we're finding is that some of those volatiles can be used by bacteria and stimulate them to grow. So long-distance communication through the smells of your cheese.

We also have examples of fungi providing nutrients for bacteria and other examples where they're really fighting for scarce resources, like metals in the cheese. It really is microbial war and peace in that rind. So the next time you go to a cheese shop, remember it's not just a static wheel of cheese — it's this dynamic microbial community that's right there before your eyes.

Microbiomes Beyond Lab Cheese

We've been working on surface-ripened cheeses, but we have a lot of other projects in the lab looking at sauerkraut and kimchi, a project looking at kombucha and interactions in that system, and a really incredible collaboration with Rob Dunn, Noah Fierer and many other folks looking at the microbial diversity of sourdough starters. And we're using this same approach: trying to understand how dispersal, interaction, and diversification come together to build a microbiome — to learn general design principles that we might be able to use to manipulate microbial communities.

We're doing this for basic science, but we can solve problems. Not every batch of cheese turns out great. You don't really see those batches of cheese because they would never go to the supermarket or to your local cheese shop, but some cheeses just get weird funky defects. And so what we can do is use our understanding of microbiome assembly to address some of these cheese problems. One of my favorite aspects of this job is helping cheesemakers understand the microbial dynamics that are really important for the quality and aesthetics of their products.

Just a quick shout out for our website, microbialfoods.org, where we digest the science of fermented foods. We have a lot of great articles that you might be interested in to learn more about how organic versus conventional agriculture affects sourdough microbiomes. You can also download posters for fermented foods like cheese and salami.