The Phight for Phage: Understanding Bacteriophage Therapy in Aquaculture and Human Health

by

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ABSTRACT

In the wake of the antibiotic resistance crisis, alternative options to prevent and treat bacterial infections are desperately needed. Researchers across the world are turning to the most abundant biological particle on our planet: bacteriophage. Often called phage, these microscopic viruses infect bacteria, and their high specificity and incredible abundance may make them viable treatment options. Scientists have known about phage for over a century, but renewed interest over the past few decades has spurred a wide variety of research into the biology and applications of these viruses. The benefits, and some of the challenges, of phage therapy for both aquaculture and human health are discussed here.

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I. Finding Cures in Sewers

Viruses could be a weapon in the war against antibiotic resistance.

Ben Chan goes hunting in sewers.

He's not looking for rats or cockroaches or creatures you might find crawling through the wasteways. He's hunting for something smaller and much more common. Chan is searching for the most abundant biological entity on our planet.

Bacteriophage—phage for short—are viruses that infect and kill bacteria. They've been around for billions of years and are found anywhere there are bacteria, which is to say, everywhere: on the surfaces of leaves, buried deep in the Arctic, and in the sewer.

Chan, the scientific director of Yale University's Center for Phage Biology and Therapy, is particularly fond of phage that exist in wet, gross places. He travels the world looking for them in ponds, puddles, and sludge, and the work has taken him from sewage pits in Kenya to muddy streams in Mexico, hauling test tubes filled with water samples back from every destination.

"If you can think of a stinky water source, we probably sampled there," he says.

Chan is searching around the Earth for phage that can potentially kill bacteria that invade human bodies. His team is one of several research groups worldwide that treat patients with phage therapy—a strategy of using these viruses to target harmful bacteria, especially strains that have become resistant to antibiotics, a problem that kills almost 5 million people annually and is estimated to double by 2050.

To study phage therapy and learn more about other uses for these viruses, the Yale team is building a phage library—a collection of hundreds of vials of frozen viruses, many from gross places. By amassing, isolating, and characterizing phage, Chan, and researchers who are forming similar libraries around the world, hope to eventually create a new model for treatment—one where patients with antimicrobial-resistant infections could provide a sample of blood, mucus, or feces to their local phage center, then medical providers could test different virus combinations and create a custom phage treatment that would either knock out the infection or re-sensitize it to antibiotics.

Yale isn't the only place doing phage therapy. The treatment hasn't been approved for medicinal use by the European Medicines Agency or the FDA, but it is allowed in compassionate use cases for patients with immediately life-threatening infections and has been employed with both success and failure worldwide. In some places like Russia and Georgia, phage therapy has been a

component of healthcare since the viruses were discovered. Clinical trials are also underway to test the therapy's efficacy against conditions like cystic fibrosis-associated infections and diabetic foot ulcers, and researchers are studying how to leverage these viruses beyond human health contexts, like for treating sick pets and reducing antibiotic use in agriculture.

In order for phage therapy to work, and to understand exactly how it works, researchers need access to a wide array of phage. For phage therapy to become a reliable weapon in the war against antimicrobial resistance, scientists like Chan will also need to overcome a host of scientific, logistical, and ethical challenges, starting with answering some fundamental questions about phage biology.

"They're like the most prevalent replicating entity on the planet, but we don't even know a fraction of a percentage of them," Chan says. "We're always finding new biology that happens when these guys are around."

Phage Phundamentals

On Yale's Science Hill, the Center for Phage Biology and Therapy is housed in a 100-year-old brick building that looks like an old castle. Inside, the lab is, unsurprisingly, full of phage paraphernalia: journals are scattered across tables, a colorful tape reconstruction of a phage sticks to the door, freezers stacked with Petri dishes contain bacteria in various stages of growth and exposure to phage, while styrofoam wells filled with vials containing sewage samples sit in fridges waiting for phage to be extracted.

The lab's 30 or so researchers are conducting a broad array of experiments that range from isolating phage from the blowholes of beluga whales to making a powdered phage drink mix. Cathy Hernandez, a third-year postdoc, is studying how marine phage are affected by changing temperatures, an early step towards understanding how climate change might impact microbial ecosystems. Other lab researchers are examining whether the nutrient source a bacteria is grown on affects whether phage can infect it, and how effectively different phage attack bacterial strains isolated from the lungs of cystic fibrosis patients.

The lab's director, Paul E. Turner, and his appreciation for biodiversity is responsible for the wide range of phage topics that his researchers explore. "He gives us a lot of freedom to explore the kinds of questions we want," Hernandez says about Turner.

Leveraging phage isn't a new idea. Phage were first discovered in 1915 and were used throughout the early 20th century to treat conditions like cholera and dysentery. But inconsistent treatment outcomes, due to a lack of knowledge about phage biology and the discovery of antibiotics that could wipe out a broad array of bacteria, infectious or not, caused phage therapy to fall out of fashion in Western medicine by the 1950s. However, phage therapy was not cast aside in other parts of the world. Researchers in countries like Georgia, Poland, and Russia all

continued treating patients. The George Eliava Institute in Tbilisi, Georgia has been using phage therapy nonstop since 1923.

As an increasing number of bacterial strains become resistant to available antibiotics, researchers worldwide are looking to phage therapy as a possible solution. Relaunching phage therapy today requires researchers to understand how bacteria evolve antibiotic resistance over and over again and how phage evolve in response. Bacteria can evolve genetically, either from spontaneous mutations—these organisms replicate a lot, which means there are ample opportunities for replication mistakes in their genomes—or through a process called horizontal gene transfer, wherein a bacterium integrates genetic material from other microscopic organisms or the environment into its own genome.

Most genetic changes don't lead to antibiotic resistance, but some do. Genomic changes can cause a bacteria to modify or get rid of its own proteins, leaving an antibiotic without anything to bind to (sort of like changing the lock on a door), or to develop enzymes that either flush out the antibiotic or add chemical compounds that render it ineffective—the biological equivalent of throwing out the key or deforming it beyond repair. Some bacteria nicknamed "superbugs" have evolved so much that they're resistant to most antibiotics, leaving patients without any medical options. Except, perhaps, phage.

"There are people with long-term infections that are extremely antibiotic resistant or close to sometimes even pan-resistant," meaning the bacteria doesn't respond to any antibiotic treatment, says Michael Baym, a microbiologist at Harvard Medical School who studies antibiotic resistance. "In those cases, phage therapy can be lifesaving."

Unlike antibiotics, which essentially bomb the gut microbiome, wiping out infectious bacteria along with beneficial bacteria that aid immune response, phage operate like snipers, targeting problem bacteria and leaving everything else alone. And while antibiotic exposure pushes lots of different bacteria to evolve resistance to a limited number of drugs, exposure to phage is much more individualized: bacteria only develop resistance to the specific phage they are exposed to, leaving doctors with an enormous number of options for different viruses to throw at the problem.

However, there are significant scientific hurdles researchers must overcome to make phage therapy work. One is simply finding the right combination of viruses. Creating a treatment cocktail requires culturing infectious bacteria and testing it against viruses from a phage library, which is why Chan's team searches high and low for viruses that can infect the specific bacteria their patients encounter. If matching phage are available, they can be infused into powders, liquids, or creams that can be inhaled, injected, or applied topically.

Phage therapy often works—Chan ballparks a 90% success rate for the hundred or so patients he's treated since 2013—but sometimes it doesn't or the infection vanishes but returns later.

Researchers can't always pinpoint why, but they suspect that specificity may play a role. Phage's incredible precision—down to the strain level—protects beneficial microbes, but the drawback is that they might not infect all bacteria if multiple strains are making someone sick. Even if providers create the perfect phage cocktail and kill all relevant bacterial strains, lasting results aren't guaranteed: "Bacteria are even better at evolving phage resistance than they are at evolving antibiotic resistance," says Baym.

In the lab, Baym has watched bacteria become resistant to phage orders of magnitude faster than they do for antibiotics, in part because they've had a lot more practice. Bacteria have fought man-made antibiotics for less than a century, but they've spent billions of years dodging and resisting phage.

To address the resistance problem, Chan and others are searching for bacteria-phage interactions with "clinically favorable trade-offs," meaning that as bacteria gain phage resistance, they lose virulence, potentially wreaking less havoc on patients or even making the bacteria vulnerable to antibiotics again. This strategy isn't a universal solution—sometimes bacteria become phage resistant and more virulent—but, Chan says, finding these tradeoffs could hopefully provide better clinical outcomes.

To answer questions about clinically favorable tradeoffs and how and under what circumstances phage therapy can work, researchers need access to a broad array of viruses, all meticulously characterized and organized. Building those collections and making them accessible comes with challenges.

Libraries of Viruses

Carl Merril has been fighting for phage for almost 60 years.

Merril first learned about phage in 1966 while attending a course at Cold Spring Harbor in Long Island that used these viruses to explore evolutionary questions about how life first arose. Then a young physician and molecular biologist at the start of what would become a 43-year career at the National Institutes of Health (NIH), Merril started thinking about a new question: "If these can kill bacteria, why don't we use them to treat infectious diseases?"

Merril couldn't stop thinking about how phage might operate inside a human body, but his colleagues weren't on board. When *Klebsiella pneumoniae*, a drug-resistant infection, swept through one of the clinical wards at the NIH in 2003, Merril saw a prime opportunity to test phage therapy. His colleagues vehemently disagreed.

“I was told that there was a person in the committee who said, ‘Carl Merril, of all people, should have known not to make this suggestion,’” Merril recounts. “After they turned that down, I was so angry, I sat down and wrote a paper.”

That paper, published in 2003, laid out a vision for building libraries of phage, so expansive, varied, and perpetually growing that as infections gained resistance, there would always be a new phage warrior ready to head in. Today, academic labs, including Chan's, as well as biotech companies and nonprofits are isolating phage and building banks and libraries across the world in places like Korea, Israel, and Canada. There’s even a Citizen Phage Library in the UK where anyone can contribute phage by sending in water samples from local streams, bird baths, or garden ponds.

Building a library isn't a simple process—phage must be extracted from a sample, duplicated until there are enough to study, then tested and characterized to answer questions about which bacteria it can infect and if it contains harmful genes. The process can be labor-intensive and time-consuming.

“There’s like a million questions you could ask about that one individual phage and we just don’t have enough human power to do it,” Chan says.

And just building these libraries won’t be enough. To help patients and propel phage research forward, libraries need to share their collections. As of now, there isn't an efficient way to do that, says Vivek Mutalik, principal investigator of the Phage Foundry, a newly funded project by the US Department of Energy that’s developing a platform that would eventually help researchers share phage and formulate phage solutions.

The problem, Mutalik says, is two-fold: first, there isn't a mechanism to quickly connect libraries that have phage with researchers and doctors who need them; second, many institutions can't, or won’t, share.

“You have to go through this multi-month, multi-weeks of material transfer agreements with each institution,” Mutalik says. “Right now, we are really stuck in this slow process.”

At Phage Foundry, Mutalik works to develop ways to characterize phage quicker, helping researchers predict which phage will be effective for a given bacterial problem with greater speed and accuracy. Mutalik hopes to one day share phage through a platform like Addgene, a website that allows scientists to send plasmids (bits of bacterial DNA) to Addgene storage facilities, where they're sequenced, categorized, and made available for researchers worldwide to order.

As of now, no central repository for phage exists and efforts to facilitate connections between disparate collections have also run into issues. Six years ago, Jessica Sacher, then a phage microbiology PhD student, met Jan Zheng, a web developer, at a swing dance class. Together, they founded Phage Directory, a website that sends“ phage alerts” from physicians seeking phage for patients. The site connects over 800 different labs, companies, and researchers, but, Sacher says, there aren’t gold standards or international regulations around how phage should be isolated, categorized, or stored.

“There is a fair amount of debate around what is the optimal safety standard for a phage,” Sacher says. “Is it okay that people are treating patients with phage that are potentially mixtures [or] potentially came from an academic lab and we don’t know much about them?"

While the FDA does set limits on the levels of toxins produced during the phage preparation process and requires labs to check the phage DNA for genes that could be harmful to humans, there is currently little guidance for compassionate phage use beyond those safety metrics. Many academic institutions send their phage to other labs or companies that are better suited to meet FDA requirements.

That landscape could change fast. If clinical trials show that phage therapy is effective, it could bring FDA approval, more funding, and better resources for sharing phage and establishing best practices. Getting there, however, isn’t easy.

The Phuture of Phage

In 2010, the US Navy was facing a pressing issue: soldiers coming home from Iraq were suffering from antimicrobial resistant infections. The Biological Defense Research Directorate (BDRD) hired Biswajit Biswas, a microbiologist formerly in Carl Merril’s lab, to look into phage therapy. It wasn't long before Merril, then retired, received a call from Biswas, asking him to come to Fort Deitrich to talk to the BDRD. Merril asked if they could come to him instead.

Over a series of lunches at Merril’s home, he discussed the phage library ideas he had outlined in 2003. With each lunch, more people showed up until, eventually, Merril was asked to give a lecture to over 200 people.

“I thought, you know, that’s nice that they’re so interested," he says. “It never really dawned on me that they were actually doing everything that I was saying.”

Inspired by Merril, BDRD scientists had been traveling the world, quietly collecting phage and building what would become one of the world’s largest and most comprehensive phage libraries. In 2016, they made headlines when they put their library to use to save a man named Tom Patterson in what became a highly publicized case and later a book that made phage therapy more widely known.

After their success with Patterson, the Navy tried and failed to find a pharmaceutical company to lead a commercialization effort for phage therapy. So soon after, Merril and his son, Greg, a life science entrepreneur, founded Adaptive Phage Therapeutics (APT) and began a collaboration with the Navy, negotiating the rights to their entire phage collection. ATP merged with a biotech company called BiomX last March.

Today, their library holds hundreds of phage to treat highly resistant pathogens. The company stocks phage in about 40 hospital pharmacies nationwide and they receive phage treatment requests daily. APT has successfully treated compassionate care patients, but without clinical trials, they can't gain FDA approval and bring these therapies to the broader market.

"Very few companies have been able to raise the capital to run the trials, which are extremely expensive," Greg Merril says, adding that prices for clinical trials can easily reach tens of millions of dollars.

APT has had ongoing discussions with pharmaceutical companies to fund trials but has not announced any partnerships so far. Greg Merril says that's partially because antimicrobials are seen as risky investments—medical providers only prescribe them when absolutely necessary, and over the years, many antimicrobial companies have gone bankrupt, making it hard to get the funding necessary for clinical trials.

But clinical trials are picking up some speed. Between 2000 and 2015, there were seven trials for phage therapy listed on clinicaltrials.gov; today, there are 39. This might sound promising, but it will still take significant time before a phage treatment is approved beyond compassionate care cases.

"Over and over and over again we've seen signs that the phage is working and we can't say that it's working because you need the clinical trials to say that it's working," says Greg Merril. "It's frustrating when you're sitting on this and you believe that it works and yet you can't make it broadly available."

Merril believes that phage therapy could be approved within the next four to eight years. If that happens, the field will still need to grapple with a separate question: who gets access to this treatment?

"We know that this is an uneven playing field," says Paul E. Turner, director of Yale's Center for Phage Biology and Therapy. "New Haven, Pittsburgh, San Diego, and Houston, Texas are kind of hot spots for where people are working on personalized medicine in phage therapy. If you're not living nearby there and you don't have the means to travel or a physician who even knows of this because it's not happening in their backyard, you're going to miss out."

Turner's lab is working on a paper that addresses ethics in phage therapy and access problems facing ordinary people, an area that he feels hasn't adequately been considered in the field. The Yale team is also experimenting with ways of expanding access to people in low-income countries and remote areas.

In 2016, Chan began a new project with collaborators in Kenya to develop a powdered drink mix that contains a cocktail of phage that target five species of bacteria that cause acute watery diarrhea. Mix with water, gulp it down, and the consumed phage could treat a bacterial infection or prevent one, as phage excreted later on might return to the water source where the infection originated and help eliminate bacteria from the water supply, too. Research is still in early stages, but with help from recent funding from the Gates Foundation, Chan hopes to begin testing the product within two years.

If successful, the project could add to the growing body of evidence that phage therapy can work. Despite the scientific, logistical, and financial challenges facing the field, Chan says that seeing this therapy change patients 'lives keeps him pushing forward.

"Some of these joint infections, you know, they were destined for amputation. It was scheduled already and we did phage as a last-ditch effort and these guys are still walking around," says Chan. "That part I love the most because we're having immediate impact and that's so cool."

II. Fishy Business

Using viruses to prevent aquaculture diseases.

In Turner Falls, Massachusetts, housed in what looks like any other corporate building, hundreds of thousands of fish live in tanks taller than two-story houses. The tanks are so large, workers don scuba diving gear to maintain the hundreds of thousands of gallons of water and fish inside each one, carefully shoving aside curious (and painfully spiny) barramundi in the process.

The tanks belong to Great Falls Aquaculture, a 35-year-old fish farm that supplies barramundi to wholesale seafood distributors. Walker Wright-Moore, the farm's nursery manager, has grown these fish for over a decade, and over the years he's witnessed how disease outbreaks across the farm can cause devastating economic losses. He's also seen how antibiotic treatments for these diseases cause massive problems as well.

"There are a lot of approved aquaculture drugs and treatments for water and fish for parasites and stuff like that, but it affects the biology and the bacterial communities of the water," says Wright-Moore. "It becomes this cycle of treating and you have stressed fish because of it."

Great Falls isn't the only fish farm despairing over disease. As reliance on aquaculture grows—more than three billion people worldwide rely on seafood from farms and capture fisheries for at least 20% of their protein intake—disease outbreaks are also growing and transforming. The Food and Agriculture Organization estimates that disease losses cost the aquaculture industry billions of dollars every year, and the problem will likely worsen as global human populations rise and terrestrial agriculture becomes increasingly unsustainable.

Antibiotics, one of the most common ways of treating and controlling bacterial disease outbreaks, can be mixed into fish food, dumped into water baths, or injected into the fish. They work by killing many different types of bacteria simultaneously, preventing harmful strains from multiplying but annihilating healthy bacteria, too. Since antibiotics were first introduced into aquaculture in the 1940s, bacteria have found ways to fight back: 90% of aquatic bacteria have acquired resistance to one or more antibiotics, leaving aquaculturists with few options for preventing entire tanks from dying off. (A similar crisis is unfolding in medicine for humans and other animals across the globe.)

Now, a handful of fish scientists and human health researchers are taking a more targeted approach to beating antibiotic resistance. Instead of killing all microbes, they're sending in bacteriophage, phage for short—microscopic viruses that infect and kill bacteria, leaving intact protective microorganisms that aid the organism's natural immune response.

Phage are the planet's most abundant biological entity, and because they're so precise and there are so many varieties of them—orders of magnitude more than antibiotics—some researchers believe that phage therapeutics could one day supplement antibiotics or replace them entirely in

certain contexts. In humans, clinical trials are underway to test phage therapy against multi-drug resistant bacterial infections associated with conditions like cystic fibrosis.

Early results in fish are promising, too. Phage products for aquaculture are already available in places like Poland and Norway as liquid additives for pond and river aquaculture, and in Denmark, efforts are underway to create phage products for fish grown in recirculating aquaculture systems like Wright-Moore's.

But there are challenges. Phage therapy doesn't always work. In people, infections sometimes return after treatment with phage. Researchers can't always pinpoint exactly why, but they do know that immune systems could potentially mount a response to these particles and that bacteria can become resistant to phage. For phage therapeutics to become viable alternatives to antibiotics, researchers will need to overcome significant scientific and regulatory barriers.

Antibiotic Overload

The aquaculture industry heavily relies on antibiotics to treat a wide range of infections. One 2020 paper published in Scientific Reports estimated that by 2030, the sector will use over 13,000 tons of antibiotics annually. These drugs are heavily monitored and regulated in the US and most of Europe, but aren't in other parts of the world, including Asia, where 92% of global aquaculture production happens.

"We don't really have a clear sense of how much, or in what context antimicrobial drugs are being used in the industry," says Dan Schar, senior regional emerging infectious disease advisor at the US Agency for International Development for Asia and the paper's first author. "You can see substantial increases year after year in aquaculture production and yet we know very little about antimicrobial resistance or drug resistant pathogens that circulate."

Antibiotics in aquaculture cause problems. Excessive antibiotic use leads to drug-resistant pathogens, which have already been identified in fish diseases like *A. salmonicida* and *Y. ruckeri*. Researchers are also concerned that this resistance could spread to people, too. Bacteria frequently share their genes with other bacteria through a process called horizontal gene transfer. If antibiotic-resistant genes from fish make their way to bacterial strains that live in humans, it could further exacerbate the antibiotic resistance crisis that's already unfolding in human medicine.

"What we want to do is to reduce the dependence of aquaculture on antibiotics," says Pantelis Katharios, a marine biologist who studies phage therapy in aquaculture at the Hellenic Centre for Marine Research in Heraklion, Greece. "One of the most promising agents are bacteriophage."

Researchers believe that phage could be viable alternatives to antibiotics, in part because they're abundant and easy to find, giving scientists lots of options to choose from when battling

antibiotic-resistant bacteria. And, Katharios adds, they could also be deployed in delicate environments where antibiotics would do more harm than good. Aquatic environments are teeming with microorganisms that help recycle nutrients, get rid of organic waste, and do lots of other jobs that keep habitats clean and piscine residents healthy. These microorganisms can also help fight off opportunistic pathogens that are harmful to the fish.

“You have billions of beneficial microorganisms that are helping clean your water and filtering,” says Wright-Moore. “These are very natural things that are consuming and helping clarify the water. I can’t stress the importance of that enough and sort of the shift in thinking of treating the fish and keeping the whole ecosystem healthy and that relies on healthy bacteria, healthy biology.”

These health-boosting microorganisms are particularly important during the larval stage when fish are especially vulnerable. Beneficial organisms protect fragile larvae from pathogens and help them grow robust immune systems. Should an infection break out, antibiotics can damage this microbial community, increasing the odds of other pathogens sneaking in and impeding the development of healthy immune systems that the fish will need once they leave the hatchery.

“If you are using disinfectants or antibiotics in the first stages, you have a problem later on,” says Katharios.

Phage could kill the problematic bacteria without disturbing the larger microbial environment. That’s true inside fish as well. When organisms ingest antibiotics, these drugs bombard the gut, disrupting the microbial ecosystems inside it that help digest food, regulate metabolism and other bodily systems, and aid immune response. In humans, even a short antibiotic regimen can have lingering effects—one 2017 review published in the Journal of Travel Medicine found that taking antibiotics for two weeks or less can disrupt the gut microbiome for a year or more. In fish, studies also show long-lasting impacts on the gut microbiome, even when antibiotic doses are low.

Phage, with their wide variety and specificity, could help preserve protective systems that nature already has in place, but to make these therapeutics reliable and available, researchers will need to overcome scientific and regulatory challenges. In Denmark, one team is trying to do just that.

Across the Sea

In the basement of the Technical University of Denmark (DTU) in Kongens Lyngby lies the DTU Aqua Fish and Shellfish Disease Facility. Biohazard signs feature bacteria warnings and an image of a clownfish wearing a mask. Doors to the facility’s infectious labs, many filled with pathogens and diseased fish, are labeled “quarantine.”

Inside, fish swim in cylindrical tanks the size of large buckets and laminated signs denote the pathogens they've been exposed to. Lone Madsen changes into sterile bright blue foot covers before coming to see her fish. Some of the tanks have been infected with *Flavobacterium psychrophilum*, the bacteria responsible for rainbow trout fry syndrome, a condition that occurs in young trout and causes significant economic loss to fish farmers.

"In small fish, it can result in mortalities up to 80% in a batch," Madsen says. "It's not just a little thing. They don't just get a little cold and then they come over it."

In adult fish, *F. psychrophilum* causes a condition called bacterial coldwater disease, which kills a broad array of commercially important freshwater species, especially in farm and hatchery environments.

Madsen points to her tanks. "I can't see that they're sick, can you?" she says. Fish with bacterial coldwater disease may exhibit pale gills, erosion on the tips of their fins, or loss of appetite, but sometimes Madsen knows if her trout have been infected simply by seeing if there are any dead fish in the tank.

Madsen is a fish and shellfish disease scientist for DTU Aqua and a collaborator on the AQUAPHAGE project, a research initiative that's developing commercial phage products aimed at preventing and treating *F. psychrophilum* infections in fish farms. Led by Mathias Middelboe, a marine biologist at the University of Copenhagen who's studied phage for over 15 years, the AQUAPHAGE team is developing two products—an edible coating for food pellets and a non-edible one for water filters in recirculating aquaculture systems. The goal is to attack *F. psychrophilum* with a one-two punch: by consuming the feed, fish ingest phage that could fight off incubating bacterial infections, and by treating the water, phage can remove harmful environmental invaders while leaving beneficial bacteria alone.

Projected to be completed in 2026, AQUAPHAGE isn't the first commercial phage product for fish. There are a small handful of companies in Norway, Poland, and China with commercially available phage products for aquaculture, but AQUAPHAGE would be the first to treat rainbow trout using a filters and feed combination approach with phage cocktails that are made specially for each farm.

But, like myriad other organizations developing phage products, they have a long way to go. One challenge is finding the right combination of phage, out of the nearly infinite number out there, that will work best for each coating. To do that, Middelboe's team has partnered with a rainbow trout farm about four hours away. The farm sends dead fish, which Madsen screens for *F. psychrophilum*, and water samples Middelboe's team uses to isolate phage and identify which ones are already effectively fighting problematic bacterial strains.

“We would like to have a collection of phage that target bacteria from that specific fish farm,” says Middelboe, adding that by doing so, his team could create treatments that are custom-tailored to individual farms. “I have this idea that we can design or target the phage for a specific community composition.” Middelboe is also working to create phage products that could be used beyond individual farms.

Finding the right phage combination requires significant time and labor on top of the resources required to amplify the cocktail on a commercial scale and test it in Madsen's lab and in fish farms. That's not the only scientific obstacle researchers are struggling to overcome.

Resistance also poses a major problem for researchers. Just as bacteria can become resistant to antibiotics, they can develop resistance to phage, too, rendering phage products ineffective. To address this issue, researchers worldwide are finding, isolating, and categorizing a wide variety of phage with the hopes that should a bacteria develop resistance to one phage, researchers will be able to attack it with another. Middelboe is also looking for instances of phage that result in favorable resistance tradeoffs, wherein the bacterium gains phage resistance, but simultaneously becomes either less harmful to fish or more vulnerable to antibiotic treatment. Middelboe has found examples of these favorable bacteria-phage interactions that work on flavobacterium, but there are other fish pathogens where those interactions haven’t been found.

As researchers grapple with these problems, fish farmers are looking for different antibiotic alternatives.

Beyond Phage

In 2016 in Turner Falls, Massachusetts, Great Falls Aquaculture received a shipment of barramundi eggs from a new supplier. Trying out a new supplier was risky—they’d been using the same one for years and were prepared for the familiar set of pathogens that sometimes tagged along with shipments. They went ahead with the new supplier anyway, and when a batch of the new fish got sick, they fixed the problem with antibiotics, or so they thought. While the immediate infection did vanish, new problems that required their own antibiotics took its place.

“It was not a good cycle,” says Wright-Moore, adding that the system of treating, then retreating went on for two full years.

It wasn’t until Spencer Gowan, now general manager of Great Falls Aquaculture, came back from grad school that the cycle was broken. Gowan suggested that the farm move away from antibiotics and towards vaccines, a disease prevention method he had used at Great Falls years ago but was abandoned when a different manager deemed the practice too expensive and unnecessary.

“It was within a year, less than a year, of starting vaccination again we stopped getting sick fish and we stopped antibiotics,” says Wright-Moore.

Since then, Wright-Moore's team has eliminated almost all antibiotics and instead relies on autogenous vaccines—a type of custom vaccine that’s designed to combat specific pathogens common in Great Falls Aquaculture tanks. The vaccination process takes weeks to do: Wright-Moore and his team inject each fish by hand one by one.

Vaccination works and is standard in some countries with robust aquaculture sectors. Norway, for example, produces more salmon than any other country—about 1.2 million tons per year, nearly all of which are vaccinated against diseases like furunculosis and vibriosis. Since Norway introduced vaccines in 1987, antibiotic use in the salmon industry has dropped by 99%.

But vaccines can’t replace antibiotics entirely. Vaccines take years to develop and often can’t keep up with rapidly changing bacterial infections. Vaccine development for aquaculture is especially challenging compared to other animal production sectors, says Pantelis Katharios of the Hellenic Centre for Marine Research. Where terrestrial animal farming might only need vaccines for sheep, chickens, pigs, and cows, it's pricier and more time consuming to develop vaccines for the extremely wide variety of aquaculture species humans consume, ranging from salmon to octopus to oysters.

“Aquaculture is fragmented,” he adds. “Pharmaceutical companies will not put money in developing vaccines—only for the very important species like salmon.”

Phage therapies could work in tandem with vaccines to reduce antibiotic use even further, but there are some serious obstacles that stand between these therapies and international commercial markets.

“The biggest problem with phage is absolutely on the regulatory side,” says Simon Brink, chief operating officer of ACD Pharma, a Norwegian company that sells one of the few commercially available phage therapeutics for aquaculture.

ACD Pharma’s product, a liquid additive used to control levels of yersinia bacteria in the water environment of salmon, is exclusively available in Norway and was only approved due to a regulatory workaround that classified it as a “biocontrol product”—the same category as pesticides and herbicides, but not for medical treatments for land or aquatic animals.

ACD Pharma can't sell its products in the US or the European Union because phage therapies aren’t fully approved there as of now. That’s partially because phage don't easily fit into a specific treatment category. Unlike traditional drugs, the composition of phage treatments may need to change as bacteria evolve resistance, making them tricky to standardize or regulate. Safety is also

a major concern: because phage are biological entities themselves, researchers can't "sterilize" them from biological contamination. There are also concerns that these viruses may contain harmful genes.

"It's a big puzzle for the FDA or the [European Medicines Agency], for the authorities, how this is going to be authorized," says Katharios.

Phage are, however, working their way into other domestic farming sectors. In 2006, the FDA granted "GRAS" status—a designation for food additives that's short for "generally recognized as safe"—for a phage product created by EBI Food Safety that was designed to treat listeria contamination in cheese products. Since then, 18 more phage products that target conditions like *E. coli* and salmonella have also received GRAS status and are currently available as additives for foods like ground beef, fruits, and vegetables without FDA review or approval.

Raj Odedra, research and development director at Carus Animal Health, AQUAPHAGE's primary commercial collaborator, thinks this is a good sign.

"That isn't a free ticket to use phage as you please, but because there is a precedent, there's a pathway, which makes entry into the American market much simpler," he says, adding that the use of phage to treat food contamination and pest control could be a good sign for aquaculture phage products in the US, which may potentially pave the way for EU approval.

While scientists race to figure out if phage can be a practical and marketable solution to aquaculture's antibiotic crisis, farmers like Wright-Moore are doing what they can to work towards an antibiotic-free future for fish. "Aquaculture is here to stay, in my opinion, and I think it's a good thing," says Wright-Moore. "I think doing it the right way is important."

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