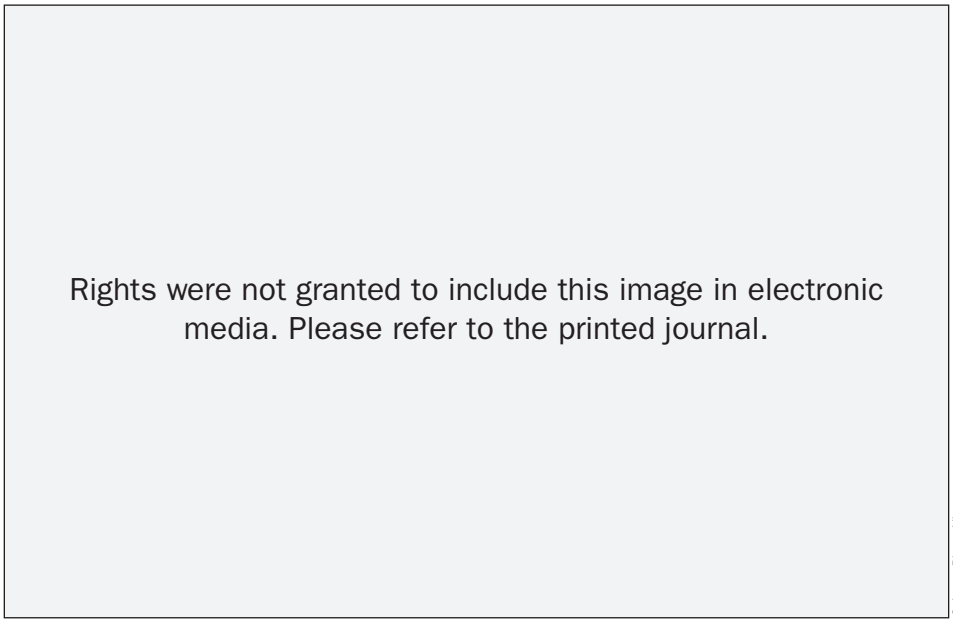
FEATURE

"My enemy's enemy is my friend"

Using phages to fight bacteria

Bacteriophages, viruses that prey upon bacteria, typically attack only a single bacterial strain. This specificity, together with the killing capacity of "phages", says phage researcher Martin Loessner, makes them the "natural enemies" of bacteria. "We are now endeavouring to make this enemy our friend", says Loessner, a professor of food microbiology at the Swiss Federal Institute of Technology in Zurich, turning phages into potentially important allies in our battle against bacteria.

Most types of bacteriophages—the term means "bacteria eaters"—use molecules on their tail fibres to recognise and attach to their target's surface. The attached phage injects its genetic material into the bacterium, in some cases killing its prey at this stage. The phage genes then commandeer the bacterium's synthetic machinery to make phage components, which are assembled into new phage particles that are released when phage-encoded enzymes lyse the bacterial cell wall.



Science Photo Library

A bacteriophage uses spidery tail fibres (orange) to secure itself to the surface of the bacterium (blue)

The idea of using phages to fight bacteria is not new. One of the first descriptions of bacteriophages came from Frederick Twort, a microbiologist at the Brown Veterinary Hospital in London, in 1915. But Félix d'Herelle of the Institut Pasteur in Paris coined the term bacteriophage in 1917 and soon afterwards began treating patients who had bacterial dysentery with an oral phage preparation. From then until the mid 1940s, hundreds of papers were published describing the use of bacteriophages for the treatment of dysentery and other human infections, and commercial companies even offered off-the-shelf phage preparations.

But results were mixed, due in large part to a poor understanding of phage biology. Phage preparations were used against bacteria insensitive to that particular phage or even against diseases that were not caused by bacteria. In some cases, to prevent bacterial contamination, manufacturers added oxidising agents to phage preparations that inactivated the phage. Enthusiasm for phage treatments soon flagged in the west. Everything that could have been done wrong was done wrong during the "first window of opportunity" for phage therapy, says Richard Carlton, president and a director of Exponential Biotherapies in Port Washington, NY, USA, an 11-year-old biotechnology company that is developing phage therapies for multidrug resistant bacteria.

These and other early errors, coupled with the anecdotal nature of clinical research at that time and the discovery of chemical antibiotics, led to the rejection of phage therapy by most western doctors by the end of the 1940s, says phage researcher Carl Merril, chief of the Laboratory of Biochemical Genetics at the US National Institutes of Health. But in eastern Europe and the former Soviet Union, phage therapy continued to be widely used. Thousands of people were treated with individual phages and phage cocktails, many of them made at the Eliava Institute in Tbilisi, Georgia —then the major phage production facility in the world. Phage preparations were used widely against dysentery by the military, for example, or applied topically to infected wounds, such as diabetic ulcers.

Phage therapy turns out to be particularly good in this latter situation, says Betty Kutter, a professor of microbiology at Evergreen State College, Olympia, WA, USA, "because phages infect the bacteria near the surface, and then multiply and go deeper for as long as there are bacteria to infect". By contrast, if antibiotics are applied locally, their concentration rapidly drops off with the distance from the wound surface, and systemic antibiotics won't work because of the poor circulation typical of this type of wound, says Kutter, who has studied phages since 1963.

Mzia Kutateladze is one of the scientists currently working at the Eliava Institute on the molecular characterisation of phages for the treatment of infections. The first step in any phage therapy is to identify the causative agent and check out which phage it is sensitive to, explains Kutateladze. Once that is done, a suitable phage cocktail, which contains multiple phage clones active against one or several bacterial strains, is usually given to the patient, often in combination with or to complement antibiotic therapy. For wounds and skin ulcers, the Eliava scientists have developed a biodegradable wound dressing into which phage cocktails can be incorporated.

The phage preparations developed in Tbilisi have been studied extensively, both preclinically and clinically, say Kutateladze and Kutter. However, little of this information has ever been published and even when details are available, reports rarely meet the full requirements of a clinical trial. Thus, says Kutter, "many US scientists believe that phage therapy has been proven not to work rather than just not proven to work, and they have rejected much that has been done in eastern Europe".

Now though, phage therapy is undergoing a worldwide renaissance, driven largely by the emergence of multidrug-resistant bacteria. "Although many people now believe it works, we still have to have the proof", in the form of extensive double-blind trials, says Kutter. Getting a phage therapy through the regulatory hurdles laid down by national regulatory bodies will not be easy or cheap but Exponential Biotherapies has taken up the challenge and is developing a phage therapy for vancomycin-resistant *Enterococcus faecium* (VRE). In 2002, Carlton, Merril, and co-workers successfully treated a bacteraemia caused in mice by a clinical isolate of VRE with a phage that kills 95% of *E faecium* strains. This phage was well tolerated in a phase I clinical trial and phase II trials should start this year.

Phage researchers are also looking for ways to use individual phage proteins as antimicrobials, particularly in the area of food safety and agriculture. Martin Loessner and his colleagues in Zurich have isolated a lytic enzyme from a phage infecting *Listeria monocytogenes*, a dangerous contaminant of some soft cheeses. This enzyme, which lyses bacterial cell walls, kills more than 99% of listeria cells when sprayed on to cheese. Loessner has put the lysin-encoding gene into *Lactococcus lactis*, bacteria that are used in cheese production, and reports that listeria growth is inhibited during cheese ripening. Carlton, meanwhile, is investigating the use of live phages for a similar application and for clearing campylobacter infections from chickens before slaughter. And Kutter is using live phages to clear *Escherichia coli* 0157 from cattle. If ways can be found to remove these human pathogens from animals, say Kutter and Carlton, it should reduce the need for antibiotics in animal husbandry.

Science Photo Library

Dozens of bacteriophages (blue) can be seen attacking an *Escherichia coli* bacterium

Vincent Fischetti, co-chair of the laboratory of bacterial pathogenesis and immunology at Rockefeller University (New York, USA) is interested in the clinical use of phage lytic enzymes. He has isolated phage enzymes directed against several gram-positive human pathogens, including streptococci, enterococci, and *Bacillus anthracis* and, because most of these enzymes act only against the bacterial species from which the phage was originally isolated, unlike antibiotics they leave normal commensal organisms untouched. Fischetti believes their main use will be in decolonising the carrier states of these pathogenic organisms. If one could treat members of a hospital community with a nasal spray of the enzyme directed against streptococci, he says, the chances of nosocomial infection by this pathogen would be reduced.

"phages infect the bacteria near the surface, and then multiply and go deeper for as long as there are bacteria to infect"

Fischetti has recently tested the lytic enzyme from a pneumococcal bacteriophage intravenously in animals with pneumococcal bacteraemia and is planning trials of a lytic enzyme specific for *B anthracis*. Although this latter enzyme would not affect anthrax spores, Fischetti believes that its use could extend the period of time over which people exposed to anthrax could be successfully treated and would be particularly useful against antibiotic-resistant strains of *B anthracis*. "We hope to do trials in monkeys this year", says Fischetti, "and clinical trials in late 2005 if these studies go well."

All the approaches described above use phage or phage proteins as antibacterial agents. But scientists at PhageTech (Montreal, Canada) hope that bacteriophages will lead them to new classes of antibiotics. The traditional approach of screening large libraries of molecules against bacteria for compounds with antibacterial activity has had limited success in recent years, says PhageTech Vice-President Jinzi Wu, "so we thought, let's use the natural enemies of bacteria to help us to identify their weak spots for target-based antibiotic development". Phages and bacteria have co-existed for billions of years so bacterial proteins that are attacked by phage proteins during infection must be critical to bacterial survival and/or growth, says Wu. If a small molecule candidate could be developed to inhibit these bacterial targets, it might provide a much-needed new kind of antibiotic.

Wu and colleagues collected phages from around the world able to infect *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* and sequenced the phage genomes. Then, polypeptides derived from predicted open reading frames (ORFs) from these genomes were tested for their ability to inhibit bacterial growth by expressing the ORFs within bugs. From *S aureus* phages alone, the researchers identified 31 distinct families of polypeptides that inhibit bacterial growth when expressed in *S aureus* (*Nat Biotech* 2004; **22:** 185–91).

"We used these growth-inhibitory phage ORFs as a bait to fish out their interactive partners in bacteria", continues Wu, and then screened chemical libraries for small molecules that inhibited these bacterial targets in biochemical and functional assays. Several of these small molecules inhibit bacterial growth and are now undergoing lead optimisation, says Wu. Importantly, although the bacterial targets that PhageTech has identified have no obvious human homologues, they are conserved between many bacteria. Consequently, this screening approach has the potential to yield a broad spectrum antibiotic, hopefully before nightmare multidrug-resistant bacteria, such as vancomycin-resistant *S aureus*, become common occurrences in hospitals and clinics.

Jane Bradbury