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Popular Article

# Bacteriophage Therapy

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## Introduction

A Virus that infects bacteria and replicates within a bacterium. Bacteriophage is derived from Greek, Backerion means bacteria, and Phage means to eat. Obligate intracellular parasites that multiply inside bacteria by making use of all the host biosynthetic machinery. It carries only the genetic information replication of their nucleic acid and synthesis of their protein coats. They require precursors, energy generation, and ribosomes supplied by their bacterial host. Phage genome+ bacterial chromosome = phage conversion.

## Types of Phages and Biology

The morphological descriptions and discussions of 6196 bacterial and 88 archaeal viruses included about 6000 distinct bacteriophages. While the majority are tailed, a tiny percentage are polyhedral, filamentous, or pleomorphic. Their morphology, genetic makeup (DNA vs. RNA), and preferred hosts can all be used to classify them. Phages display different life cycles within the bacterial host: Lytic, Lysogenic, Pseudo-lysogenic, and Chronic infection. For phage therapy, mainly lytic phages were preferred which represented 3 families of the Caudovirales order: The Myoviridae, The Siphoviridae, and The Podoviridae (1).

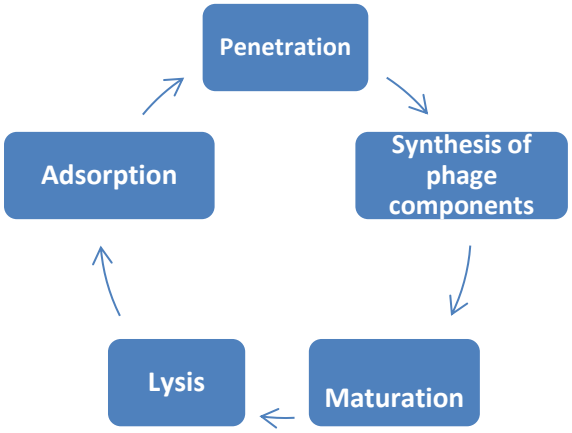
## Structure

A capsid, a protein covering that encases the genome, is what makes up a bacteriophage. Its shape options include icosahedral, filamentous, and head-tail. The bacteriophage has a helical tail, a short collar, and a polyhedral head. 2000 capsomeres that are double-stranded DNA-enclosed make up the head. Tail: The tail is composed of an inner hollow tube that is

encircled by a contractile sheath that has 24 annular rings. The majority of phages have a length of 24–200 nm. From the head to the tail's tip, it goes on, forming a canal. Nucleic acid invades the host cell as it travels through. The protein sheath is hexagonally shaped and spirally coils around a thin disc-like structure called the "collar" at the base of the head and a tail-end "end plate." Small "spikes" at each of the hexagonal plate's six corners are attached to very long fibers known as "tail fibers."

**Life-Cycle (Multiplication or Infection Cycle)**

1. **Lytic Cycle**: The process can be classified into




2. **Lysogenic Cycle**

Phage follows the same process up to penetration, after that it exhibits its infection strategy. Phage enters into a symbiotic relationship with the host cell, temperate phage nuclei acid becomes integrated with bacterial chromosome. Integrated phage nucleic acid (PROPHAGE). Prophage acts like a segment of the host chromosome and replicates synchronously with it.

**Isolation of bacteriophage**

Samples were taken from many sources, including river water, municipal sewage treatment plants, hospital sewage wastewater, soil samples taken from outside the hospital, marine water from coastal regions, and fermented foods such as kimchi, shrimp, and sauerkraut. To isolate bacteriophages, the collected water or soil samples were processed right away. They may have been filtered through 0.45-micron filters and kept at 4°C. The phage enrichment technique uses a 1 ml bacterial culture with an OD600 of 0.6. 9 mL of water samples were added, and they were shaken at 120 rpm for 24 hours in a 37°C incubator. After enriching the bacteria with bacteriophage for 24 hours, the supernatant was collected after a 15-minute centrifugation at 6000g. At least three milliliters (ml) of phage filtrate from each



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sample was collected after filtering using 0.22-micron syringe filters. The filtrate was stored at -20°C until further use.

**Spot Test**

The sample and host bacterial culture are combined in a certain volume, and then the mixture is incubated with vigorous shaking for 24 hours. Filtering to decontaminate a fraction, then centrifuging it. Prepare a confluent monolayer of the host bacterium, place a drop of around 10µ l on top of it, and incubate. Lysis confirms the presence of phages in the tested sample, whereas no lysis indicates the lack of phages in the tested sample (2).

**Double-Layer Agar Test**

Phage lysate, bacterial culture with an OD 600nm of 0.5, and soft agar (0.7%) are added to agar plates with 1.5% agar, which is then gently mixed. After incubation, solidification at room temperature and visualization of phage plaques on the bacterial lawn follow.

Choose a single phage through phage enumeration after the double-layer agar test, concentrate it, and then perform a transmissible electron microscope analysis on it by staining it with uranyl acetate (3).

**Phage Therapy**

The most prevalent bacterial predators in nature are phages utilized in the Soviet Union since the 1920s and still it is in use today in former Soviet states including Georgia, Poland, and Russia. Phage therapy as an antimicrobial agent has emerged as a possible treatment for bacterial infections that are persistent and resistant.

To ensure the lack of potentially hazardous genes, such as virulence factors, toxin-producing genes, and antibiotic resistance genes, as well as to establish phage phylogeny, the bacteriophages present in the banks should be thoroughly described. This includes whole genome sequencing. Bioinformatically testing a bacteriophage's lysogenic potential or just its lytic potential. There are recommended phage purification techniques and Good Manufacturing Practices (GMP) for phage manufacturing and quality control. These purification procedures involve testing the finished products to determine the bacteriophage's identity, viability, potency, and purity (i.e., the absence of bacterial or chemical residues) (4)

**Strategies for Phage Therapy**

**1. Phage Cocktails** – A combination of phages that can target one or more types of bacteria, postpone the development of phage resistance in bacteria, and strengthen the antibacterial impact (5).

**2. Phage-Derived Enzymes**-The lytic bacteriophage encodes the enzymes virion-associated peptidoglycan hydrolases (VAPGH), endolysins, and polysaccharide depolymerase. VAPGH are positioned on the phage base plate and they are capable of directly destroying the



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peptidoglycan of a bacterial cell wall and are thus thought to be a viable antibiotic substitute. Depolymerases encoded by phages hydrolyze bacterial polysaccharides such as capsules, lipopolysaccharides (LPS), and extracellular polysaccharides found in biofilms (6)

**3. Phage and Phage Enzymes Combined with Antibiotic**- The term "phage-antibiotic synergy" (PAS) describes the phenomena wherein sub-lethal dosages of some antibiotics can considerably boost the proliferation of lytic bacteriophages in the host bacterium, resulting in faster cleavage of host cells and rapid diffusion of progeny phages (7).

**4. Phage Engineering -** One of the most popular techniques is to engineer genes expressing receptor-binding proteins (RBPs) in the tail fibers or spikes of phages. The anti-receptor gene orf18 of the *Streptococcus thermophilus* phage DT1 and the siphovirus MD4 were homologously recombined to create the chimeric phage, which acquired the host range of the phage MD4 as one example (8).

**5. Phage Combined with CRISPR-Cas System**-Bacteria and archaea that use the clustered regularly interspaced short palindromic repeats-associated (CRISPR-Cas) system have sequence-based adaptive immunity against mobile genetic material including viruses and plasmids. The CRISPR-Cas system functions by integrating the brief nucleic acid fragments from viruses or plasmids into the CRISPR array to produce short RNA sequences complementary to them (referred to as CRISPR RNAs, or crRNAs), and these crRNAs direct the Cas protein complex to specifically target invasive foreign genetic elements for eradication (9).

**Properties of encapsulating phages for therapy versus the deployment of freely diffusing phages**

"Protection" against factors like enzymes and an acidic pH that render the phage inactive. The encapsulating material's makeup provides the ideal circumstances for ensuring "stability" while phages are stored or administered. By employing liposome-encapsulated phages, which permit penetration into tissues, "active site delivery" is enhanced. When phages are implanted in a three-dimensional network, which keeps the phage at the site of infection, "availability" is ensured. By utilizing appropriate encapsulating materials that permit interaction with the tissue, "adhesion" can be created (10).

**Phage Neutralization**

Anti-bacteriophage activity mediated by humoral response was noticed during or after phage therapy. Most of the healthy population has antibodies against the T4 bacteriophage. Since it has been observed that the presence of antibodies does not always guarantee



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therapeutic effectiveness, it is unclear whether the use of bacteriophages in treatment should be prohibited in the presence of such antibodies. Without checking for antibodies against bacteriophages specifically, it is crucial to screen for phage neutralization (11).

**Why Would We Need Phage Therapy?**

The extensive appearance and dissemination of AMR bacteria over the previous 2 to 3 decades has posed a significant treatment challenge. For instance, in 2005, there were around 1,00,000 significant MRSA infections reported in the US, which led to 20,000 fatalities. The limited therapeutic options remaining to treat major multi-drug resistant (MDR) bacteria, known by the acronym as the ESKAPE pathogens (for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter sp, Pseudomonas sp, E. coli.* It is unquestionably necessary to treat bacterial diseases with a completely new, non-antibiotic strategy. In the current era of the progressive development of MDR bacterial pathogens and the scarcity of new antibiotics to tackle these diseases, an alternative to antimicrobial chemotherapy. Phage applications undoubtedly outweigh its role in causing human infections. Food safety, agriculture, veterinary applications, business, and clinical diagnostic applications like the identification and characterization of bacteria causing human infections have all been mentioned as possible uses for bacteriophages.

**Biofilm Degradation**

A biofilm is a community of microorganisms made up of bacterial cells enclosed in an extracellular polymeric substance (EPS) made of polysaccharides, teichoic acids, proteins, and extracellular DNA and can be either a biotic or abiotic surface.

Lytic phages are a tactic for AMR biofilm-associated infections because they can effectively infect and kill bacteria that form biofilms. By ways by which bacteriophage destroys the biofilm 1. Penetration across the biofilm, 2. Propagation inside the biofilm, 3. Infection of biofilm cells including persister, 4. Production of exopolysaccharides depolymerase, 5. No inactivation by matrix component, 6. No induction of biofilm formation.

**Synergism With Antibiotics**

Despite the difficulties posed by AMR and MDR infections, antibiotics will continue to be the clinical standard of care for treating bacterial illnesses. Phage-antibiotic combinations should be used to both enhance the therapeutic effects of antibiotics and introduce effective combination medicines into the clinical toolbox. Sub-lethal concentrations of numerous kinds of antibiotics have been shown to have a favorable impact on the size of phage plaques and the effectiveness of phage propagation, which is known as phage-antibiotic synergy (PAS) in both



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Gram-positive and negative bacteria.

**Phage Resistant**

Phage therapy has the potential to cause host bacteria to develop bacteriophage resistance (BPR), which is similar to the selective pressures brought on by antibiotic use in bacteria. Bacteria can develop resistance by inhibiting various steps in the phage replication cycle, including phage adsorption, DNA entry into the bacteria, phage DNA destruction, and a system that kills the infected bacterium. Phage adsorption may be prevented by altering the surface receptors of bacteria. By changing the bacterial surface through glycosylation or acetylation, mutations in the genes encoding the O-antigens that phages employ as receptors might confer resistance to particular phages (12).

**Conclusion**

In summary, bacteriophages have many functions which make them effective against superbugs. In the era of burgeoning antimicrobial resistance in animals as well as humans, phage therapy plays a major role in Western countries. In addition, a large number of publications suggested that phages may be replacements for antibiotics in AMR-producing bacteria. Exploiting the combinations of phage with antibiotics appears to be a promising solution and decreases antibiotics usage as well as AMR in animals, humans, and agriculture.

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