For Safer Food, Just Add Viruses

By [Cassandra Willyard] on Wed, 25 Sep 2013

http://www.pbs.org/wgbh/nova/next/nature/phages-for-food-safety/

In March 2012, inspectors from the U.S. Department of Agriculture uncovered a problem in Elgin, Texas. Beef sausage from a small family-run meat processor appeared to have been contaminated with a nasty bacterium called *Listeria monocytogenes*. The bug can make people sick and, in rare cases, be deadly. The processor had to recall more than a ton of sausage. It's the kind of story that strikes terror in the hearts of sausage peddlers, including Mike Satzow.

Satzow owns North Country Smokehouse in Claremont, New Hampshire. It's a small operation; the smokehouse, the packaging facility, and Satzow's office are packed into a low brown building located near the town's tiny municipal airport. Satzow comes from a long line of local meat purveyors. His grandfather, a Russian immigrant, sold meat from a horse-drawn cart before setting up a butcher shop in Claremont in the 1930s. Satzow's father inherited the shop, though Satzow had other plans. "I went away to college," he says. But when he finished school in 1970 there weren't a lot of opportunities. Eventually he took over the family business and transformed it into an upscale smokehouse.

Despite precautions, contamination is still possible. That's why food producers are looking for new ways to ensure safety.

Satzow takes pride in his sausages. He uses pork from a special breed of pigs and marinates it in a slurry of dark maple syrup and spices. Apple wood supplies the smoke. "When you've got a small company like this, you just want to make the best you can make," Satzow says. "It's all about the brand," Satzow says. Chefs trust North Country's products, and a recall could deal a devastating blow to the company's reputation. So *Listeria* is a constant worry. "It's everywhere," Satzow says.

The bacterium thrives in cool, damp environments and resists both heat and salt, tried and true methods for wiping out other pathogens. Satzow cooks his sausages at temperatures high enough to kill off *Listeria*, but a small window of opportunity still exists: "Between the end of the heat treatment step and the packaging, that's where *Listeria* can be introduced into the product," he says.


Mike Satzow uses phages to keep his small company's sausages safe to eat.

Over the years, Satzow has adopted a variety of antimicrobial products and processes to try and close that window. In 2011, he added a new weapon to his bacteria-fighting arsenal, a spray that contains billions of virus particles called bacteriophages—"phages" for short—which target and destroy bacteria, but not human or animal cells. "We try to be on the cutting edge of everything," Satzow says. So now each package of chorizo or smoky maple links that rolls down the smokehouse's spotless conveyor belt gets a squirt of a bacteriophage product called Listex before being sealed.

Inside that liquid are billions of phages that bind to bacteria and inject their genetic material. These molecular instructions direct the cells to make more phages that produce an enzyme that "breaks open the cell wall from the inside out," says Olivia McAuliffe, a senior researcher at the Teagasc Food Research Centre in Ireland. The bacterium bursts and dies, and the phages escape and infect other bacteria.

Doctors began using phages to treat bacterial infections nearly a century ago, but the idea that phages could protect against food-borne pathogens came about in the past decade. "The food industry isn't known for its quick adaptation for new innovations," says Dirk DeMeester, director of business development for Micreos Food Safety, the Dutch company that developed Listex. Yet the idea seems to be slowly gaining traction. DeMeester declined to provide sales figures, but he hinted that business is booming. "Our growth is exponential," he says. "People are starting to understand that it's more than just a good idea. It's going to be an industry standard."

Discovering Phages

Evidence of the existence of phages surfaced in the late 1800s, but it wasn't until the 1900s that scientists began to understand what they were seeing. In 1915, dysentery broke out among French troops stationed on the outskirts of Paris, and Felix d'Herelle, a microbiologist employed at the Institut Pasteur in Paris, went to investigate. When he cultured bacteria taken from the soldiers' stool on a plate, he noticed strange clear spots where bacteria had been destroyed. "I have shown that the disappearance of the dysentery bacilli is coincident with the appearance of an invisible microbe endowed with antagonistic properties against the pathogen," he wrote in a 1917 paper. Similar spots had been observed before, but d'Herrele proposed that these bare patches were caused by viruses and dubbed them bacteriophages.

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By the 1930s, researchers in the Soviet Union were conducting trials to see whether phages might prove an effective therapy for all kinds of infections. When World War II broke out, Soviet doctors used the viruses to treat wounded soldiers on the battlefield. Even scientists in the U.S. experimented with phage therapy. "But people didn't really understand how they worked," says Lawrence Goodridge, a microbiologist at McGill University in Montreal. "Sometimes they could cure people, other times they couldn't."

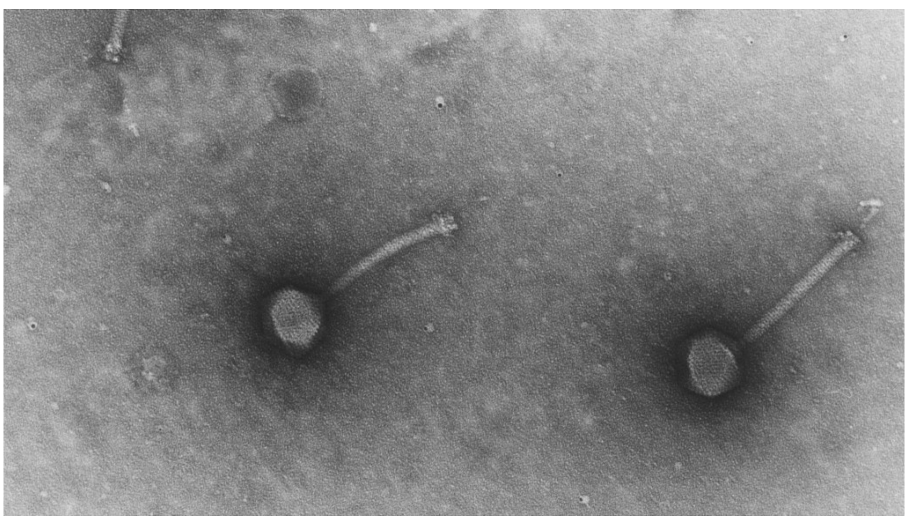
With the advent of penicillin and other antibiotics, however, phage therapy all but died out. “Pharmaceuticals were so much easier to use and predictable,” says Todd Callaway, a microbiologist with the USDA in Texas. In the Soviet Union, however, where antibiotics were costly and difficult to procure, scientists continued to investigate the power of phages.

Importing Old Ideas

In Georgia, a former Soviet state, phages remained a key strategy in the fight against infection. When Georgian microbiologist *Alexander* Sulakvelidze arrived at the University of Maryland Medical Center in 1993, he assumed other countries still used phages, too.

Sulakvelidze came to the U.S. to work with infectious disease specialist J. Glenn Morris, who was grappling with the emergence of antibiotic-resistant bacteria. When Sulakvelidze arrived, Morris told him about a particularly troubling case—a patient with an *Enterococcus* infection that failed to respond to standard antimicrobials. “How come the bacteriophages didn’t kill the bacteria?” Sulakvelidze asked. Morris had heard of phages, but he didn’t know about their therapeutic potential.

Yet Morris found the idea intriguing. The pair began sifting through Baltimore’s harbor in search of bacteriophages that might be useful in the clinic. In 1998, they founded a company called Intralytix, but they made frustratingly little headway. Isolating phages proved relatively easy, but how would they get them into the clinics in the West? Drug development is a costly, risky business even for companies pursuing conventional medications.



A close-up view of bacteriophages

“While we were debating what would be the next proper steps, we got involved with a large poultry company that saw this as a remarkable opportunity to deal with food borne infections,” Sulakvelidze says. “This was a brand new concept.” He and Morris launched a pilot project and shifted the company’s focus to food safety, hoping to make swifter progress. “It’s much more difficult to approve something as a human drug than it is as a dietary ingredient or food additive,” he says.

Intralytix may have fell into its niche because the path seemed simpler, but products to enhance food safety are also desperately needed. While today’s food supply is safer than ever, outbreaks and recalls are still a common occurrence. The U.S. Centers for Disease Control estimates that roughly 48 million Americans fall ill and 3,000 die as a result of food-borne pathogens each year. Farmers, ranchers, food producers, and federal regulators are seeking new ways to reduce the risk of contamination.

Phages have become one of those new tools. Today, Intralytix has three products on the market: ListShield targets *Listeria*; EcoShield curbs *Escherichia coli* O157:H7, a toxic pathogen that grows in the guts of livestock; and SalmoFresh, approved earlier this year, kills some of the most deadly strains of *Salmonella*, a bacterium found on everything from chicken to cantaloupe and spinach. All three have been designated "generally recognized as safe" by the U.S. Food and Drug Administration. Researchers at the USDA's Agricultural Research Service have tested EcoShield and ListShield on both fresh cut lettuce and cantaloupe. Their research suggests that the products work. Micreos, the company that makes the Listex treatment which Satzow uses, is also conducting trials of its own *Salmonella*-killing product: Salmonelex.

The phage products developed by Intralytix and Micreos are meant to be spritzed on food or squirted into the packaging but other researchers aim to deploy phages much earlier in the path from farm-to-fork. Listeria is omnipresent in the environment, but the toxic *E. coli* O157:H7 are mostly confined to cattle, so Callaway set out to develop phages to curb these pathogens at the source.

Phages also have the potential to reduce the use of antibiotics in agriculture.

"This would not be a replacement for anything that's out there currently. This would be a completely new step, a new approach," Callaway says. A few years ago, he isolated several phages that grow well in anaerobic environments, such as animal's guts, and developed phage combinations designed to be fed to cattle in feedlots. These phage cocktails reduced the amount of toxic *E. coli* in the animals' feces, a critical source of contamination in the slaughterhouse. Another company, Elanco, sells a liquid phage product called Finalyse that is sprayed on the hides of cattle before they're slaughtered.

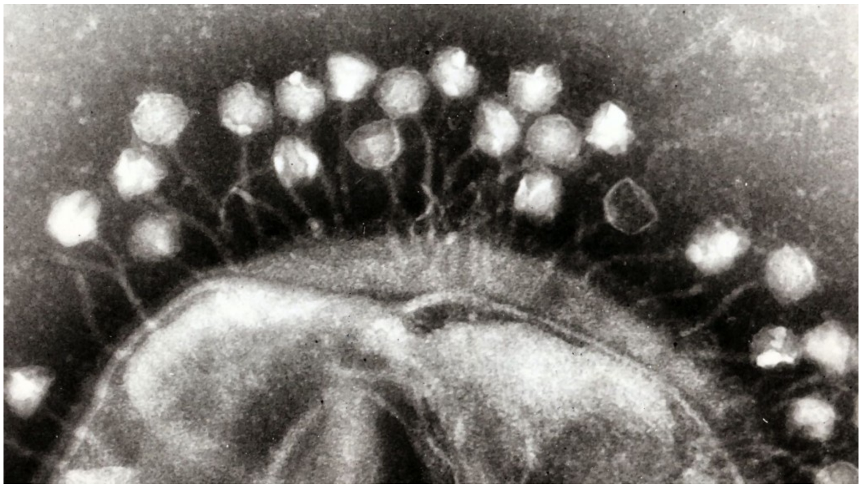
Other companies are using phages to detect harmful bacteria rather than destroy them. The French company bioMérieux has developed a diagnostic test that uses man-made mimics of certain phage proteins to detect *Listeria*. "The phage proteins that we're using are, in a lot of ways, like antibodies," says Stan Bailey, director of scientific affairs for bioMérieux Industry. "It not only recognizes the bacteria, it attaches very tightly." The test is simpler than some other methods being used to detect bacteria, and it's more sensitive, which reduces the amount of time needed to complete the test.

"One of the problems with detecting foodborne bacteria is that they are typically present in very low numbers," says Goodridge, the McGill microbiologist. In many cases, food must be cultured for a day or two in order for the pathogens to reach detectable levels. "By that time, the food could already have been shipped. If it's a fresh food, some people may have already consumed it," he says. bioMérieux's test takes just 18 to 24 hours.

Bacteria Killers

Phages also have the potential to reduce the use of antibiotics in agriculture, but it could be some time before that happens. Food-producing animals receive the bulk of antibiotics sold in the U.S. Producers often administer the drugs to prevent or treat infection. They also frequently give "sub-therapeutic" doses to promote weight gain. "Some pathogens may simply reduce growth efficiency of the animal," Callaway says.

If researchers could find phages that wipe out those same pathogens, they might be able to develop cocktails to help cattle and other livestock beef up faster. Callaway hasn't heard of anyone using phages in such a way, but he says companies are likely investigating the possibility. "That's the holy grail," he says, "to find something as good as antibiotics that's not antibiotics." That would be a boon to both agriculture and medicine, where antibiotic resistance is a growing concern. Just last week, the CDC definitively said that agriculture plays a role in antibiotic resistance.


Phages attack a bacterium

While phages may be finding myriad uses, they aren't a panacea. They can't eliminate harmful pathogens entirely, and bacteria can develop resistance to phages just as they can develop resistance to antibiotics. "Bacteria are very clever," Callaway says. "Once they figure out this guy has the key to my house, they'll change the locks."

To circumvent this, most companies use cocktails of phages. ListShield, for example, contains six bacteriophages. "We have not yet seen resistance," Sulakvelidze says. If they do begin to see evidence of resistance, they can always swap out one or two of the phages in the cocktail. That process is both simpler and cheaper than creating a new antibiotic.

The cocktail has another benefit. It increases the products' killing power. "No single phage will kill, say, 300 strains of *Listeria*. It might kill 100, and then the other phage might kill 80," Sulakvelidze says. By combining all six, "you can kill all of them, or as close to all of them as possible."

One of the biggest challenges to the widespread use of phages seems to be fear of public backlash. Although bacteriophages can't infect human cells, that message may be difficult to convey to consumers. "People hate viruses," says Goodridge. According to Sulakvelidze, larger companies are impressed by how well Intralytix's products work, but worried about the risk.

"I know two or three very large companies that would like to use [our product], but their lawyers tell them to be cautious," Sulakvelidze says. "The backlash is unknown." A 2007 fact sheet from Food & Water Watch, a nonprofit that advocates for safe food and water, calls for clear labeling of products that contain bacteriophages. In the U.S. and Europe, Listex is considered a processing aid, which means it doesn't need to appear on the list of ingredients. Food additives, on the other hand, must be listed.

"Bacteriophages are everywhere in the environment."

Still, researchers remain optimistic, in part because phages offer a new way to keep food safe in an era of antibiotic resistance. Among their numerous advantages, phages are exceedingly specific: the viruses possess tail fibers that only bind with a particular strain of bacteria. That gives producers the power to target only pathogenic bacteria and leave those that don't cause illness unharmed. That's especially important for foods that depend on bacterial cultures to develop—for example, yogurt or some pungent cheeses like Munster. Phages also appear to be safe, as long as the strains are carefully selected. "What you don't want is the phage spreading genetic material around that could potentially make nonpathogenic strains of *E. coli* for example pathogenic," McAuliffe says. That risk can be minimized by choosing phages that can't integrate into the bacterial genome.

Phages have a leg up on other chemical additives and disinfectants which curb bacterial growth because they don't alter a food's flavor or texture. "A lot of the *Listeria* inhibitors are vinegar based," Satzow says. They're effective, but they must be mixed into the sausage. Satzow tried using one such product, but it gave his sausage an off taste. "I've worked hard for 40 years building a really great flavored product. So I don't want to put anything in there that's going to alter the flavor," he says.

Perhaps phages' best selling point is that we already ingest millions of them every day. "Bacteriophages are everywhere in the environment. They're in our water, they're in our soil, they're in the food that we eat" say Manan Sharma, a microbiologist at the USDA in Maryland.

Satzow doesn't hide the fact that he uses phages, and he has received little pushback. His real concern is keeping his sausages safe. The phage spray he uses "is natural, it's organic, and it really enhances food safety," he says. "Once you know that you can employ something that will inhibit the growth of *Listeria*, morally you really should do something."