



Alternative to Antibiotic Resistance: Bacteriophages

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Globally, antimicrobial resistance is a matter of concern for clinicians and researchers. It is increasing despite several awareness programmes and new treatments being employed. With decrease in discovery rate of novel antibiotics, threat to take humankind back to a “pre-antibiotic era” of clinical care is obvious. There are approximately 10^{30-31} bacteriophages (phages) in the biosphere [1,2], which is estimated to be 10-fold higher than the total number of bacterial cells [3]. This makes it the most ubiquitous organism on earth. Phages are also an inherent part of the human microbiome, and so are usually well-tolerated when used in phage therapy [4,5,6]. Phages are one of the most promising alternatives to antibiotics, which can be used for medicine, agriculture, and related fields [7]. The practice of using bacteriophages did continue in some countries such as Georgia (as part of the former USSR), where they were, and continue to be routinely isolated and used to treat a large number of diseases [8]. The evolution of multidrug-resistant and pan-drug-resistant bacteria (Superbugs) poses a real threat to the control of infectious diseases globally, so it is urgent to have new therapeutic tools available. Bacteriophages (phages) are one of the most promising alternatives to antibiotics for clinical use.

History:

In 1896, Ernest Hanbury Hankin reported from the waters of the Ganges and Yamuna rivers in India, that had marked antibacterial action against cholera and it could pass through a very fine porcelain filter. In 1915, British bacteriologist Frederick Twort, discovered a small agent that infected and killed bacteria.

The Structure of Bacteriophage:

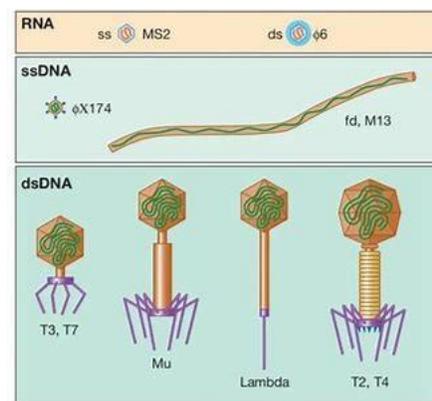
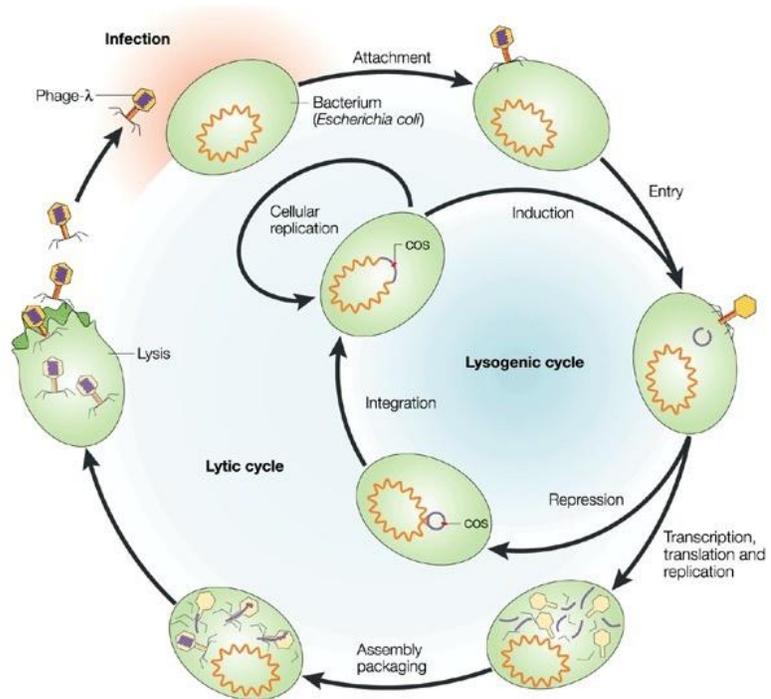
The structural composition of bacteriophages exhibits significant diversity, albeit with a discernible convergence towards certain shared attributes. Bacteriophage T4, specifically infecting *Escherichia coli*, exemplifies this trend through its possession of an icosahedral head structure comprised of recurring protein subunits denoted as the capsid, housing a linear double-stranded viral genome. The genomic size of phages such as Phage λ (lambda) spans a range from approximately 2 to 200 kilobases per strand of nucleic acid. Noteworthy heterogeneity characterizes the nucleic acid constitution of phages, encompassing both double-stranded and single-stranded configurations of DNA and RNA. Predominantly, bacteriophages harbor double-stranded DNA genomes.

Furthermore, the structural arrangement of bacteriophage T4 involves the connection of its head to a helical tail via a collar structure, with the tail featuring an array of specialized appendages including tail fibers and tail pins at its terminus. These specialized syringe-like structures facilitate the binding process to receptors located on the cellular surface. It is pertinent to note that while a majority of bacteriophages exhibit a tail structure, such architectural elements are not universally present across all phage types.

The Life cycle of Bacteriophage:

To comprehensively elucidate the ecological functions of bacteriophages, a comprehensive understanding of their potential interactions with host organisms becomes imperative. The myriad of life cycles exhibited by phages, in conjunction with their interface with the surrounding physicochemical milieu, intricately determine their impact on the dynamics of bacterial or archaeal ecosystems.

If a phage invades a susceptible bacterial cell, the phage (or at least its nucleic acid) enters the cell and triggers a cycle of phage production. During this cycle, the cell is destined to become a phage factory in which the components of the cell's biosynthetic apparatus (such as ribosomes and ATP



generators) are diverted from their normal tasks in bacterial growth. The various pathways for reprogramming are initiated by phage-specified proteins, which are translated from phage mRNA that is made after infection. Generally, nucleic-acid replication occurs first, followed by the synthesis of structural proteins of the phage particle. New phage particles are then assembled, and are later released from the cell by the disruption of the cell envelope and sudden lysis of the cell (LYTIC). The contents of which (including the new phage particles) spill out into the medium. The whole cycle can last for nearly 40 minutes and produce as much as 100 new phages.

This type of lytic cycle is the only mode of reproduction for many phages. Other phages have an alternative life cycle in which a fraction of the infected cells go into the lytic cycle, whilst other cells survive infection and permanently harbour the phage genome in a quiescent form (PROPHAGE). Cells that harbour a prophage are known as LYSOGENIC. Certain agents (such as ultraviolet light) can induce lysogenic cells to re-enter the lytic cycle. The process of both lytic and lysogenic cycles is presented in the figure above.

The systematic Plan:

Bacteriophages exert a substantial influence on global geochemical cycles and serve as a repository harboring the most extensive genetic diversity found on the planet. The investigation of phages has been pivotal in catalyzing seminal advancements within the biological sciences, ranging from the pivotal elucidation of DNA as the hereditary material to the elucidation of the genetic code and the subsequent evolution of molecular recombinant technologies.

Mode of action:

Bacteriophages are viruses that infect and kill bacteria. When used as an alternative to antibiotics, they work through a process known as the lytic cycle, which involves several key steps:

- [1] **Attachment:** Bacteriophages have specific proteins on their surface that enable them to recognize and attach to specific receptors on the surface of bacterial cells. This attachment is highly specific and allows the phage to target only certain types of bacteria.
- [2] **Penetration:** Once attached, the bacteriophage injects its genetic material, typically DNA, into the bacterial cell. This DNA carries instructions for the phage to replicate itself.
- [3] **Replication and Transcription:** Inside the bacterial cell, the phage's genetic material takes over the host's machinery. The phage's DNA is transcribed into messenger RNA, which is then translated into proteins. These proteins include enzymes necessary for phage replication.
- [4] **Replication of Phages:** The host bacterial cell's resources are redirected to produce new phage particles, including the viral DNA and proteins. These newly synthesized phages assemble in the cell.
- [5] **Cell Lysis:** As the phage replication progresses, the bacterial cell becomes filled with newly formed phage particles. Eventually, the cell reaches a point of lysis, where it ruptures or bursts open due to the accumulation of phages. This releases the newly formed phage particles into the surrounding environment.

[6] **Release and Infection:** The released phage particles can then go on to infect other bacterial cells of the same species, starting the cycle again. The newly infected bacteria are usually killed during this process.

Advantages:

- 1) **Targeted Action:** Bacteriophages can offer highly specific targeting of bacterial pathogens, which is a significant advantage in preventing and treating bacterial infections in animals. This precision can minimize the disruption of beneficial gut bacteria and reduce the risk of antibiotic-resistant bacteria developing.
- 2) **Reduced Antibiotic Use:** Substituting antibiotics with bacteriophages has the potential to reduce the reliance on antibiotics in animal agriculture. This is critical in addressing the global concern of antibiotic resistance, which is a result of overuse and misuse of antibiotics in both human and animal medicine.
- 3) **Safety:** Bacteriophages are generally considered safe for animals and humans, and they do not harm non-bacterial organisms. This safety profile is an essential consideration in food production and for public health.
- 4) **Natural Occurrence:** Bacteriophages are naturally present in the environment, and they have been used safely for various purposes, including food safety, in some countries for decades.
- 5) **Food Safety and Animal Farming:** Bacteriophages can play a vital role in improving food safety by reducing the prevalence of harmful bacteria in animal products. In animal farming, they can be used to prevent and control bacterial infections, thus enhancing animal health and reducing the need for antibiotics. This application contributes to the safety of food products derived from animals.
- 6) **Probiotics with Phages:** Incorporating bacteriophages into probiotic preparations for animals can be beneficial. These probiotics, often referred to as "phage-enriched probiotics," help maintain a balanced microbial community in the animal's gut by targeting pathogenic bacteria. This approach has the potential to enhance digestive health and overall well-being in animals.
- 7) **Disease Prevention:** Bacteriophages can serve as a preventive measure to reduce the risk of bacterial infections in animals. This proactive approach is especially valuable in livestock farming, where disease outbreaks can have significant economic and animal welfare implications.
- 8) **Aquaculture:** Bacteriophages are essential tools in aquaculture to manage and control bacterial infections in fish and other aquatic organisms. Their targeted use can help reduce the use of antibiotics, which is essential for sustainable aquaculture practices.

Challenges and Critical Considerations:

1. **Specificity Limitation:** Bacteriophages are highly specific, which means that they target a narrow range of bacterial strains. This specificity can be a limitation when dealing with complex infections involving multiple bacterial species or strains.
2. **Resistance Development:** Bacteria can develop resistance to bacteriophages, similar to antibiotics. While resistance to phages tends to develop more slowly, it remains a concern. Phage cocktails can help mitigate resistance issues but are not always a foolproof solution.

3. **Regulatory Approval:** The use of bacteriophages in animal agriculture may require regulatory approval in many countries. The approval process can be costly and time-consuming.
4. **Lack of Standardization:** There is a lack of standardized methods for the production, purification, and application of bacteriophages in animal feed. This can result in variability in effectiveness and safety.
5. **Cost Considerations:** The production of bacteriophages can be more expensive than antibiotics. This cost difference can impact the overall cost of animal feed, which is a concern for producers and consumers.
6. **Research Gaps:** There is a need for more research to understand the long-term effects and potential ecological impacts of using bacteriophages in animal agriculture, including the potential for horizontal gene transfer and unintended consequences.
7. **Cultural Acceptance:** Cultural and consumer acceptance of phage-treated animal products may vary by region and require educational efforts to explain the safety and benefits of this approach.

Conclusion:

The use of bacteriophages as an alternative to antibiotics in animal feeding is a promising concept that can help address the antibiotic resistance crisis and promote more sustainable animal farming practices. However, it is not a one-size-fits-all solution, and its successful implementation requires addressing the critical challenges and limitations discussed above. Extensive research, standardization, and regulatory oversight are necessary to ensure the safe and effective use of bacteriophages in animal agriculture. It work as an alternative to antibiotics by specifically infecting and killing bacteria through the lytic cycle. They do not harm eukaryotic cells (including animal and human cells) and are highly targeted, as each phage typically infects only a specific type of bacteria. This specificity can help reduce the use of broad-spectrum antibiotics and minimize the risk of antibiotic-resistant bacterial strains developing. Bacteriophages are a natural part of the ecosystem and have been explored as a potential tool to combat bacterial infections, especially in situations where antibiotics may be less effective or to address antibiotic resistance concerns.

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