

Companies seek food safety using a microbiome approach

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In a pet food factory in Reno, NV, researchers from IBM Research and Mars, Inc. are conducting an experiment that may someday revolutionize food safety. For more than two years, they've taken regular samples of poultry meal—a protein-rich mash of chicken parts—as it enters the factory on its way to becoming pet food. Each sample of this raw material contains a vast community of living bacteria and viruses—the poultry meal's microbiome—that either originated with the chicken parts or entered at some point along the food processing chain. The researchers then use genetic sequencing to determine the identity and relative quantities of each microbial species.

The hypothesis is that safe batches of poultry meal all have a fairly standard set of microbial residents. A shift in the microbiome, then, could signify that something is amiss—a pathogen has spiked, a toxin is present, or the item labeled poultry is actually from another animal altogether. By routinely sampling the microbiome of this raw pet food material, researchers could pinpoint and stop safety issues before the food

leads to sick pets. And if a microbiome surveillance approach works for poultry meal, it might just work for the rest of the food supply. But if the approach is ever to replace more traditional food surveillance methods, the team must categorize a vast amount of bacterial diversity.

Sequencing the Food Supply

Food suppliers and government agencies such as the Food and Drug Administration (FDA) and the US Department of Agriculture (USDA), regularly monitor food in the production chain to limit the spread of foodborne illnesses. And yet, roughly 48 million Americans get sick from a foodborne illness each year, according to an estimate from the Centers for Disease Control and Prevention (CDC). Part of the problem is that traditional food surveillance methods typically involve detecting individual pathogen species one at a time by culturing microbes found in food samples (1).

These time-intensive culturing methods have been used for more than 50 years, says Bob Baker, director



Researchers with the Consortium for Sequencing the Food Supply Chain ultimately want to explore the microbiomes of food in each stage of the supply chain—from the farm, to the factory, to the supermarket. Image courtesy of Shutterstock/Alf Ribeiro.

of the Mars Global Food Safety Center. “You take samples, you wait for results, you react to results,” he says. “We wanted something more predictive, a preventative approach rather than reactive.”

In 2015, Mars teamed up with IBM to launch the Consortium for Sequencing the Food Supply Chain. The group’s ultimate goal is to harness recent advances in next-generation genomic sequencing to develop a detailed understanding of the microbiomes in each stage of the food supply chain—whether the farm, the factory, or the supermarket.

Working with microbial physiologist Bart Weimer of the University of California, Davis, the Consortium is using whole-genome sequencing (WGS) to create a reference database of pathogen genomes. The team then uses metagenomics to sample the genomes of all the bacterial species in a given food. With the aid of deep sequencing—around 500 million reads per sample—they can detect extremely low levels of microbes.

By comparing the genomic sequences from the metagenomics analysis to the reference database, the Consortium can identify and calculate the relative quantity of each individual microbe in the community, hence highlighting problem pathogens. By focusing on RNA rather than DNA, the team gets a better sense of which microbial species are actively producing proteins and, therefore, still alive. And within this same analysis, the team can also test for genomic material belonging to animals and plants, which could provide direct evidence that, say, fish had somehow ended up in the poultry meal.

Although lacking a precise definition, the term “microbiome” typically describes a living community of interacting microbial species. Food habitats often support particularly rich microbial communities, says Weimer. In poultry meal, for example, the team is finding on average 800 to 1,000 genera of bacteria in a single sample. “That’s as complicated or more complicated than stool samples. Not quite as complicated as soil,” he says.

The poultry meal study attempts to test a microbiome surveillance system that would be applied to the many different stages of food production. The approach hinges on the concept that a food, when safe, should have a microbial baseline—that is to say, a fairly standard microbiome. “When we went into it, I wasn’t sure we were going to see a baseline,” says Baker. “I thought it would change with time.”

But after the team analyzed dozens of samples entering the factory from different poultry meal suppliers over the course of more than two years, a pattern emerged. The identity and relative abundance of each microbe in the poultry meal was similar for most samples. And the samples that deviated were telling. In one case, Weimer intentionally introduced a pathogen as a test. Other members of the Consortium, unaware of the pathogen’s identity, not only accurately identified the pathogen but also showed how it triggered a shift in the types and relative quantities of other microbes.

In another case, a sample with an atypical microbiome tipped off the team to cross contamination,

which sometimes occurs inadvertently when different raw materials share the same shipping and handling systems. A direct analysis of the sample’s animal genomic material confirmed that there was indeed something nonpoultry in the mix.

The microbiome surveillance approach is based on the idea that new microbial species added into the food should compete for resources such as oxygen and nutrients, affecting other species that rely on those same resources and causing a shift in the microbiome. “That was our hypothesis,” says IBM’s Kristen Beck, research technical lead for the Consortium. “But it was excellent to be able to confirm that.” (The team says a publication is forthcoming.)

Right now, these microbiome surveillance tests take about four days to complete. The most commonly used culture-based *Salmonella* tests currently take four to five days according to the FDA, but Weimer says that some labs use methods that can take up to nine days. And the power of the Consortium’s approach, Weimer contends, is that a single analysis gets you “*Salmonella* and the other 999 organisms with it.” Soon, he predicts, the analysis will also tell researchers the specific serotype of each pathogen; traditional serotyping of *Salmonella*, for example, entails an extra step that can take up to another two weeks according to the CDC, or, Weimer estimates, multiple months for scientists who must send the sample away to reference labs. Weimer and his team are currently build-

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ing the genome reference databases so they can confidently reach beyond the genus level.

Next-Generation Food Surveillance

Others are also teasing out microbiome changes in hopes of pursuing problem pathogens. Microbiologist Andrew Benson of the University of Nebraska-Lincoln, used DNA sequencing of the 16S rRNA gene to track the microbiome of refrigerated fresh pork sausage over an 80-day period (2). “We picked up ecological successions—waves of growth of organisms and the demise of others,” he says. In one treatment group in which the sausages contained shelf-life extenders, for example, a sour odor emerged at around day 30, accompanied by a 300-fold increase in the relative abundance of a species of *Lactobacillus*. Benson is now the managing partner of MetaGenome Analytics, LLC, which designs software for interpreting genomic and metagenomics food surveillance data.

At the University of Wisconsin-Madison, plant pathologist Jeri Barak found that tomato plants infected with the bacterial pathogen *Xanthomonas* were more likely to harbor larger populations of *Salmonella* (3). Barak and her team are beginning to categorize the microbiome of healthy versus diseased tomato plants,

part of a recently launched Tomato Microbiome in Sickness and in Health project.

Although government agencies have yet to adopt microbiome surveillance strategies, they are using new genomic techniques that are already starting to revolutionize food surveillance. In 2013, researchers at the CDC teamed up with scientists at other agencies including the FDA and USDA to test whether a WGS technique could help them more rapidly identify *Listeria* strains from infected patients. The approach was so successful that the CDC now uses WGS in its tests for other pathogens as well (4), and other agencies are following suit. "We very quickly built our capacity for whole-genome sequencing," says Uday Dessai, senior public health advisor in the Office of Public Health Science at the USDA's Food Safety and Inspection Service. For now, his group still cultures the microbes one potential pathogen isolate at a time before performing genetic analyses. But Dessai expects his group will turn to microbiome methods once the technology matures.

Putting Research into Practice

If microbiome surveillance is ever to become a legitimate food safety approach, researchers will have to grapple with multiple challenges. For one, there is the deluge of data. "IBM's interest in food safety is, in part, because of its impact on human health through food sustainability and food security," says Beck. "But also because [food safety] is a big data problem." The Consortium has already generated more than 500 terabytes of data. Beck and her team are currently developing a web-based platform to store, process, and analyze this data, laying the groundwork for quick, easy-to-read food safety reports.

But to accurately identify a pathogen from amongst the astounding diversity of microbial culprits, researchers will actually need substantially more data. In 2012,

Weimer launched the 100K Foodborne Pathogen Genome Project in an effort to increase the number of publicly accessible pathogen genomes. In the past five years that number grew from 2,500 to 365,000, with 35,000 genomes coming out of Weimer's lab alone. The group, which consists primarily of universities across the United States, Europe, China, Mexico, and South Korea, is not slowing down. "Genetic variation is excruciatingly high," says Weimer. When he started the project, he thought having 20 sequenced genomes for a single bacterial serotype would be good enough reference. "It's clearly not," he says.

The Consortium also needs to test whether their early findings hold true in different foods and locations. Under a new partnership with Cornell University, which has its own dairy plant and dairy supply chain, the Consortium plans to delve into milk. "It allows us within a very controlled pipeline to use some of the tools we've established already and see how milk behaves," Baker says.

But it's not yet clear how the current regulatory regime would handle microbiome results. Right now, regulations are geared toward culture-based pathogen tests. Does a handful of short sequences of genomic material from *Salmonella*, for example, indicate a public health risk? "Having traces of DNA or RNA of *Salmonella* doesn't necessarily mean it is able to grow or produce toxins, and that is what makes people sick," says Beck. But she and others at the Consortium believe that in time they will learn to integrate this new data into regulatory practice.

"The methods that we use have to be dependable, definitive, and defensible because our actions have a lot of impact," Dessai says. Referring to a microbiome approach, he sounds an optimistic note. "Is omics or metagenomics there yet? No, it's not. Will it get there? Absolutely."

- 1 Weimer BC, et al. (2016) Defining the food microbiome for authentication, safety, and process management. *IBM J Res Develop* 60:1–13.
- 2 Benson AK, et al. (2014) Microbial successions are associated with changes in chemical profiles of a model refrigerated fresh pork sausage during an 80-day shelf life study. *Appl Environ Microbiol* 80:5178–5194.
- 3 Potnis N, Colee J, Jones JB, Barak JD (2015) Plant pathogen-induced water-soaking promotes *Salmonella enterica* growth on tomato leaves. *Appl Environ Microbiol* 81:8126–8134.
- 4 Centers for Disease Control and Prevention (2017) AMD projects: Learning from Listeria. Available at <https://www.cdc.gov/amd/project-summaries/listeria.html>. Accessed August 1, 2017.