HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 1 (2022)

Exploring the Hidden Potential of Bacteriophages: A 3-part Miniseries

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ABSTRACT

Bacteriophages are naturally abundant viruses that specifically infect bacteria. These organisms can be found throughout the ecosystem wherever bacteria are found, including in the oceans, on plants, and as part of the human microbiome. Bacteriophage fall into two categories: lytic and lysogenic. Lytic phages have been used for the treatment of various bacterial infections, to the neglect of lysogenic phages. However, lysogenic phages have the potential to act as a vector for bacterial genetic manipulation through the introduction of key genes via horizontal gene transfer. This process would result in the introduction of engineered genetic material to bacterial hosts, for instance to change the behavior of gut bacteria without impacting the human host or requiring complex procedures. Therefore, bacteriophage could be used for the targeted treatment of a multitude of complex diseases, including antibiotic resistant bacterial infections, sexually transmitted infections, bacterial dysbiosis, cancer treatment, and potentially even hormone or metabolite replacement therapy. Similarly, bacteriophages are in use for the prevention and eradication of agricultural pests, e.g. Pierce's Disease, and offer relief from the heavy antibiotic usage by the meat industry. These organisms could even be used to stave off parasite-induced honeybee colony collapse. Indeed, genetic manipulations of bacteriophage could be employed as a novel method to increase oceanic carbon capture, methane fixation, and plastic degradation to reduce the levels of carbon emissions and plastic pollution contributing to environmental toxicity and climate change. This three-part mini review series highlights phage biological processes and the correlated potential areas of application, making an argument for an increase in phage research while presenting the field's current limitations and the future of innovative solutions for some of the most pressing problems of the 21st century.

Bacteriophages and the Medical Field

Bacteriophages have the potential to solve numerous problems related to human health. This article will propose new potential utilizations yet to be researched as well as review pre-existing uses. To understand the importance of phages in public health we must first know what these creatures are and how they work.

Bacteriophages can be found anywhere in nature: in water, on land, and even within human and animal bodies. They are viruses that infect bacteria in order to replicate. Although there are types of phages that replicate inside bacteria without killing the host (the lysogenic phages) the modern utilizations of phages highly revolve around lytic phages: phages that lyse and thus destroy their host bacteria while releasing new phage particles. Phages are very "picky" about their hosts; they mostly infect single bacterial species or a limited number of bacterial strains. In other words, each phage has a limited selection of hosts that they can infect. For example, the Escherichia virus T4 only infects *E. coli* bacteria. This is very beneficial for targeting pathogenic bacteria without wiping out commensal bacteria.

Bacteriophages evolve along with bacteria making it significantly harder for bacteria to develop resistance. A study at University of Pennsylvania discovered that the bacterial immune memory appears to only have space to hold merely a few dozen viral DNA strains. As bacteriophage viromes mutate, they can evade bacterial immune detection. Likewise, genetically engineered phages by definition would not be recognized by the bacterial immune system, thereby creating an opportunity to effectively target specific bacteria for a specific purpose. Let us dive more into some of those purposes here.

Combatting Septic Antibiotic Resistance:

The research and usage of bacteriophages as a treatment for infections is starting to gain traction worldwide. However, innovative uses for phage therapy continues to be used most prevalently in Eastern Europe and the former Soviet Union. There is a good reason why phages are becoming more and more popular within the medical field.

Antibiotic overuse has led to the development of multi-drug resistant (MDR) pathogenic bacteria. As bacteria develop resistance to antibiotics, it is increasingly more difficult to treat their infections. People infected by MDR bacteria or who are incapable of taking antibiotics for health reasons (for instance, if they are allergic to the drugs) cannot depend on antibiotic treatment. This is where phage therapy has been helpful.

Phage therapy takes advantage of the lytic bacteriophage-bacteria interaction. Phages or phage cocktails that target the specific infecting pathogen are administered to the patient. Phages lyse the pathogenic bacteria and reduce infection; the treatment continues until the patient has fully recovered from the infection. Phage therapy is a very effective bacterial infection treatment, which can be inferred from its high success rates.

There is a broad spectrum of pathogen prevention utilizations for phages, including the bacterial decontamination of public spaces like gyms, hospitals, and public transportation, however the methodology of such is still in its early stages.

Phage Therapy for Mucosal & Skin Barrier Infections:

Another area of health where phages could be game-changers is in the prevention and treatment of sexually transmitted bacterial infections, which is crucial for the well-being of sexually active adults and newborn babies. Bacterial infections (ex. *Chlamydia*, *Gonorrhea*, *Syphilis*) can cause severe health problems if left untreated, and on top of that, bacterial STIs increase the risk of acquiring HIV. However, the most concerning characteristic of bacterial STIs is that in most cases they are asymptomatic, which results in a fraction of the people infected being aware of their STI. Left untreated, bacterial STIs can cause severe disease; for instance, *Chlamydia* infections can result in severe, long-term complications such as pelvic inflammatory disease in women that can lead to infertility, epididymo-orchitis, and reactive arthritis. This is particularly concerning as fewer people are reportedly seeking regular STI testing as a result of changes in standard medical care in the wake of the SARS-CoV2 outbreak.

Preventative treatments that can be used topically after engaging in potentially-infectious sexual intercourse may be one way to ensure populational health, especially with the rise of antibiotic-resistant STIs. For example, Chlamydia phages Chp2, Chp3, φCPG1 φCPAR39 (φCpn1), and Chp4 could be utilized as a means of preventative care if a topical ointment with stable phages can be designed and made commercially available. Another STI with similar complications to *Chlamydia* is *Gonorrhea*. The World Health organization (WHO) has raised the alarm to the emerging resistance of *Gonococci* to a number of antibiotics; the particularly high resistance to quinolone is very troubling. Indeed, *Gonococci* are known to rapidly develop resistance to antibiotics and therefore pose a very real threat to global health.

Another potential area of innovation is to use phages to amplify the activity of helpful bacteria that reside on our bodies. The vaginal microbiome, particularly the *Lactobacilli* and the acidic environment that they create, have been proven to significantly decrease *Gonococci* growth, and overall the mucosal microbiome takes a big part in preventing STIs and fungal infections. A new avenue of sexual health that is worth exploring is the use of a process called horizontal gene transfer to enhance the work of the vaginal microbiome and thus decrease the risk of STI transmission. This avenue could be used in conjunction with phage-containing ointments and lubricants for bacterial STI prevention.

In a similar vein, phages could also be used as a means to prevent and treat skin infections, such as acne, wounds from scraped knees to surgical incisions, or bacterial conjunctivitis, particularly for pus-forming infections. As part of pus is made up of bacteria, phages have the potential to decrease the size of pustules and lessen the risk of contaminating the bloodstream. Phage-infused ointments and creams can be used to disinfect wounds or treat acne to lessen the formation and size of pustules. To ensure the safety and efficacy of such topical ointments, further testing is needed both in the lab and in clinical trials.

Phage Usage to Target the Gut Microbiome:

It is well known that antibiotic treatment kills most species of bacteria during treatment, including the beneficial bacteria in the human microbiome. The gut bacteria play an essential role in the body. They take a big part in the digestion process, control hormone levels, help prevent infections, and may help cause or even fight cancer. And in a vicious loop, antibiotics apply selective pressure that permits the survival and growth of antibiotic-resistant bacteria, which can lead to problems like recurrent *C. difficile* infections. In other words, antibiotic treatment can have a multitude of long-lasting harmful effects on the microbiome that negatively impact the health of the patient.

However, bacteriophages do not pose this concern due to their precision. In fact, many species of phages already exist in large numbers in the microbiome, and most appear to be dsDNA Caudovirale and ssDNA Microviridae and Inoviridae. Although the human “phageome” has been poorly studied, there are many known ways bacteriophages contribute to the well-being of gut bacteria, like maintaining species diversity, and there are even more ways in which they could contribute to enhance the actions of the microbiome.

The microbiome holds a wide range of bacterial species. Although gut-bacteria predominantly are beneficial for the human body, there are certain groups of bacteria that produce toxins that could have harmful effects if dysbiosis, a reduction in species diversity, were to occur in the body. When dysbiosis occurs, the efficacy of the main functions of the microbiome in the human body is reduced, which can lead to hormone instability, digestion problems, and more. Dysbiosis is caused by many factors such as an unhealthy diet, antibiotic treatment, alcohol intake, and exposure to environmental pollutants. Childbirth through Caesarian section (C-section) has been linked to dysbiosis and changes in microbiome development in children. Moreover, studies suggest the existence of genetic dysbiosis, a change in the composition of the microbiome caused by specific versions of the human genetic code that alters our body’s response to and recognition of bacteria. As roughly 7.47 million Europeans have gluten sensitivity and/or carry versions of the genes that are thought to cause it, the effects of a gluten-free diet on the microbiota should also be considered. The impact of dysbiosis can be quite severe: certain bacterial species found in the microbiome may increase risk of thrombosis, inflammatory bowel disease, and colorectal cancer. As mentioned before, phages already play a major role in managing species diversity in the microbiome. With their unique traits, phages could prevent dysbiosis and clear the microbiome from overabundant harmful bacterial groups.

Phages as Drivers for Bacterial Engineering:

Indeed, the phenomenon known as horizontal gene transfer, mentioned briefly above, could be key to potentially developing novel medical uses of bacteriophages. Bacteriophages transfer fragments of bacterial genomes from one bacterium to another. This process is called transduction. Ultimately, phages transfer bacterial DNA by mistake.

While replicating inside the host, a phage capsid assembles around a fragment of a bacterial DNA due to an assembly error. When the phage particle containing the pieces of bacterial DNA infects another host, those captured bacterial DNA pieces are then injected and transferred into the new host. As a result, phages play an essential role in bacterial evolution. Although this raises a major concern about the transfer of antibiotic resistance genes to susceptible bacteria, it also opens the door for new creative solutions to various pressing medical problems. Engineering phages with genes to manipulate bacterial genetics could open endless opportunities to enhance the work of beneficial bacteria and re-purpose harmful bacteria within human bodies.

As previously stated, most uses for phages nowadays are highly focused around lytic phages that destroy the host while replicating. However, when working with the microbiome, it is important not to disrupt species diversity and the population numbers of bacteria. Here is where lysogenic phages can come into play: lysogenic phages do not harm the host in which they replicate and therefore provide an opportunity to conduct gene transfer without losing the engineered bacteria via viral lysis, thereby reducing the risk of patient dysbiosis while altering very specific bacterial behaviors.

The human microbiome plays a key role in metabolism and stabilizing hormone levels. Research suggests that bacteria are able to signal for specific cells to produce specific hormones. A study led by Elaine Y. Hsiao and Thomas C. Fung from University of California Los Angeles indicated that a specific group of bacteria, predominantly consisting of *Turicibacter sanguinis* and *Clostridia*, produces molecules that signal gut cells to increase serotonin production. Later studies showed that *Turicibacter* thrives in high serotonin levels, which is why it signals the gut cells to produce this particular hormone. More research has identified that the growth of other bacteria (ex. pathogenic *E. coli*) increases in the presence of certain hormones, and some bacteria are able to produce hormones like dopamine and norepinephrine. Bacteria have even been found to produce mammalian neurotransmitters. Further understanding of the bacteria/somatic cell hormone communication can make possible the tackling of a broad range of endocrine dysfunctions in humans via phage therapy.

Let's imagine a scenario: the estrogen levels in a woman become unstable, which affects her weight and reproductivity and can cause additional health-related problems. The estrobolome, the gut bacteria group that is responsible for regulating estrogen levels, has become less effective at their tasks. This is commonly caused by gut dysbiosis. To solve the issue, we engineer a phage to introduce a gene that enhances the work of the estrobolome. Engineered phages can be introduced into the gut, and through horizontal gene transfer the engineered gene can integrate into the bacterial genome. Following this treatment, the phage-treated estrobolome theoretically works at satisfactory levels and the estrogen levels in the woman's body return to normal baseline.

The same can potentially be applied to any group of bacteria that are linked to hormone production to help treat or hopefully cure associated hormone dysregulation diseases and disorders. For example, ghrelin, so called "the hunger hormone," has various functions related to endocrine and metabolic activity. Being able to control the amount of ghrelin released in the body could help in the treatment and prevention of obesity and eating disorders. Serotonin, estrogen, and ghrelin are merely a handful of potential hormones that could be targeted for medical interventions through engineered phage therapy.

Phages in Cancer Therapies:

By far the most fascinating aspect of bacteriophages is their potential to contribute to the prevention and treatment of cancer. Some bacteria, such as *Fusobacterium nucleatum,* increase the risk of developing colon cancer and of chemotherapy resistance. Indeed, one research group has proposed decreasing or removing the population of harmful bacteria through bacteriophages to decrease the risk of colon cancer development and enhance the efficacy of chemotherapy. While this research is still in preliminary stages, the innovative approach to cancer prevention could prove valuable both for cancer prevention and treatment for countless patients.

Interestingly, although a few gut bacteria increase the risk of cancer, other species may actually help with cancer treatment. With the recent discovery of *Bifidobacterium's* ability to promote antitumor immunity *in vivo*, increasing this specific function of *Bifidobacterium* could enhance the antitumor immune response for more effective cancer therapies. Likewise, gut microbiota species *Enterococcus hirae* and *Barnesiella* have a demonstrated impact on the efficacy of tumor immunotherapy. Horizontal gene transfer could be implemented to engineer the bacteria in order to improve their immunotherapeutic efficacy. Therefore, modifying gut bacteria through bacteriophages could lead to a scientific breakthrough in cancer treatment.

In order to implement the aforementioned proposals, further research is required to better understand the toxins produced by phages as well as long-term phage behavior within the human body. For instance, the human immune response to each utilized phage must be assessed and taken into consideration when choosing a course of treatment. In the countries that use phage therapy as medicine, this therapy either is paused every 21 days or is replaced with a different phage cocktail because the human body starts to develop an immune response to the phage, which can nullify the effectiveness of the treatment. This could limit the mass use of a certain phage for long periods of time or require a specific regimen for medical usage across the board. As well, further study is required to understand how phage treatment affects people who are immunocompromised, in particular people with autoimmune diseases. Even with its current challenges, phage therapy provides hope and excitement on the horizon for novel and effective therapies for some of the most pressing medical problems of our time.

Bacteriophages and Agriculture

Now that we have a sense of how phages can contribute to public health and medicine, let's talk about the opportunities that phages can provide in a different field: agriculture. Phage treatment can be implemented to benefit many aspects of agriculture like the meat industry, plant agriculture, and beekeeping as well as to prevent the spread of foodborne disease.

Agriculture and public health constantly intersect, as public health often is dependent on agriculture, whether that be food politics or foodborne illnesses. While phages in medicine tackle mainly already acquired health problems, phages in agriculture could prevent the development of such problems. There are two specific subsets of agriculture for which phage applications could prove beneficial – animal farming and plant production.

Animal Agriculture:

With the population of the world growing, the demand for animal food products is drastically rising. The animal industry answers that demand by innovating ways of mass production. However, this "efficient and low-cost" production has led to animals being housed in highly densely populated environments, which in turn leads to the rapid spread of various diseases. So far, the meat industry heavily relies on antibiotics to prevent disease and promote animal growth. Moreover, most antibiotics used by these industries are the same antibiotics used for human infections, for example, tetracyclines, an antibiotic class active against human pathogens like *Mycoplasma*, *Chlamydia*, *Pasteurella*, *Clostridium*, and *Ornithobacterium rhinotracheale*. Tetracyclines are used as growth promoters in animal agriculture. Although tetracyclines have been banned from industrial use by the USA and Europe, they are still administered in other countries with fewer regulations on antibiotic use. Likewise, according to 2019 data collected by a survey monitoring the quantities of antimicrobial agents used in animals, the use of bacitracin, a disinfectant for minor skin damage for humans, is authorized for use as a growth promoter in 22 of the 30 countries surveyed.

So what does this mean for humans?

The most pressing problem on a global scale resulting from this overuse of antibiotics is its contribution to the development of antibiotic resistance in pathogenic bacteria. At the moment, bacterial pathogens like *S. aureus* are already resistant to multiple antibiotics, with 83.1% of *S. aureus* strains resistant to penicillin. The data from the previously mentioned survey indicated that virginiamycin, an antibiotic that may be responsible for developing cross-resistance to quinuspristin/dalfopristin in vancomycin-resistant *Enterococci* is used in 13 of the countries surveyed as a growth promoter in animal agriculture. Vancomycin-resistant *Enterococcus* (VRE) infections are listed in the "serious" category in the Centers for Disease Control and Prevention's 2019 Antibiotic Resistance Threats in the United States Report as these infections are resistant to a number of antibiotics. With few effective treatments for VRE infections available, developing cross-resistance to quinuspristin poses a serious global public health threat.

If bacteria develop resistance to the antibiotics used for human medical treatments due to overuse in animal agricultural practices, then the antibiotics will no longer be useful to treat infections in humans. Indeed, one of the few last-resort antibiotics used to treat humans, Colistin, is already in use in animal agricultural practices. The inevitable antibacterial resistance that has started to crop up in various *E. coli* populations internationally has led to a ban on using this class of drugs in livestock in India, although not all countries have followed suit. Thus, the usage of antibiotics in mass meat production is creating a ticking time bomb that will eventually cause an explosion of multi-drug resistant (MDR) bacteria. And with the largest pharmaceutical companies of the world, for instance Novartis, leaving the antibiotic industry due to the high cost and low yield of novel antibiotic classes (no new antibiotic has entered the medical industry since 1987), this can only mean a catastrophe for humanity.

In 2017 the Preservation of Antibiotics for Medical Treatment Act was introduced to the US Congress. This bill took aim at the nontherapeutic use of antimicrobials, including antibiotics, in animal husbandry and meat production. It would require heavier FDA oversight of the off-label use and a requirement than there be no reasonable chance that its nontherapeutic use would lead to antimicrobial resistance. While various political bodies recognize the incredible harm that can be done to human health through the overuse of antibiotics, the bill has yet to be introduced to the floor for a vote in Congress, much less be ratified into law. In fact, no action has been taken in the past three years, leaving constituents to wonder just when such legal protection for the foods we consume will be put into law.

A novel solution needs to be found to prevent a dramatic increase in bacteria-related human deaths. Already, 700,000 people die from drug-resistant bacterial infections each year; with the rise of MDR bacterial strains, this number will skyrocket. The World Health Organization (WHO) predicts that the increase in MDR bacteria will result in 10 million bacterial infection related deaths per year by 2050. To put this number in perspective, 9.9 million people worldwide died from cancer in 2020.

People are starting to look into using phages both for human treatment and also in the prevention of infections in the livestock industry. Using phages in animal food production has multiple critical advantages. Firstly, most phages already exist within nature and are part of the natural microbiome of animals and humans; for example, at least 69 morphologically distinct phages were identified in horse feces, including coliphages that infect *E. coli*. Phages evolved along with bacteria, and thus evolved mechanisms to evade bacterial immunity. Secondly, phage utilization can reduce antibiotic resistance because fewer antibiotics will be used in the process of food production. And lastly, phages are highly specific and therefore significantly less toxic compared to antibiotics both for animals and the humans that will later consume the animal products, which ensures the safety and the well-being of the human microbiome and the liver that metabolizes the drug. Phages can kill bacteria strains that are resistant to antibiotics, so using phage therapy in animal and plant production has the potential to significantly reduce infection rates in humans, particularly for the common pathogens described below.

Plant agriculture:

So what does this mean for preventing plant foodborne bacterial infections?

Plants not only cause bacterial infections in humans when contaminated, but they are also the victims of bacterial infections. As a result, roughly 10% of global food production is lost to plant disease yearly. In conjunction with other types of parasites, there are over 200 plant pathogenic bacterial species that are responsible for crop loss. Similar to meat industries battling bacterial infections, plant agriculture also uses antimicrobial substances to prevent infections, often utilizing human antibiotics that are usually sprayed on plants like pesticides. Antibiotics serve a range of purposes in plant farming, including serving as growth promoters, herbicides, and insecticides.

Data indicate that a number of plant-pathogenic bacteria (ex. *E. amylovora*, *P. Syringae)* that can cause substantial crop loss have developed significant resistance to streptomycin, a commonly used human antibiotic in plant agriculture. While bacteria that infect plants have rare crossover to human infections, it is not outside the realm of possibility that one of these bacteria may become pathogenic to humans. Likewise, bacteria like *E. coli* may grow on crops as a result of their presence in fertilizers; they are also exposed to antibiotics and can develop resistance prior to infecting unsuspecting consumers.

As phages occur within nature, they are an eco-friendlier solution to plant infections. What's more, phages have been found to be more effective towards certain pathogens. Different phages have varying degrees of efficacy against common crop pathogens; for instance, phages 8PD10.3 and 8PD23.1 may reduce the degree of soft rot by 80% on potato tuber slices and 95% on whole tubers. The performance of phage treatment against *Xanthomonas campestris pv. Vesicatoria* was proven identical to the widely used bactericide copper-mancozeb. A series of biocontrol experiments have shown overall positive results on the majority of the studies, although a few treatments required specific phage cocktail formulation or alterations in treatment for enhanced effectiveness.

Industries have already started to look into phages as treatment for plant disease. A recently engineered bacteriophage XylPhi-PD has already been approved for use in organic production and is marketed through Inphatec LLC. In short, Xylphi-PD is already approved for commercial use and can be purchased. The phage targets Pierce's Disease, an infection that affects vineyards across the U.S. The approval of this phage for use in agriculture provides hope and opportunity for other types of phages to be utilized in the same way. Phage utilization in plant agriculture could considerably decrease crop loss caused by bacterial infections. Indeed, this product is only the beginning.

Beekeeping:

An overlooked application for phages in agriculture is the use of phages in place of antibiotics in beekeeping practices. Beekeepers regularly use antibiotics on their hives to prevent bacterial pathogens from infecting bees. In many cases, antibiotics are overused on hives. As a result, honeybee gut bacteria are disrupted, which makes bees more susceptible to infections that are often fatal. This leads back to the main advantage that phages have over antibiotics: their precision. Bacteriophages are unlikely to disrupt the bee microbiome, and their effectiveness against bacterial pathogens makes them a perfect option to prevent or mitigate honeybee infections.

The biggest fear of beekeepers is the American Foulbrood (AFB), a fatal bacterial disease caused by *P. larvae* merciless to bees, even ones with the strongest immune system. It can kill off entire colonies. Due to the lack of available treatment, beekeepers are forced to exterminate the entirety of the infected hive. In 2017, Dr. Sandra Hope proposed the usage of bacteriophages to combat AFB. Dr. Hope stated that phages against *P. larvae* have already been identified and proven by the FDA to be safe for humans and bees. The introduction of phage treatment into beekeeper practices could prevent large colony die-off and negative effects of antibiotic use on bees. Since the phage has been FDA approved to be safe, then phages used against AFB in the US is a very real possibility.

The introduction of phage therapy into agriculture has limitations, and a number of experiments are required in order to identify appropriate formulations of phage cocktails for each bacterial target. That means each phage treatment needs to be tested for its impact on human and animal health prior to its implementation in the field. Indeed, some treatments may even require engineered phage production to adequately address the target bacteria, which could take many years to perfect. While some infections may be relatively straightforward to address with phages, others may take considerably longer to perfect and delay the transition off of antibiotics.

The response of consumers to the use of viruses in food production must also be taken into account. As we know, genetically modified foods are not viewed positively by consumers. A survey conducted in China indicated that 46.7% of the surveyed people had a negative view regarding GMOs and merely 11.9% of those surveyed had a positive view. Similarly held views can be observed in regards to most scientific manipulations of food production. The utilization of viruses in agriculture may be met with a similar distrust. The widely developed negative association of viruses as a result of the COVID-19 pandemic may largely interfere with the utilization of phage treatment in agriculture by consumers. For phage utilization to reach its full potential, it is necessary to educate people on the subject and prove the safety of phages to the public.

Salmonella infections:

According to the CDC, roughly 1.35 million people per year get infected with *Salmonella* mostly acquired from food, resulting in approximately 26,500 hospitalizations and 420 deaths per year. Salmonellosis can lead to complications such as reactive arthritis, and if passed into the bloodstream, it can infect brain tissue, bones, spinal cord, and blood vessels, especially in immunocompromised people, pregnant women, and children. The most common causes of *Salmonella* infections are animal food products such as poultry, eggs, raw meat, and seafood. Excitingly, relevant phages for *Salmonella* treatment have already been identified. Research by Kangwon National University indicated that phages phiSE 7, 16, 18, 36, and 43 are effective against seven types of *Salmonella*, one of them being the most dangerous: typhoid fever-causing bacteria *S. typhi*. Typhoid fever in humans has a high mortality rate ranging from 12 to 30%. For comparison, the mortality rate for one of the most fatal diseases, Ebola, is roughly 50%.

As phages have been proven to be effective against *Salmonella*, their utilization in the poultry industry is essential. Even though people are told to avoid eating raw eggs for fear of *Salmonella* contamination, some common cultural cuisines include dishes containing raw egg. A personal example is a Georgian dish called Ajaruli Khachapuri. The dish is very important to Georgian culture, as it remains one of the main symbols of the Ajarian Region. You can find the dish depicted on a broad range of souvenirs from Georgia, including sock designs. This is a pastry dish resembling a boat which has cheese inside (similar to a cheese bowl). However, the most authentic and traditional way that it is served includes a raw egg poured inside the “bowl” for the consumer to mix into the cheese. A lot of people, especially in the Ajaria region, have this dish as their everyday meal. Given the risk of bacterial contamination and the rise of MDR bacteria, utilizing phages to decontaminate the eggs before they are used in similarly prepared dishes could prevent a major number of Salmonella infections worldwide and provide an opportunity to hold on to cultural authenticity.

E. coli infections:

Another bacteria that cause foodborne infections is *E.coli*. Some strains of *E.coli*, like Shiga toxin-producing *E.coli* (STEC), can cause severe stomach cramps, diarrhea, and vomiting. Other strains can lead to severe complications such as hemolytic uremic syndrome that may result in kidney failure. *E.coli* is found in animal food products as well as vegetables that become contaminated in the process of farming, for instance the application of feces-based fertilizers. Green leafy vegetables and sprouts can be contaminated with STEC from water sources. Decontaminating water sources or using active phages against *E. coli* while washing vegetables could significantly decrease infection rates in humans.

B. cereus infections:

Plants are often the cause for food poisoning. For example, plants can carry bacteria like *B. cereus,* which can cause human infections upon plant consumption. *B. cereus* form dormant spores and are often found in rice, a product consumed on a massive scale. These spores are able to survive the cooking process and may give rise to active bacteria when rice is left at room temperature. Infection from *B. cereus* follows the general symptoms of food poisoning: diarrhea and vomiting.

B. cereus is commonly known as a short-term and largely non-lethal gastroenteric infection. However, the bacterium is able to produce tissue-destroying enzymes and harmful toxins which may be cause for concern. This microbe can invade the GI tract in specific instances, get into the bloodstream, and then spread throughout the body. Once disseminated, the microbe can replicate and colonize parts of the body, for instance a susceptible cardiac valve where its colonization can lead to endocarditis. Immunocompromised people are at the highest risk of complications from B. *cereus* infections. Of the patients with hematologic malignancies that developed *B. cereus* sepsis, one third died within a matter of days, indicating the severity of *B. cereus* sepsis and the foods we eat that make these infections possible.

Taking into consideration that roughly 504.3 million metric tons of rice were consumed through the years 2020/2021, a decontamination method is necessary to prevent unnecessary illness and death. There are a number of phages that are effective against *B. cereus*, like the PBC1 phage. Food product prophylaxis from pathogenic bacteria such as *B. cereus* could decrease food-borne illness infection rates.

Bacteriophages Benefitting Ecology and Environment

Species that play essential roles in world ecology are drastically decreasing in population, the environment is becoming more and more polluted with plastic waste, the oceans and seas are poisoned with chemicals and toxic algae, and global warming is becoming an inevitability - these are all problems that will very soon have a catastrophic impact on the world. Humanity ought to seek solutions for problems that it has created, and bacteriophages and the bacteria they infect have the potential to assist in fixing many of these aforementioned problems.

Bacteriophages and plastic:

It's hard to imagine a modern life without plastics. After its advent, plastic became an inalienable part of our everyday lives. However, only recently has the detrimental impact of plastic on the environment and public health been discussed.

Plastic takes more than 400 years to break down in the environment, and with companies constantly aiming to create more durable plastic, the degradation of these newer plastics may take even longer.

How does plastic affect the environment?

The world produced more than 367 million tons of plastic in 2020, and the number is expected to skyrocket; all of these tons of plastics end up as litter, with only a fraction getting recycled. That litter then escapes into the ocean, creating a threat to the lives of many species. Plastics kill large amounts of animals, including endangered species, either through the consumption or fatal wounds caused by plastics.

How does plastic affect me?

Wild animals are not the only organisms consuming plastic. The plastic that has escaped into the ocean has is broken down into microplastics that can be found everywhere: in food, water, and air – even inside our bodies. Recent research at the University of Victoria estimated that an average American consumes and inhales 74,000 particles of microplastics a year. Some of this plastic contains chemicals such as Bisphenol A and phthalates, which are considered endocrine disruptors in the human body. Controversy surrounds whether microplastics may be carcinogens. With so many concerns about plastic, the decomposition of plastic should take center stage alongside the bacteria and their phages that could be central to decomposition efforts.

In 2016, a team of researchers in Japan was able to isolate *Ideonella sakaiensis*, a bacterium capable of producing plastic-degrading enzymes to consume plastic waste. Although only able to degrade a single type of plastic, polyethylene terephthalate, the bacteria became a worldwide symbol of hope that one day we could decompose all of the plastic waste that has been destroying the environment for decades. This dream may very possibly come true.

To expand upon this possible solution, phage-bacteria interactions and horizontal gene transfer may become key. The process of horizontal gene transfer via bacteriophages has been viewed with some fear, as it is behind the spread of antibiotic resistance in pathogenic bacteria. However, horizontal gene transfer could be key to engineering plastic-eating bacteria to enhance their ability to degrade plastic, expand their application to additional plastics, or control their population to appropriately target plastic waste. Moreover, with plastic-eating bacteria potentially becoming mainstream, a concern arises of the bacteria unnecessarily consuming plastic still in utilization. For example, if the bacteria accidentally get into a technological device, they could consume the device's parts. This may also become a problem for essential items like medicine and food packaging, medical devices, and various parts of vehicles. With so much plastic integrated in our everyday lives, the plastic-eating bacteria population needs to be kept under control. And what better biocontrol can there be than bacteriophages? Engineering phages against plastic-eating bacteria will provide a possibility to create plastic-eating bacteria-free zones and focus the populations on their main task: cleaning our environment from plastic.

Carbon fixation:

Another key component to environmental destruction is the rise of carbon and methane in the atmosphere that contribute to global warming. Of note, the ocean sequesters roughly 25% of the world's carbon dioxide. Interestingly, current phages that target these bacteria actually seem to decrease carbon fixation to some degree by diverting the metabolic pathways of the bacteria to promote virome replication. This process could be targeted via modern genetic engineering techniques to increase carbon fixation and address one of the most pressing problems of our generation.

Likewise, some bacteria are able to perform methane fixation; this process appears to be possible due to genes that originally belonged to bacteriophages that were transferred to the bacteria, and not from the bacteria themselves. So it may be possible to insert these relevant genes into the virome of other phages to introduce into new bacterial species with the goal to expand methane fixation. Importantly, methane is a much more potent contributor to global warming than carbon dioxide, though methane fixation causes release of carbon dioxide into the atmosphere, indicating the value of increasing both carbon and methane fixation capabilities.

Cyanophages against toxic algae:

As agriculture industries are mass producing enormous amounts of food, the utilization of fertilizers has grown significantly. In particular, nitrogen fertilizers often run off into bodies of water, such as lakes, rivers, or oceans and create ecological catastrophes in these waters. A prime example of the catastrophes to be caused by fertilizer runoffs is the state of watery affairs in Turkey in June. The Marmara Sea bordering the largest Turkish cities was covered in brown-gray mucus that reached record levels in size. The responsible flowering plankton and algae, or the so-called "sea snot," is a large threat to marine life. Although most algae have an important role in marine ecology and are not harmful (ex. blue-green algae produce oxygen and intake carbon), large outbreaks of toxic algal blooms are able to kill marine populations like fish, can infect shellfish, and pose a health threat for humans as well as marine mammals. In fact, blue-green algae have been linked to a disease similar to Alzheimer's in dolphins. Likewise, the outbreak of toxic algae known as Red Tide has caused the loss of millions of dollars and shuts down sectors of the shellfish economy each season. But what is even worse, it kills hundreds of tons of marine life: in 2018, Red Tide led to the death of 600 tons of marine life just along the coast of Florida alone.

Global warming and constant water pollution make for a perfect environment for algal growth, and both vastly increase the risk of outbreaks. A method of controlling algae populations should be implemented. Excitingly, such a method may already exist. Cyanophages can be used as biocontrol for blue-green algae outbreaks, which largely consist of cyanobacteria. Using phage/bacteria interactions, algal blooms can be biocontrolled by engineering algicidal *Pseudoalteromonas sp*. As we learn more about phage-algal dynamics and interactions, we can design better and more targeted methods to prevent algal bloom while maximizing the amount of carbon sequestration performed by the very same family of bacterial species.

Wastewater cleaning and filtering water for consumption:

Phages are already used as water pollution indicators because they have many advantages: they are exact and eco-friendly, as most phage types already exist in water. Water ecology constantly suffers negative consequences due to the pollution caused by wastewater contamination. This type of pollution causes toxin build-up and provides the perfect environment for pathogenic bacteria to thrive.

Wastewater contains a lot of dangerous pathogenic bacteria that affect not only marine life, but also humans. A disease caused by contaminated water includes Typhoid Fever, (caused by *S. typhi*) with a mortality rate of 12-30%, Cholera (caused by *V. Cholerae*) with a mortality rate of 25-50% if left untreated, and Bacillary dysentery (caused by *Shigella*), which accounts for 50% of diarrhea-related deaths in some parts of the world.

According to a WHO/UNICEF report of 2019, roughly 785 million people do not have access to clean water. In fact, contaminated water causes more death than war and human conflict each year. The deaths caused by contaminated water due to pathogenic infections reach more than 3.4 million per year. To put these numbers into perspective, an estimated 3.3 million people worldwide died of COVID-19 in 2020.

As bacteriophages are already used as contamination indicators, they also could be used essentially as water filters by lysing pathogenic bacteria commonly found in water supplies. As mentioned in the Bacteriophages in Agriculture article, multiple bacteriophages against *S. typhi* already have been isolated. Similarly, a company called Intralytix has developed a phage cocktail, ShigaShield, that is effective against *Shigella* pathogens. Intralytix also distributes phage cocktails for decontamination of food products from bacterial pathogens. Phages that are effective against waterborne illnesses can be used for decontamination of wells and other sources of drinking water.

Limitations might arise with how the human immune system reacts to a change of phage level in drinking water or how these phages may interact with our gut microbiomes. Further research is necessary to provide insight into novel phage behavior within the human body. But with many species of phages already known that can target specific pathogenic bacteria, we are in a position of power to innovate and to test the limits of just how much we can do with bacteriophages to improve the world and the lives of the billions of people living in it.

Bacteriophages and bees:

The Bacteriophages in Agriculture article mentioned using bacteriophages as a replacement for antibiotic treatment used by beekeepers and a treatment for one of the most dangerous bee infections, however, these are not the only ways bacteriophages could benefit honeybee populations. Honeybees are the world's main pollinators; many plant species depend on bees. If bees were to die off, essential agricultural products would disappear from the market and cause mass economic loss. With the growing demand for food products, the decrease in food availability would drive up hunger rates among the world's growing population.

Aside from the anthropogenic factor, the bee population encounters a number of dangers from parasitic organisms. The varroa mite is one of the most common parasites for honeybees and one of the deadliest ones. Accounting for 85% of colony deaths in a 2009 study conducted in Ontario, Canada, the varroa mite is a dangerous and easily spread threat for the bee population.

A team of researchers at the University of Texas engineered bacteria that produced double stranded RNA that triggered the varroa mite RNA interference immune response that led to mite death. The study indicated that the mites were 70% more likely to die in bees treated with the bacteria. If honeybee populations were to be treated with the bacteria, this could potentially put an end to honeybee die-off from varroa mites and similarly targetable parasites. Bacteriophages could be used to transfer genetic material to commensal bacteria in the honeybee microbiome in order to enhance the bacteria's ability to induce varroa mite death. A global implementation strategy may prove fairly complicated, especially to treat honeybees in the wild. Creativity and ingenuity will be at the forefront of addressing honeybee colony collapse and saving global food production.

Bacteriophages and dolphins:

Similarly to honeybees and global food supply, marine mammals are a cornerstone for the maintenance of the ecology of various water bodies including oceans and seas, and population decrease can have disastrous effects. Dolphins serve a very important role in the food chain and ecosystem. As dolphins predominantly prey on old and ill fish and therefore prevent disease spread within fish populations, these dolphins thus are crucial for the health of marine ecosystems. Indeed, many studies indicate that removing predators from any marine environment leads to adverse consequences, including a large decrease of fish populations that could cause an ecological catastrophe.

However, the waters that serve as a home for marine mammals are getting more and more polluted with pathogenic bacteria, toxic wastes, agricultural fertilizers, and thus, outbreaks of toxic algae. These pollutants impair reproduction and kill dolphins, putting these endangered species at a risk of extinction.

Dolphins, just as humans, are susceptible to pathogenic bacteria. In fact, dolphins and humans often share the same pathogenic bacteria. For example, *S. aureus* causes furunculosis both in humans and dolphins, and *Brucella* causes brucellosis in both species, although rare in humans).

Brucellosis in dolphins is caused by a *coccobacilli* bacteria *Brucella* (mainly *B. ceti* dolphin type and *B.ceti* porpoise type). It affects endangered species such as harbor porpoises, short beaked dolphins, and bottlenose dolphins. For that reason, *Brucella* is a particular concern. The host cells for the bacteria are the immune cells known as macrophages and trophoblasts. As a result, the bacteria can cause meningitis, neurobrucellosis, orchiditis, male infertility, fetus abortions, various types of lesions, arthritis, cardiopathies, chronic illnesses, and in many cases can lead to death. In addition, dolphins with brucellosis have been observed to suffer from disorientation, loss of buoyancy, muscle spasms, and seizures. Brucellosis poses complications in its diagnosis as it appears to be asymptomatic in dolphins until severe pathologies occur.

The research on transmission routes of *Brucella* in marine mammals is scarce, however, it is known that *Brucella* are zoonotic, which means the bacteria can be passed on from different animals, including humans. In terrestrial animals, *Brucella* can be transmitted through sexual intercourse, maternal feeding, and exposure to infected birth products including the placenta, fetus, fetal fluids, and reproductive organ fluids. It can also be passed on from mother to fetus. Unfortunately, there have been no successful reports of brucellosis treatment in dolphins. However, bacteriophages against *Brucella* have been isolated and could be designed to treat aquatic infections. Their use as treatment and prophylactics in marine mammals could be essential for maintaining stable population numbers among these endangered species.

Additionally concerning, a group of Italian researchers recently investigated cases of dolphin strandings and found that all autopsies that they performed identified *Salmonella* within the corpses, a pathogen commonly associated with human foodborne illnesses. *Salmonella typhi*, a serious human pathogen, has been linked to contribute to 10% of 2018 strandings in the Ligurian Sea. The previous Bacteriophages in Agriculture article provides more insight into the potential to prevent *Salmonella* with bacteriophages, including phiSE 7, 16, 18, 36, and 43 that are active against *Salmonella typhi.*

A study in the Black Sea Flora and Fauna Scientific Research Center conducted on Black Sea dolphins showed details on the microbiome of their upper respiratory systems. The most commonly isolated bacteria were *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Acinomyces*, and *Enterobacteriacea*. As with humans, the microbiome can play an important role in preventing infections, although more research is necessary to understand the extent of this role in dolphins. Like with humans, bacteriophages could be implemented as a preventative for microbial dysbiosis among other illnesses. Thus, bacteriophages could be an important tool for promoting biodiversity and the health of various ecosystems in additional to the many possible roles outlined above.

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