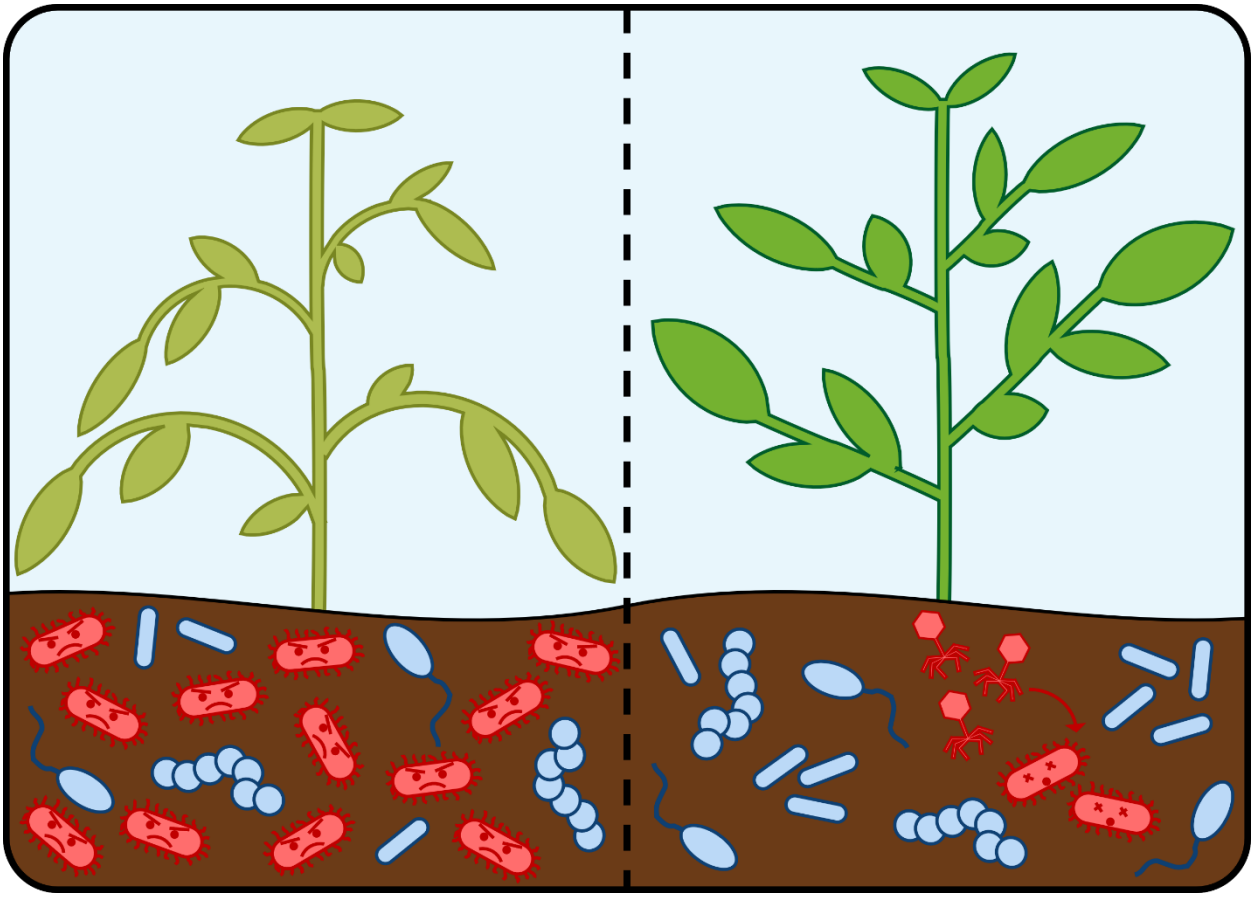
A learner-centric microbiology education framework

Viruses as Biocontrol Agents: Harnessing Bacteria's Natural Predators



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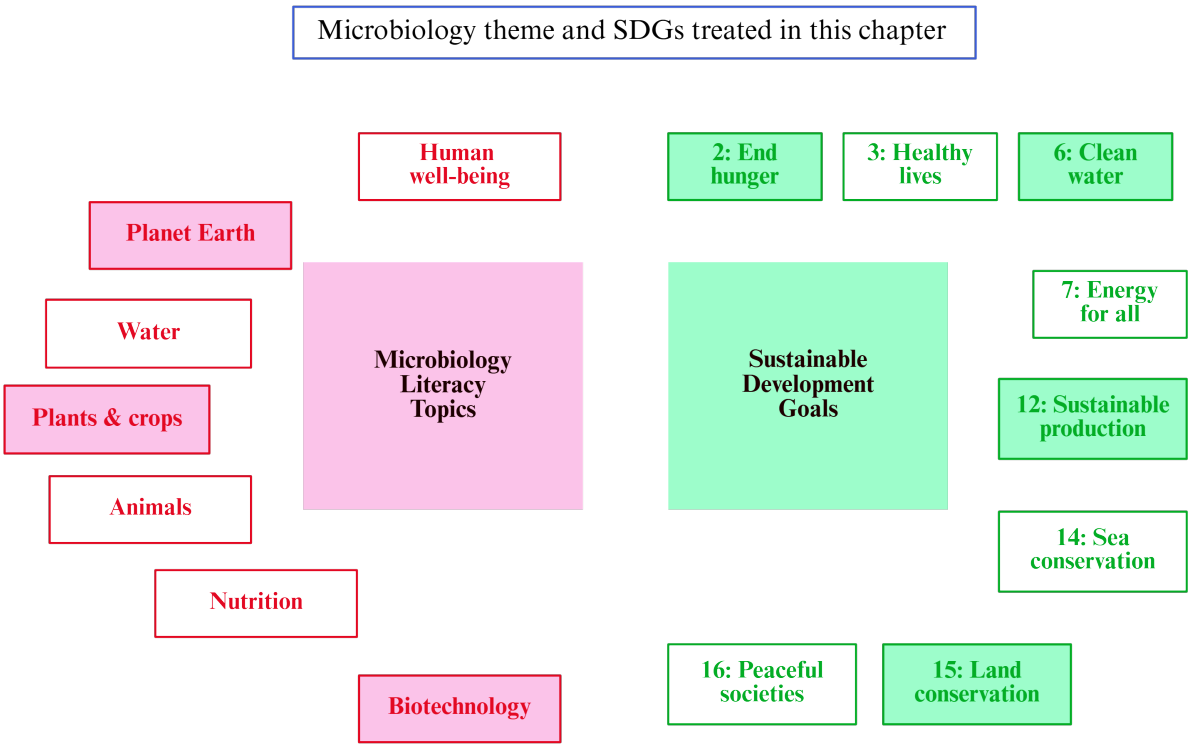
Viruses as Biocontrol Agents

Storyline

Bacteriophages, or phages, are viruses that infect bacteria. After their discovery more than 100 years ago, phages have been used to fight the bacteria that make humans and animals sick. Phages can also be used to fight against bacterial diseases of plants. Plants are regularly attacked by a wide range of disease-causing bacteria. Bacteria attack plants throughout their life cycle, from growing in the field to in storage after harvest. If left untreated, these diseases can be a huge problem for farmers, as they either lose money due to dead plants and damaged produce, or they must use chemical pesticides that can have harmful effects on the surrounding environment. These effects can range from destroying or disrupting the native microbiome, to the spread of antibiotic resistance genes, to harming farm workers and nearby communities. Phages provide a potential method for avoiding these effects because they can be targeted only to the bacterial species that cause disease. Besides being specific, phages also multiply in the presence of their target bacteria, producing more phages to target the bacteria still present. Altogether, phages have tremendous potential to lessen the burden of bacterial disease and increase the efficiency of food production for a growing global population.



The Microbiology and Social Context

Microbiology: Bacteria-infecting microbes and the plant disease-causing bacteria that they infect.
Sustainability issues: Providing food security and reducing the use and reliance on broad-spectrum chemical pesticides. Improving the health and function of plant microbiomes.

The Microbiology of Viruses as Biocontrol

1. What are phages? How are they different from other microbes?

In the 1910s, the scientists Frederick Twort and Félix d'Hérelle noticed a similar pattern – their bacterial cultures were being killed by an unknown "agent". Twort wasn't sure what was causing this issue. He hypothesized it might be a natural stage of the bacterial life cycle, an enzyme produced by

bacteria, or a virus that grows on and destroys bacteria. Meanwhile, d'Hérelle was convinced he had discovered "a virus parasitic on bacteria". He named these viruses "bacteriophages" from the Greek for "bacteria eater". As research on phage continued, scientists realized that they may be used to treat bacterial infections in humans, a treatment called "phage therapy". D'Hérelle and his colleague Giorgi Eliava started a phage therapy research institute in Tbilisi, Georgia, in 1934. While many phage therapy treatments were administered, they were not always successful. At this time, clinical trials were not standardized and lacked key controls. Additionally, antibiotics were becoming more popular in North America and Western Europe, especially as distrust of the Soviet Union grew. For a long time, phage therapy was ignored outside of the former Soviet Union. Now, interest in phages is growing, and new applications are being discovered for phages based on their unique biology.

Living things such as people, animals, plants, and even bacteria can reproduce on their own. Phages and other viruses cannot reproduce on their own – as parasites, they require a host. During infection, phages use the resources of their bacterial hosts to replicate. Instructions for phage proteins (genes) are encoded by nucleic acids like DNA and RNA. The phage carries its own replication instructions (genome) from bacterium to bacterium as it infects. Upon infection of a new bacterial cell, a phage takes over the host, turning the infected bacterium into a phage-making factory. Often, phages will produce proteins that stop the bacteria from using any of its machinery to make its own proteins or even degrade the bacterial proteins it does not need. Phages are very efficient at taking over their hosts. Depending on the phage, they can infect, replicate, and burst from a cell with hundreds of new phages in less than 10 minutes.

2. How do phages live in the environment?

Phages come in a variety of shapes and sizes, but they all have a nucleotide genome made of either DNA or RNA, and a capsid made of proteins. As discussed above, the genome contains the instructions for making new phages. The capsid protects the genome from damage from things like sunlight, heat, or chemicals while phages await a new host. Phage capsids are usually polyhedral (like a 20-sided die) with a connected tail or filamentous (like a piece of thread), although they can have other shapes. To recognize the right type of bacteria to infect, phages also have a receptor-binding protein (RBP) included on their capsid. The RBP allows a phage to recognize bacteria that it can infect and attach to the outside of the cell.

After attaching, phage genetic material (either a DNA or RNA genome, depending on the type of phage) is ejected from the capsid into the bacterium. The bacterial replication machinery replicates the phage genome so that each new phage has its own copy to pass to future hosts. At the same time that the genome is being replicated, phage genes are being transcribed into messenger RNA (mRNA) and translated into proteins. This process also uses the host machinery. As the proteins that make up the structure of the capsid are translated, new phages are assembled. The last phage proteins to be made are those used to damage the host cell walls and membranes. This results in the bacterium bursting open, releasing the new phages into the environment. This life cycle is called the lytic cycle, named for the process of phages lysing their bacterial hosts (Figure 1).

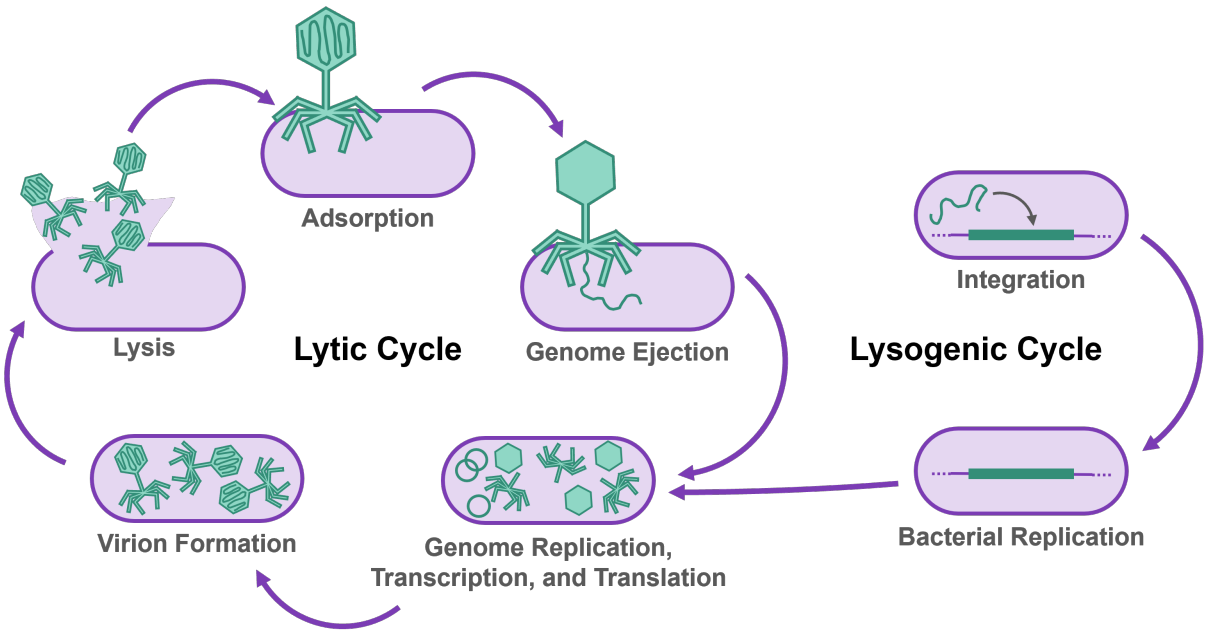


Figure 1. Bacteriophage life cycles. The lytic life cycle starts with phage adsorption to a bacterium and ejection of the phage genome. Using host machinery, phage genes are transcribed into RNA and translated into proteins and the genome is replication. Lytic replication concludes with virion formation and lysis. In the lysogenic cycle, ejection of the genome is followed by integration of the phage genome into the bacterial host genome. The phage genome (called a prophage in this integrated form) replicates as the host

However, there is another major phage life cycle, called the lysogenic cycle (Figure 1). All phages can go through the lytic cycle, but only some, called temperate phages, can go through the lysogenic cycle. In the lysogenic cycle, phages do not start to make new phage capsids. Instead, the phage DNA is integrated into the DNA of its bacterial host. The integrated phage, called a prophage, can stay

within a host for multiple bacterial generations. Each time the bacterium cell replicates, it makes a copy of the phage DNA along with its own bacterial DNA. Eventually, a signal from the environment will cause the prophage to "activate" again. There are lots of different signals that can cue prophages to cut themselves out of the bacterial genome, like changes in resource availability, an increase in bacterial population density, and damage to the phage DNA. At this point, the phage DNA is excised from the bacterial genome, and the phage restarts the lytic cycle right where it left off. The phage DNA is replicated, genes are transcribed into mRNA and translated into proteins, capsids are assembled, and new phages burst out of the bacterial cells.

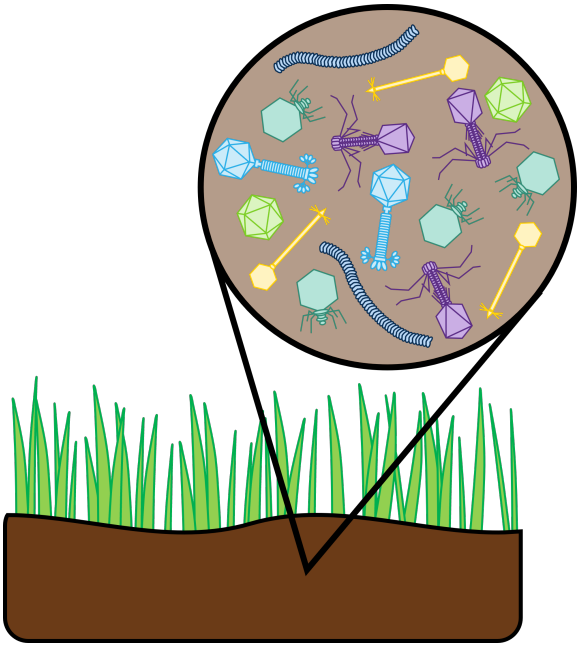


Figure 2. Bacteriophage types. While many shapes and sizes of phage can be found in the soil, some are more abundant than others.

Phages are incredibly abundant in the soil. Scientists estimate that one gram of soil (about ¼ teaspoon) contains between one thousand to one billion phages (10^3 to 10^9), depending on the environment. Many different phage types can be found in the soil, which reflects the differences in their microbiomes. Soil can have different particle sizes, pH, moisture, and temperature. Additionally, different species of bacteria grow in different regions and different types of soil. All of this contributes to a high diversity of phages in the soil. The presence of plants in soil can increase the phage diversity even more because they provide nutrients and a niche for bacterial growth. When bacteria are more abundant, the phages that infect them also have more opportunity to grow.

3. Why are phages good biocontrol agents?

Scientists have found that phages can be used to fight disease on a variety of plants, including potatoes, tomatoes, citrus, grapes, and many more. Several aspects of phage biology make them potentially effective and environmentally friendly biocontrol agents.

1. Specificity: In contrast to antibiotics and chemical pesticides, which tend to affect broad spectrums of microbes, phages are much more limited in what they can attack. In general, phages can only infect a small number of potential hosts. Their RBPs recognize their preferred host and do not attach to unrelated hosts. Therefore, phages will only kill the type of bacteria they are meant to target, reducing the damage to the native microbiota, which may contain beneficial bacteria.

2. Lack of effects on eukaryotes: Antibiotics and chemical pesticides can sometimes be toxic for animals and plants. Therefore, we must be careful of the dosage or accept any negative effects. Phages, in contrast, cannot infect human or plant cells. This reduces the potential for negative side effects.
3. Amplification in the environment: When phages infect and kill their hosts, they also replicate. If a phage encounters their target bacterial host, they not only kill the bacteria cell, but also produce more phages, allowing them to seek out and infect more hosts. This increases the potential effects of phage treatments and means that they can persist in the environment if their host is present, reducing the amount of treatment that is needed.
4. Self-limitation: Phages will only replicate and persist if their host is present. If the target disease-causing bacteria are not present, the phage will die out, reducing the possibility of treatments lingering in the environment.
5. Evolution: One of the greatest challenges for conventional control of bacterial diseases is the evolution of resistance. Bacteria can adapt and become resistant to chemical treatments, leading to serious global problems like the emergence of antibiotic resistance. Because phages are also microorganisms, they can evolve, too. For example, if bacteria evolve resistance to phage (see next section), phages have the potential to counter this resistance with additional adaptations that allow them to regain infectivity.
6. Ubiquity: Phages are common in the environment, and more phages are being isolated every day. This provides an enormous resource to develop further phage treatments.

All these advantages for phages are important, but it is also important to consider the potential downsides of any potential biocontrol agent. What are the potential downsides of phages?

1. Specificity: A highly specific phage could have little or no effect if the bacteria that is causing the problem is not the correct host. Using phages for treatment requires specific knowledge of the bacterial species that is causing the given disease, in contrast to conventional antibiotics which can kill a wider range of bacteria.
2. Self-limitation: Phages are vulnerable to many things in the environment. Phages can be killed by extreme temperatures, drying out, UV radiation from the sun, and a variety of other environmental factors. Low phage survival in the environment can limit the efficacy of phage treatments.
3. Evolution: While some phage evolution is beneficial (as detailed above), evolution is not a directed process, and we cannot control evolutionary outcomes. Just as a phage can evolve to become more effective, phage could just as easily evolve to become less virulent. For phage, if they can survive longer without killing their host, they could produce more offspring, potentially increasing their fitness without controlling the bacteria. An example of this is lysogeny, where phages coexist with their bacterial hosts without lysing them.

4. Ubiquity: Phages are everywhere, which means that bacteria have lived with, evolved alongside, and developed strategies to escape phage for millions of years. This means that, if we want phage to effectively control plant diseases, we must carefully choose our biocontrol agent and design our treatments to consider their past evolutionary history.

4. Phage and bacteria coevolution

As discussed above, phages are very common and diverse in environments like the soil. For bacteria that also live in these environments, this provides a challenge. Bacteria must avoid, escape, or fight off phages that can infect and kill them. This provides pressure to direct bacterial evolution. Presumably, this evolution has been occurring for as long as phages have existed. We can observe the evolutionary race between phage and bacteria through analysis of their genomes. On the bacterial side, one way we know that phage have been important for bacterial evolution is the presence of phage defense systems in bacterial genomes. These defense systems are genes that encode proteins that attack or defend against phage. Most bacteria encode many of these defense systems, which shows that having a diverse defense toolkit has been important for bacterial life. These defense systems are also important for humans, as many of them have proved to be extremely useful tools for biotechnology. Some defense systems have revolutionized genetic engineering and are providing new ways to improve human health and society. For example, CRISPR, often discussed for its gene-editing applications, is a type of phage defense system used by bacteria to destroy phage DNA. More and more defense systems are being discovered every day, and they use many different strategies to target phage DNA.

In addition to phage defense systems, bacterial genomes also contain many phages. As described above, some phages can incorporate their genomes into bacterial genomes in a process called lysogeny. While these lysogenic phages can sometimes reactivate and reenter the lytic cycles, others stay as prophages for long periods of time or have even lost the ability to excise. Most bacterial genomes contain many of these prophage regions, providing a genetic record of phage infections that occurred in the distant past.

In phage genomes too, we can observe the evolutionary race between phage and bacteria. Just as bacteria encode defense systems, phage encode anti-defense systems, which counteract defense systems and allow phage to successfully infect. Some phages have even stolen defense systems from bacteria, and can use them against their hosts!

Sustainability Impact of Phage-Based Biocontrol

Phage biocontrol could offer a sustainable, environmentally friendly alternative to chemical pesticides. Phages would likely be less damaging to the environment, the soil microbial community, and farm workers. They could allow farming practices to be less impactful while also providing more adaptive methods to fight against important plant diseases.

Pupil Participation Activities

- **Watch a video to learn more about viruses, including phages:** Video is also available in Spanish and Portuguese (links to each in description of the original video).
    - *What are some similarities between phages and viruses that infect animals or plants? What are some differences?*
    - *The video mentions that using viruses to target pests can have harmful consequences to the ecosystem. What are some potential downsides of using phages as biocontrol?*
    - "Viruses (Updated)" by Amoeba Sisters on YouTube: https://www.youtube.com/watch?v=8FqlTslU22s
- **Visualize phage infection using an epidemic simulator:** Adjust settings to reflect bacteria in different environments. This tool was originally made to model human epidemics, so some variables may make more sense if renamed. For example, "village" could mean "bacterial density". The simulator is not compatible with Internet Explorer 8 but does work on mobile devices.
    - *What do each of the adjustable factors mean in the context of phage infection?*
    - *What factors can you change to make the phages infect bacteria more quickly?*
    - *What factors can you change to best protect the bacteria?*
    - *How could you design a biocontrol method to be most effective?*
    - Epidemic Simulator by Tachyondecay https://tachyondecay.github.io/epidemic-simulator/
- **Design and build your own phages:** Students can design their own phages and test their ability to bind different types of bacteria using building materials like tape, string, toothpicks, pipe cleaners, *etc.* Instead of giving students a goal of being able to attach to all "bacteria" models, assign the models to one of two categories: beneficial bacteria and harmful bacteria. Students should aim to design phages that bind the harmful bacteria but leave beneficial bacteria uninfected. Consider modifying the activity difficulty level based on student age.
    - *Why do phages only bind certain bacteria and not others? How does this affect their replication?*

    - *How can bacteria protect against phages binding them? How might phages overcome bacterial protections?*
    - *Phages in nature are very diverse, and many unrelated phages can infect the same bacterium. Choose 2 different phage models from this activity that both attach to the same "bacteria". What is different about the way in which they bind the bacterial model?*
    - Bacteriophage Builder Challenge, developed by the Alvarez Lab at Rice University with the mentorship of Pingfeng Yu and guidance of Christina Crawford: https://www.teachengineering.org/makerchallenges/view/rice2-2501-bacteriophage-virus-bacteria-builder-challenge
- **Dive deep into phage research:** For groups with available time and resources, check out the HHMI Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program. Programs are generally organized for high school and community college level students in the United States. However, their resources are publicly available online. Students isolate novel phages from soil samples and characterize them as far as time and resources allow. Depending on the bacterial host used, this can be done in a BSL-1 level environment. Safety details should be coordinated with your institution.
    - SEA-PHAGES Homepage: https://seaphages.org/
    - SEA-PHAGES Phage Discovery Guide: https://discoveryguide.seaphages.org/

Further Reading

History of Phage Research

https://asm.org/articles/2022/august/phage-therapy-past,-present-and-future

https://pmc.ncbi.nlm.nih.gov/articles/PMC3442826/

Importance of Phages in the Soil Microbiome

https://pmc.ncbi.nlm.nih.gov/articles/PMC11065364/

Experiments on Phage Biocontrol (Review Article)

https://pmc.ncbi.nlm.nih.gov/articles/PMC5247434/

Glossary

Biocontrol – The use of one organism to kill or limit another (usually a pest or disease-causing organism that humans want to avoid)

Capsid – The 'body' of the phage. Made of protein, the capsid forms a protective shell around the phage genome

DNA – The genetic material used by most organisms to encode all the genes that make up the genome

Genome – The collection of all the genes and other genetic material necessary for an organism to live and replicate. Can be composed of DNA or RNA

Host – An organism that serves as the environment of a smaller parasitic organism. The parasite takes its energy and nutrients from the host organism

Lysis – The breaking of a cell to release its contents. In the context of phage biology, lysis is the process where the phage, after having replicated themselves, break out of the host bacterial cell and are released into the environment

Lysogeny – When a phage, instead of reproducing itself and lysing the host bacterium, hides its genome in the host genome, where it can be carried along inside the host as it grows and divides

Lytic cycle – The process by which a phage reproduces and breaks out of its host through lysis

Parasite – An organism that lives on and depends on another organism for its nutrition and home. Typically, parasites also harm their hosts as they live on them

Phage – A virus that infects bacteria

Phage defense system – A method used by bacteria to detect and destroy invading phages. Typically composed of one or more genes that make proteins that are used to find phage proteins or genetic material, and then either kill the phage or kill the bacteria itself to prevent the spread of the phage

Protein – Large molecule composed of amino acids that can be used for many jobs within a cell. Proteins can, among other jobs, form the structure of cells and viruses, move material around the cell, help chemical reactions occur, or allow the cell to sense and communicate with their surroundings

Receptor-binding protein (RBP) – Phage proteins that attach to specific molecules on the outside of their hosts. This is the first step in the phage's infection of its host

RNA – A biological molecule very similar to DNA. RNA is most commonly used to translate information from the DNA genome into proteins. However, some viruses use RNA as their primary storage molecule for their genome instead of DNA