

Review Article

Review of bacteriophage and its applications

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Summary

The most prevalent and ubiquitous organisms in the world are bacteria-killing viruses called bacteriophages. The aim of this paper is to highlight some application areas of bacteriophages. There are both virulent and temperate bacteriophages in the environment, but only virulent bacteriophages are used for treatment, known as phage therapy. Since their discovery, bacteriophages have been considered a vital weapon to fight human and animal illnesses of bacterial origin. Currently, the emergence of growing microbial resistance to antibiotics and attention to bacteriophage use in treatment has all but grown up again. Correspondingly, the applications of bacteriophages for biocontrol purposes have become a remarkable opportunity in a number of fields, including farms and food production. Despite their treatment effects, bacteriophages also maintain a vital relationship with their hosts through moderating microbial populations and promoting their evolution by horizontal gene transfer. Additionally, they serve as vehicles for the transfer of vaccinations, the detection of dangerous microorganisms and systems for displaying proteins and antibodies. Bacteriophages are a varied collection of viruses that are simple to handle, making them suitable for use in treatments and biotechnology research. Hence, the expansion of various phage companies for phage production and the use of phage cocktails for the treatment of various bacterial diseases at different stages is recommended.

Introduction

Viruses are regarded as intracellular parasites that require a specific host cell for replication. A type of virus that only targets and replicates within bacterial cells is called a bacteriophage [1]. Bacteriophages consist of a genetic material, which can be either deoxyribonucleic acid or ribonucleic acid, surrounded by a protein coat, or capsid. Phages are structurally simple and extremely diverse, representing the most abundant living organisms in the environment. It's expected that there are between 10^{31} – 10^{32} bacteriophages helping to manage bacteria populations within natural ecosystems within the world at any given time [2]. Additionally, it has been claimed that they are in charge of eliminating 20% – 40% of the bacteria on marine surfaces once every 24 hours [3].

As natural adversaries of their host, bacteria, bacteriophages are helpful to humans in the fight against bacterial illness.

Bacteriophages can infect bacteria either lytically or lysogenically after adhering to receptors on their surface and introducing their genetic material. A lytic infection occurs when a bacteriophage multiplies, lysing the bacterial target while continuing to attack additional bacteria. During a lysogenic infection, a DNA bacteriophage inserts its genetic material into the bacterial genome and also passes on its genome to daughter cells as the bacterium grows. The incorporated phage DNA may extract itself from the bacterial chromosomes in response to changes in the environment, creating lytic phage particles [4-6].

After bacteriophages were developed in the early 20th century, several researchers speculated about their capacity to destroy bacteria, which would surely make them useful therapeutic agents. But after World War II, when antibiotics were found, this natural potential medicinal agent received little attention and was only used as a research tool several



times (Clark and March 2016). Bacteriophages are accepted as harmless, effective [7] and innovative alternatives to the use of chemotherapies nowadays [8,9]. This would make it possible to stop the development of bacterial antibiotic resistance. Additionally, bacteriophages are recognized as effective agents for bio-decontamination against undesirable bacteria in hospitals, along the food supply chain and in flocks and livestock. [10,11].

Even though they are solely bacteria's natural enemies, bacteriophages still have a vital relationship with their hosts because they control microbial populations and encourage horizontal gene transfer to speed up bacterial evolution [12]. Bacteriophages have contributed significantly to the fields of molecular biology and biotechnology and continue to do so. They have helped to unravel many molecular biology riddles. Bacteriophages are receiving greater attention than ever before in today's technologically advanced world due to their potential for use as antibacterials, diagnostic tools (phage typing), phage display systems and delivery methods for vaccines (Clark and March 2016).

Phage molecular biology research has recently fueled numerous biotechnological applications in a wide range of sectors, such as vaccine development, medicinal administration, bacterial detection systems, novel antibiotics against antibiotic-resistant bacteria, etc. Another possible area of use is the use of bacteriophages as natural antimicrobials in food to prevent undesirable microorganisms, which is probably acceptable to consumers [10,13]. Hence, the objectives of this paper are:

Hence, the objectives of this paper are:

- To review the role of bacteriophages in the treatment of diseases caused by different bacteria as well as in the bacterial diagnosis,
- To review the role of bacteriophages in vaccine administration.

Literature review

Taxonomy

In order to approve and coordinate the taxonomic coding and nomenclature of viruses, the International Committee on Taxonomy of Viruses (ICTV) was established in 1973. Notably, classification is crucial for the following reasons: finding novel bacteriophages, figuring out how phages are related to one another and maintaining bacteriophage databases and collections. Additionally, it can be utilized to identify either hazardous bacteriophages in the biotechnology and fermentation industries for control and eradication purposes or beneficial bacteriophages with medicinal and industrial applications [14].

Based on the type of encapsulated nucleic acid and the appearance of their virion, bacteriophages are divided into 13 families [15]. There are nine families with double-stranded DNA: the *Corticoviridae* (icosahedra capsid with lipid layer),

Fuselloviridae (pleomorphic, envelope lipids, no capsid), *Lipothrixviridae* (enveloped filaments, lipids), *Myoviridae* (contractile tail), *Plasmaviridae* (pleomorphic, envelope, lipids, no capsid), *Podoviridae* (short, non-contractile tail), *Rudoviridae* (helical rods), *Siphoviridae* (long, non-contractile tail) and *Tectiviridae* (icosahedral capsid with inner lipoprotein vesicle). *Microviridae* (icosahedral capsids) and *Inoviridae* (rod-shaped with helical symmetry) were seen in single-stranded DNA. *Cystoviridae* (enveloped, icosahedral capsid, lipids) and *Leviviridae* (quasi-icosahedral capsid) are two families that have double-stranded RNA and single-stranded RNA, respectively. According to Ackermann [16], tail phages, which are a subclass of the *Caudovirales* order, account for more than 96% of all bacteriophages. Additionally, they have a wide range of characteristics, including size and fine structure, DNA content and composition, the way proteins are made, serology, host range and physiology [14].

The three major families that make up the *Caudovirales* account for 60% of the described phages: *Siphoviridae*, *Myoviridae* and *Podoviridae* [14,17,18]. However, only three to four percent of the phages studied (polyhedral, filamentous and pleomorphic) belong to the ten families studied and some of these families are extremely small [16].

Bacteriophage life cycle

Because they lack their own metabolism, bacteriophages must totally rely on the machinery used by their bacterial hosts to produce energy and proteins in order to reproduce [19]. Adsorption (attachment), infection, multiplication and discharge (release) are typically the four steps that distinguish bacteriophage replication cycles from those of their bacterial hosts [20].

The attachment phase relies on the presence of specific receptors on the surface of the bacterial host cell, to which the phage can attach. The phage is delivered to the host receptor by random diffusion. Following attachment, the infection phase occurs because the phage genetic material passes into the host cell by a mechanism likened to injection [21]. After-infection, the beginning of the multiplication phase relies on the phage species. Virulent bacteriophages are known to require bacterial replication machinery for the assembly of the progeny of the phage within the bacteria. Several copies of those new phages are produced until a critical mass is reached, triggering the lysis of the bacteria's semipermeable membrane and therefore the release of the new phage progeny to reinitiate the lytic cycle (Figure 1) [19,22]. This critical mass frequently depends on factors such as specific bacteriophage characteristics, the strain of bacteria infected by the virus, and therefore the environment within which the bacteriophage-bacteria interaction occurs [19]. Phage lytic enzymes (endolysins or lysins) play a major role in breaking down bacterial peptidoglycan during the ultimate stage of the lytic cycle. Additionally, some phages with filamentous morphology are capable of fleeing from the host cell via cell membrane extrusion without causing the destruction of the host [15].

On the other hand, lysogenic phages are found to integrate their genetic material into that of the host, forming an entity

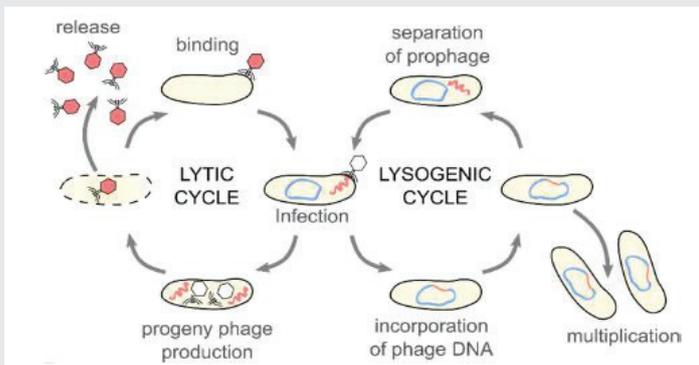


Figure 1: Lysogenic cycle of phages. First, viruses bind to receptors on bacteria and inject genetic materials into the bacteria. Lysogenic phages incorporate their chromosomes with the genome of the host, forming a prophage. A prophage duplicates upon the division of the bacteria. The prophage might be activated by external factors and thus start the lytic phage life cycle [154].

known as a prophage (Figure 1). This results in the vertical transmission of the virus's genetic information to daughter cells of the bacteria through a biological process and it is also possible for viral genes and proteins to be expressed. Occasionally, the lysogenic phage's genetic material stays inside the host bacteria's cells as a distinct plasmid rather than integrating into its chromosome, where it might continue to pass from one bacterial generation to the next. The cycle can change from lysogenic to lytic as a result of stresses such as chemicals, UV light, or damage to the host DNA [23].

Because of the difference between phages, it's important to see which are the most appropriate bacteriophages for every potential application [24]. Given the antibacterial activity of virulent phages, they're considered suitable for biocontrol purposes, whereas temperate phages don't seem to be usable because of the high probability that they're going to cause horizontal gene transfer between bacteria [25], which is potentially related to the chance of favoring the spread of antimicrobial resistance or other dangerous genes between microbes through bacterial transduction. Non-lytic phages, on the other hand, are frequently as important in biomedical research, being used in phage display techniques [26] and potentially providing the ability to observe tumors [27] and treat some diseases [28].

Application of bacteriophages

Therapeutic agents: In order to combat bacterial illnesses or to cure environments contaminated with dangerous bacteria, viruses' host specificity offers an alluring approach. Although there is little research on the potential benefits of viral treatment, studies have demonstrated that illnesses in humans, plants, livestock, and aquacultured fish can be successfully treated using this technology [29,30].

The ability of bacteriophages to infect and kill bacteria led to the exploration of their therapeutic potential against bacterial pathogens in a clinical approach known as bacteriophage therapy/phage therapy, almost immediately after their innovation, with the first therapeutic use in humans described in 1919, just two years after their innovation by d'Herelle [30,31]. Phages can support the progress of an inflammatory response

against bacteria via the breakdown of bacterial cell walls, which triggers the immune system [32]. Thus, bacteriophage therapies, i.e., the administration of bacteriophage cocktails to patients infected with bacteria, besides the direct removal of bacteria cells, further stimulate the immune system to fight against infection [33].

A wide variety of bacterial infection diseases, including cholera, dysentery, typhoid fever, skin and surgical site infections, peritonitis, septicemia, and external otitis, have been treated by phages before the discovery of antibiotics [34]. Nevertheless, an incomplete understanding of bacteriophage biology, together with the discovery and increased use of antibiotics during the 1940s and 1950s, as well as several other factors, led to degeneration in the clinical use of bacteriophages in Western Europe and North America. In contrast, phage therapy continued to be employed in the previous Soviet Union and certain Eastern European countries (e.g., Poland), where therapeutic phage preparations are still readily available in pharmacies today [30,33,35].

Two main advances played a major role in reviving the interest in bacteriophages as antimicrobial agents. First, the appearance and extensive distribution of antimicrobial resistance (AMR) bacterial pathogens have narrowed our therapeutic options. The situation is further worsened by the limited number of new antimicrobial drugs entering the market. Also, most novel antibiotics approved in recent years are modifications of existing drugs and, therefore, are at advanced risk of being rendered ineffectual in a short period [36]. Secondly, there has been an enlarged appreciation of the injury broad-spectrum antibiotics can impose upon the microbiome. In fact, antibiotic-mediated microbiome agitation is now believed to contribute to several non-communicable and chronic diseases [37].

Bacteriophages are classes of viruses that can attack and destroy bacteria without any impact on human or animal cells. As a result, it is supposed that they can be used, alone or in combination with antibiotics, to treat infections caused by bacteria [38]. Hyman [39] suggested the following features of bacteriophages be used for therapeutic purposes: (a) the bacteriophage should be lytic and be able to cause broad cytotoxicity to the target bacterium. (b) It should be completely lytic and should not become a lysogenic phage. (c) The bacteriophage should have the ability to transduce the host bacteria. (d) It should have the desired host range. (e) It should be checked for toxin genes that can affect the patient. *Myoviridae*, *Siphoviridae*, and *Podoviridae* families are used frequently for bacteriophage therapy [40,41]. There are around 800 phages against pathogens like *Escherichia*, *Klebsiella*, *Enterococcus*, *Pseudomonas*, *Staphylococcus*, and *Salmonella*. [42].

Phage therapy may not completely replace antibiotics at this time, but there is optimism that it will be used in conjunction with antibiotics, particularly for strains that are resistant to antibiotics. Phages will be much more reliable when used externally and where the immune system gives them a chance by favoring them to persist within the body for a little while (Clark and March 2016).



Clinical Cases Treated with Bacteriophages

In human medicine, bacteriophages are utilized both topically and internally to treat various problems and diseases that are resistant to antibiotic treatment. Patients suffering from ulcers received effective treatment from bacteriophage moistened in biodegradable films and the wounds swiftly healed [43]. Numerous studies have demonstrated the effectiveness of local and topical phage treatments. Phage cocktails have been used locally to treat infections such as conjunctivitis, otitis, gingivitis, furunculosis, decubitus ulcer, open wound infection, burns, osteitis (caused by fractures) and chronic suppurative fistulae caused by *Staphylococci*, *Klebsiella*, *Pseudomonas*, *Proteus* and *Escherichia* [44-47]. A commercial product called PhagoBioDerm, which targets *P. aeruginosa*, *S. aureus*, and *Streptococcus* spp. and contains phages as well as ciprofloxacin, can be applied directly over infected wounds. Goode, *et al.* [48] eliminated *Salmonella* contamination on chicken skin by using a lytic bacteriophage.

A *P. aeruginosa* that had caused skin infections was treated with phage therapy by Vieira, *et al.* [49]. Phage treatment resulted in a four-orders-of-magnitude reduction in *P. aeruginosa* 709 levels in human skin. *S. aureus*, which produces wound abscess, may be successfully combated by sewage-derived phage [50]. According to studies, mice that get an intraperitoneal injection of a bacteriophage to treat *P. aeruginosa* infection experience less infection [51]. *E. coli*-induced diarrhea in calves can be avoided by giving them bacteriophages orally. Similarly, oral phage therapy could help stop the sepsis brought on by *P. aeruginosa* [52]. Additionally, intramuscular treatments against *E. coli* in chickens have been performed, and they were successful in preventing septicemia [53]. Mice were saved from death by phages against vancomycin-resistant *Enterococcus faecium* [54]. In mouse models, phages against *Acinetobacter baumannii*, *P. aeruginosa*, and *S. aureus* also performed well [55]. As a result, *Salmonella Typhimurium*, one of the main threats to the swine sector, has been reduced in prevalence as a result of the use of phage [56,57].

Advantages and limitations of phage therapy

Bacteriophage treatment has been used in animals, plants, and humans with a diverse degree of accomplishment. Bacteriophages have various potential benefits over antibiotics, but at the same time, they do have drawbacks as well (Table 1). The main advantage of bacteriophages is their specificity for target host bacteria, which significantly reduces the destruction of the host's normal flora. The bacteria to be targeted must be recognized first, or else a mixture of bacteriophages should be used. Phages are self-limiting, i.e., they require their hosts to be continually growing; their persistence in the environment for a long period of time depends on their specific host availability (Clark and March 2016). Duplication at the site of infection is another merit of bacteriophages. They are safe with no or few side effects [35]. If bacteria become resistant to bacteriophages, bacteriophages will naturally change to infect the resistant bacteria, reducing the likelihood of bacterial escape (Hausler, 2007).

Table 1: Potential benefits of bacteriophage therapy against antibiotic therapy [153].

Characteristics features	phage therapy	Antibiotics treatment
Specificity	Highly specific	Wide range of action
Toxicity	Almost totally non-toxic	Variable levels of toxicity ranging from mild to severe
Biofilm penetration	Effective penetrating ability	Unable to penetrate except in high doses
Possibility of resistance	Almost non-existent	High possibility of resistance
Simplicity of administration	not require repeated doses	Require repeated doses
Cost of treatment	Cheaper	more expensive

After their administration, bacteriophages can disperse very rapidly in the body, reaching almost every organ; but the immune system quickly clears systemic bacteriophages, which poses yet another problem for their acceptance as therapeutic agents [58,59]. One of the serious concerns about the use of bacteriophage therapy *in vivo* is a strong antibody response, which would clear the bacteriophages more quickly and thus the use of bacteriophages for a prolonged period of time would not be possible (Clark and March 2016).

Other limitations of bacteriophages as therapeutic agents are their narrow host ranges and the fact that bacteriophages are not permanently virulent under certain physiological situations. During the preparation of bacteriophage stocks, it must be ensured that bacteriophage preparations are free of bacteria and bacterial toxins in order to prevent secondary infections. But sterilizing bacteriophages could deactivate them. Bacteriophages may convey toxic properties to the bacteria, resulting in virulence [60]. One way around this is the use of the bacteriophage lytic enzyme endolysins, rather than administering the entire virion [61,62]. Likewise, genetically improved bacteriophages can be used, which will only deliver the DNA essential for making antibacterials that would be specific to the target bacteria [63].

Status of phage therapy in Ethiopia

Although bacteriophages are widely used as treatment agents for pathogenic bacteria in developed countries, this application potential is not widely practiced in Ethiopia. Only limited investigation has been conducted in our country regarding the therapeutic potential of bacteriophages, focusing on the isolation of bacteriophages and assessment of their activity against biofilms of uropathogenic *Escherichia coli* in Jimma Town, South Western Ethiopia (Gudina *et al.*, 2018).

As-biocontrol agents in food industry

Foodborne infections from microbial sources remain a severe food safety problem worldwide. In addition to being of substantial public health significance, the economic impact of foodborne bacterial illness is significant [64]. Contamination prevention and the endowment of a safe food supply is one of the extraordinary priority areas to control and bound the foodborne pathogen outbreaks under the "One Health" approach [65]. Nevertheless, the challenge is not a direct one to solve quickly



or simply. The epidemiology of foodborne pathogens is difficult and involves multiple routes of transmission from food animals and agricultural produce to consumers. For example, animals are often asymptomatic carriers of Shiga-toxin producing *Escherichia coli* (STEC), *Salmonella*, *Campylobacter* and *Listeria*, and they can spread the pathogens to other food animals, crops, slaughter facilities, and, in some instances, directly to humans. Food processing facilities can also harbor foodborne pathogens as biofilms, which can potentially transfer to food products and reach consumers [66].

Traditional pathogen sanitization procedures in food processing facilities emphasize the use of chemicals, physical destruction techniques, and irradiation to reduce the microbial load in those facilities and the foods produced in them [67]. For instance, numerous harsh chemical sanitizers, such as chlorine and peracetic acid, are commonly used to reduce microbial contaminants in many fresh fruits and vegetables as well as ready-to-eat (RTE) food products [68]. Heat pasteurization is often used to decrease bacterial numbers, generally in liquids and dairy items, such as milk. High-pressure processing (HPP) is also used to successfully reduce pathogens in liquid products, as well as pre-cooked, meant to be frozen meals [69,70]. This technique exposes foods to high pressure to deactivate microbes. Irradiation has been accepted as a means of reducing the burden of pathogenic organisms in foods since 1997 [71].

Conversely, no single approach is 100% effective, and the aforementioned methods also have some substantial disadvantages. For example, many chemical sanitizers corrode and harm food processing materials [72,73] and may have toxic chemical residues that may damage the environment. Pasteurization and HPP are not appropriate for fresh produce and meat products as they can harmonically affect the organoleptic properties and/or the nutritional content of some foods [69,70]. Irradiation, which can deleteriously affect the appearance of some foods, also has low customer acceptance, which is compounded by a labeling requirement for many food items treated with radiation [74]. Food sustainability and safety are challenges that continue to dominate the food industry worldwide. The shift in Western countries towards consuming foods that are produced by natural means adds significant pressure to produce foods that are safe, natural, free from chemical preservatives, and of acceptable quality to meet consumer demands [75].

The food industry has repeatedly had to contribute to efforts to avoid infectious diseases and the problems associated with antibiotic resistance in human pathogens originating in food animals. Regardless of the many advances in technological methods for the detection and removal of foodborne pathogens at each stage of the food production process, good manufacturing practices, quality control and sanitation and changes in animal agriculture and agronomic processes, microbial safety problems are still predominant. In addition, the controlled use of certain antibiotics during food animal production, together with the lack of development of novel antimicrobials, has put further strain on the food production sector and as such, there is a need for the development of

alternative antibacterial approaches at the production level to maintain safety standards, control foodborne pathogens and limit their negative impact on the food industry and on human health [76].

The natural specificity of phages to attack and destroy their target bacteria, in addition to the fact that they are abundant in the surrounding environments and are harmless to humans and animals, makes them valuable candidates for use in both the detection and control of pathogens at each stage of the food production process from farm-to-fork. In recent years, a number of bacteriophage-based products have gone into commercial use to control some of the leading foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* serovars [76,77]. The application of bacteriophages is harmless and related to the use of antibiotics [78]. Phages have been used since the 1980s to control and exclude bacterial contaminants from food surfaces, food-borne spoilage bacteria, and bacteria causing gastrointestinal diseases [57], as well as to disinfect raw food. Due to their specificity, phages are smart for the sanitization of ready-to-eat foods (RTE) such as milk, vegetables, and meat products [79].

In addition to direct treatment of food, bacteriophage use is also indicated for decontaminating material surfaces in farms or food-processing facilities to significantly decrease pathogen colonies on surfaces and the development of biofilm, helping to reduce the risk of transmission of pathogens along the supply chain. In fact, biofilm represents one of the most important sources of contamination with pathogens in farms or industrial settings, contributing to the transmission of pathogens to dairy products and, along the food chain, to consumers [78].

The idea of preventing pathogens in food by means of bacteriophages can be achieved at all stages of production in the classic 'farm to fork' approach throughout the complete food chain. Phages are suitable (i) to prevent or reduce colonization and diseases in livestock (phage therapy), (ii) to decontaminate carcasses and other raw products, such as fresh fruit and vegetables and to disinfect equipment and contact surfaces (bacteriophage sanitation and biocontrol); and (iii) to extend the shelf life of perishable manufactured foods as natural preservatives (biopreservation). Phages should also be considered in hurdle technology in combination with different preservation methods [80,81].

Merits and limitations of using bacteriophages as bio-control agents

The fact that bacteriophages are presently being used as bio-control agents in sectors of food production [82] shows their advantage as efficacious complementary approaches for controlling specific dangerous pathogens in food in many circumstances. The fact that they have GRAS status supports their safety for food applications, and indeed, there is no known negative side effect of using lytic bacteriophages on humans or animals. Bacteriophages are natural, and low-cost to produce [83,84]. While a suitable propagating host must be selected to ensure endotoxin and virulence factor contamination of the preparation does not occur, the commercialization timeframe



is less stringent than what might be required for human therapeutic applications [85].

Additionally, bacteriophages are highly specific for their target bacterial host and, as such, have no substantial impact on consumers' resident microflora. Bacteriophages also don't impact the sensory and quality characteristics of food [73]. Contrasting chemical biocides or antibiotics that have the ability to leak into food products and persist in the environment; bacteriophages persist in high numbers for a short time without their host. Commercial bacteriophage products are 100% natural and non-genetically modified organisms (GMOs). They're generally kosher, halal, and permitted in organic foods, with several officially certified as such [73].

Generally, there are two main scientific challenges in bacteriophage-mediated biocontrol in food. Initially, the constituents of the bacteriophage product must have a wide enough host range to kill all members of the target pathogenic genus or species. Secondly, the bacteriophages need to be applied such that the particles physically come into contact with all or most of the target bacterial cells in order to work. It is also important that users of bacteriophage products in the food sector understand that individual products don't guarantee the full well-being of foods if the foods are contaminated by a different foodborne pathogen (e.g. a pathogen not targeted by the phage product applied to the food) [86].

Although the biological characteristics of virulent phages provide benefits for improving food safety, these characteristics also lead to some of the limitations and disadvantages of bacteriophage biocontrol. As stated before, phages are highly specific and, as such, they are only active against the pathogen of interest. Still, if foods are contaminated with a group of different pathogens, a mixture of bacteriophage biocontrol products could be used to target more than one pathogen. Meanwhile, phages themselves are also microorganisms. Commonly used disinfectants or chemicals could deactivate them, so their use needs to be cautiously coordinated within the processing line. Additionally, the currently marketed bacteriophage preparations require refrigerated storage (typically 28°C). Hence, a good understanding of the biological properties of phages and designing optimal application regimens that consider those properties is essential for the best possible efficacy of phage biocontrol intervention. The main advantages and disadvantages of bacteriophage biocontrol are indicated below in Table 2 [86].

Bacterial detection (phage typing)

Bacteriophage-based approaches for bacteria detection were developed using the bacteriophages' typical capacity to grab host bacteria. Only the recognition of a suitable and workable host offers the chance for virion proliferation and virus life cycle completion. This offers an advantage over other widely used techniques, such as PCR or mass spectroscopy-based ones, which might produce false positive results when dead bacteria are present. Bacteriophages are far less expensive and simpler to prepare than antibodies, while conventional

Table2: Benefits and limitations of bacteriophage as a biocontrol [86].

Pros	Cons
Phages are a natural product	Not entire bacteriophages make a good biocontrol agent, e.g. temperate phages.
Specific only targets problem foodborne bacterial pathogens	May not guarantee full well-being of foods if the foods are contaminated by a mixture of pathogen
Effective in killing targeted bacteria	Their theoretical use is constrained by a small host range; A mixture of bacteriophages can be used to get around this restriction
Single dose application	Despite lytic phages' capacity to infect new hosts, residual activity has not been seen on food; this may be because food surfaces are physically inaccessible to lytic phages.
limited possibility for resistance and absence of antibiotic cross-resistance	bacteriophage resistant bacterial strains can emerge, but using a cocktail is shown to reduce the phage resistance
Comparable simplicity of preparation and application and rapid discovery	
Little inherent toxicity and no negative effects on the environment	
does not affect the food's rheological, nutritional, or organoleptic qualities	

biochemical or culturing approaches take a lot longer to produce results [87,88].

Due to the fact that bacteriophages only infect live hosts, bacterial cells may be quickly and precisely identified using bacteriophage detection methods. Bacteriophages can be adjusted, lysed, isolated, and extracted from their hosts to permit detection in a variety of methods. The three main categories of phage-mediated detection techniques are reporter bacteriophages, bacteriophage amplification, and bacteriophage capture [89].

Genetically modified reporter phages allow the entry of reporter genes into the bacterial host, where the gene is produced, a signal is picked up, and the pathogen is recognized. In order to enable gene expression when the phage infects live hosts, reporter genes encoding luciferases or fluorescent proteins have been introduced into the phage genome [90].

The formation of offspring phages or the demise of the bacterial host is used as a detection signal in bacteriophage multiplication assays [91]. Typically, the development of plaques on a Petri plate is used to gauge the bacteriophage's growth. Plaques develop when infected hosts lyse, releasing offspring phage that allows bacterial reinfection. Bacteriophage amplification requires phage infection, extracellular phage chemical inactivation using virucide and plaque detection using a rapidly proliferating "reporter" organism/lawn. Each plaque is thought to represent the initially infected bacterium. Plaques can then be removed and analyzed using PCR for further specificity [92,93].

Phage capture uses a phage's features to specifically bind bacteria, such as endolysins or tail spikes. Endolysins are phage-produced enzymes that help lyse bacteria by dissolving

their cell walls. They have two domains: one breaks down the cell wall, while the other, the cell wall binding domain (CWBD), selectively identifies regions of the host cell wall. To enable infection, phage tail spikes bind and selectively attach to host cells. Tail spikes have been used to collect particular bacteria from a variety of matrices, such as food and processing machinery [89].

Phage display system

Despite the fact that natural phages have been successfully used to lower bacterial resistance, some researchers have gone above and beyond to uncover additional antibacterial strategies based on bacteriophages. This results in bacteriophage modifications by gene engineering, the main benefit of which is increased precision [94]. However, bacteriophage engineering has further benefits, such as obtaining phage elements that can recognize bacterial hosts, altering phage hosts, or enhancing their activity (Hauser, 2016).

George P. Smith introduced the idea of bacteriophage display for the first time in 1985 (Figure 2) [95]. It is a molecular technique used to produce polypeptides with novel properties. The desired protein is expressed on the surface of the bacteriophage particle when the DNA encoding the polypeptide is linked with genes for the bacteriophage coat protein [95,96]. Although the *E. coli* filamentous phage M12 is frequently utilized, the phage display technique also makes

use of lambda and T7 [97,98]. Detecting and isolating peptides with high specificity and affinity for target proteins can be done using bacteriophage display libraries. These peptides can be employed in medication development as tools for comprehending molecular recognition and reducing receptor mimics [96].

Possibly different phage kinds are utilized in phage display systems, but filamentous phages are the most beneficial because they enable the genetic material extension by merely enlarging their filament size. The phage's interior structures are unharmed during the process of delivering genetic material. Lysogenic filamentous phages do not kill bacterial cells in order to complete their life cycle and release new virions. The standard phage for this technique is M13. The most intriguing gene in phage display is gene VIII, which codes for important structural proteins and can show short peptides and produce a large number of desired compounds. In contrast, gene III only produces a small number of minor proteins and is more suited for producing big peptides than other genes [99].

The development of vaccines, the advancement of new medications, the study of protein-protein interactions, the selection and modification of substances of interest, the development of monoclonal antibodies with the desired specificity for therapeutic use, the creation of libraries of peptides and other substances, in epitope mapping (as antivenom), or the production of food as biocontrol, all benefit

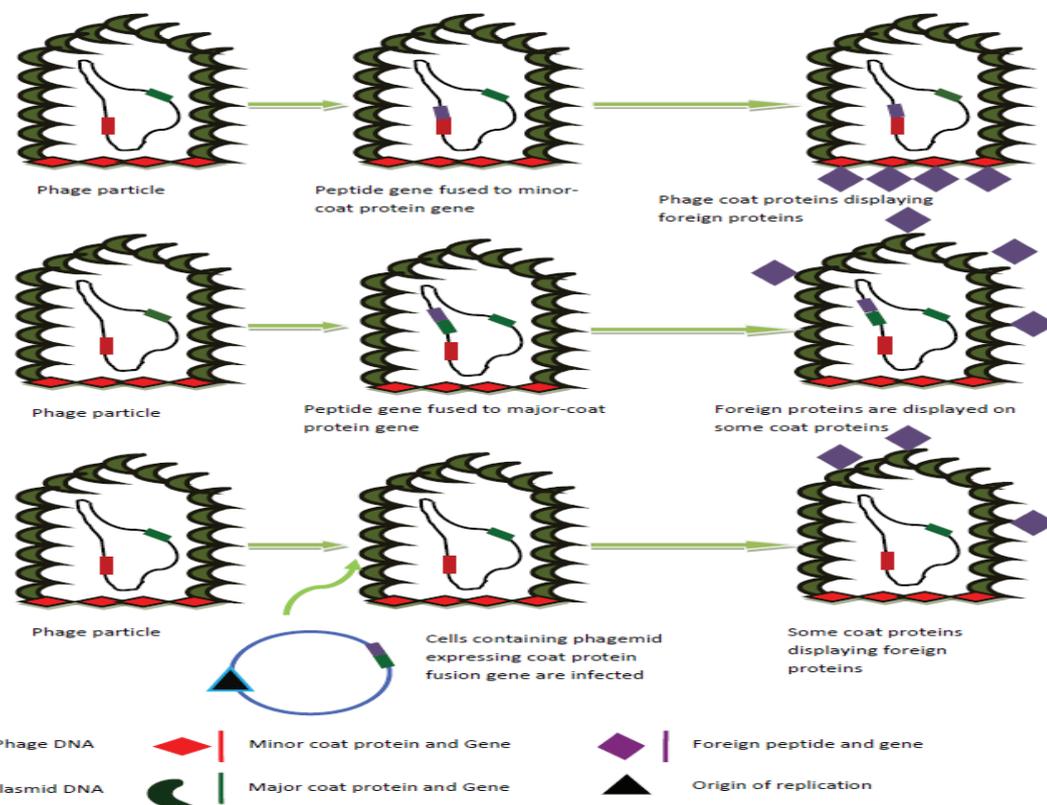


Figure 2: Some methods of phage display (A) The minor coat-protein gene is directly linked to the gene encoding a foreign peptide. The foreign antigen is present in all minor coat proteins. (b) A foreign peptide gene is fused to the gene for a major coat protein, and the major coat protein gene itself is also present. Some significant coat proteins exhibit foreign proteins. (c) A helper phage that hasn't changed is introduced into cells that have phagemids, which are plasmids with bacteriophage and plasmid origins of replication. These cells then express the foreign peptide or protein. Some coat proteins exhibit foreign antigens.

greatly from the use of bacteriophage display. Similarly, selecting and isolating antibodies against desirable antigens or other targets, followed by the creation of an antibody library, is a highly helpful strategy [100].

Bacterial evolution vehicles

Bacteriophages play a significant role in horizontal gene transfer among bacteria. When bacteriophages multiply, they can occasionally encapsidate host bacterial DNA to generate transducing particles. Transduction is the process by which foreign DNA (plasmid or bacterial) is transmitted into bacterial cells with the help of a bacteriophage. In theory, transducing particles are similar to mature bacteriophage particles, but when they infect other cells, they release bacterial DNA rather than a viral genome. The DNA can then replicate as a plasmid or be recombined into the chromosome in the new host cell. Genetic transduction is the term for this process of bacterial DNA transfer from one bacterium to another [101].

Transducing particles' genetic cargo can have a significant impact on the bacteria they transfer to. For instance, genes encoding for virulence or antibiotic resistance might confer new traits and open up new ecological niches, hastening the rise of new strains that are progressively more virulent and resistant to drugs. Although there are a number of horizontal gene transfer processes, phage transduction is frequently considered to be the primary method by which bacteria obtain the genes necessary for quick adaptation to changing environmental obstacles [101].

Generalized transduction

In *Salmonella* phage P22, generalized transduction was the first mode of bacteriophage-mediated gene transfer to be identified and discovered [102]. It is how bacteriophages package and transfer any type of bacterial DNA (chromosomal or plasmid) to another bacterium. These so-called pseudoviruses are nevertheless capable of binding to cells and ejecting packaged DNA into a new host. Depending on the kind of donor

DNA, the transduced molecules can either be integrated or stay free to continue reproducing as plasmids in the cytoplasm (plasmid or chromosomal). This form of transduction can take place during the lytic phase of both the lytic and lysogenic life cycles (Figure 3) [103].

Specialized transduction

The second transduction mechanism described and discovered in the coli phage was specialized transduction [104]. Specialized transduction, in contrast to generalized transduction, is unique to lysogenic bacteriophages and entails the prophage DNA excision from a particular integration site as well as a neighboring region of the host genome (Figure 3). Site-specific recombination (mediated by phage integrases) or homologous recombination (mediated by bacterial recombinases) causes the integration of the phage DNA into the host genome, typically at specified places, after packing into virions and infection of a new host cell [105]. The term "lysogenic conversion" refers to the phenotypic alterations that occur as a result of the prophage's integration into the host genome [106]. Typically, lysogenic conversion increases the pathogenicity of bacteria to improve fitness [107]. The environment's presence of bacteria, bacterial viruses and bacteriophages as well as horizontal gene transfer enables the bacterial host to diversify its gene pool, which aids in environmental adaptability [108]. Therefore, bacteria can inhabit new ecological niches thanks to bacteriophage-mediated gene transfer, which influences subsequent gene transfer with local populations and increases bacterial diversity [19].

Vehicles for vaccines delivery (vaccination tools)

accines are biological agents that stimulate the immune system of the host to provide both therapy and disease prevention. They are made from disease-causing agents (bacteria, viruses, etc.). Conventional vaccines of the live attenuated or inactivated microbe variety have advanced greatly and some of them have been applied in clinical settings [109]. Newly developed vaccines are based on selected target

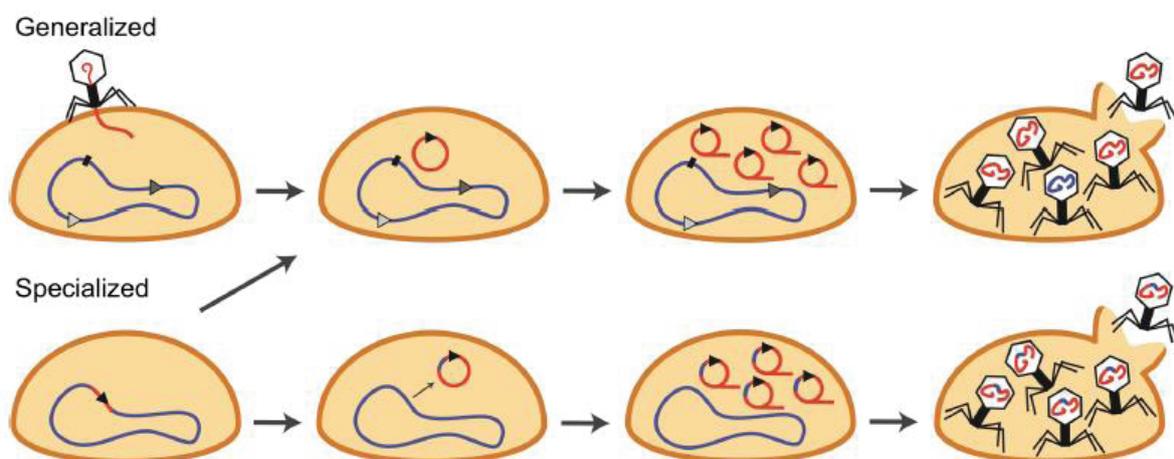


Figure 3: Bacterial genetic transduction. In generalized transduction (top) and specialized transduction (bottom), the viral genome (in red) first undergoes theta replication, followed by rolling circle replication [111].

antigens, derived from an infectious microorganism, a tumor cell, an allergen, or an auto-antigen. The target molecule may be administered as a purified protein or as a peptide (s) or may be expressed from plasmid DNA or a recombinant virus [110].

Bacteriophages have been shown to have the capacity to deliver vaccines both directly and indirectly. They can first display vaccine antigens as fusions to their coat proteins utilizing bacteriophage display, which causes the desired immune response [111]. Additionally, a DNA vaccine expression cassette can be delivered to eukaryotic cells using the phage particle as a gene delivery vehicle [112].

When compared to phage DNA vaccines, bacteriophage display vaccines are more stable since they are based on a virion that contains the gene that codes for the antigen being exhibited. On the other hand, phage DNA vaccines contain DNA that has the antigen gene cloned in a eukaryotic cassette inside the virion. These vaccinations produce a stronger immunological response than traditional vaccines do. These vaccinations are being studied for use against things including bacteria, autoimmune disorders, cancer, fungi, parasites and even contraceptive vaccines [113]. Studies have also looked into using vaccines to prevent or lessen the development of antibiotic resistance. This may be novel because when a patient is exposed to a pathogen, they trigger an immune response that prevents or lessens the sickness. Additionally, if vaccination rates rise among the populace, herd immunity may result, in protecting others who aren't vaccinated [114]. Phage display, like other techniques, has several shortcomings that need to be examined. The peptide's ability to bind to its target may be lost during the transfer to a soluble medium. Additionally, there is a chance that peptides' in vivo and in vitro functionalities will differ, which could have an adverse impact on the patient. Due to proteolysis, peptides are also unstable and their capacity to trigger immunological reactions might provide a challenge for their use. However, various new methods are being explored to address these obstacles. Many of them try to reduce immunogenicity and increase peptide affinity and half-life using protein engineering or nanotechnology [115].

Purified phage particles are administered to the host after the vaccine gene is cloned in a lambda bacteriophage under the direction of a eukaryotic expression cassette. The coat shields DNA from deterioration, and because it behaves like a virus particle, it would direct the vaccination toward the cells that present the antigen [116]. In mice and rabbits, the antibody response was vastly superior when compared to the usual DNA vaccine [117]. A DNA vaccine encapsulated in a phage particle under a eukaryotic promoter and a phage display version of the same antigen present on the phage surface have recently been suggested as potential components of a hybrid phage [116]. A vaccination of this type would successfully target the humoral and cellular immune systems [59]. By adding particular protein sequences to target particular immune cell types, such as galactose residues that will target galactose-recognizing hepatic receptors in the liver, it can also be extended to the alteration of the phage vaccine's surface. By separating peptides from the phage display libraries, Langerhans cells and dendritic cells [118] may both be targeted [119].

Gene delivery

The possible therapeutic gene delivery agents are phages [120,121]. Phage delivery of DNA vaccines, in which the phage coat shields the DNA inside from degradation after injection, is comparable to the use of phages for targeted gene delivery. Both, however, are conceptually distinct. Phages can target particular cell types because of their capacity to show foreign proteins on their surfaces, which is a requirement for effective gene therapy [116]. To show the targeting and processing molecules on the surfaces of phages, researchers have employed phage display and artificial covalent conjugation [122,123]. Targeting sequences, such as fibroblast growth factor, have been utilized to deliver phages to cells with the necessary receptors [124,125]. Protein sequences like the adenovirus penton base, which mediates entry, attachment, and endosomal release, are employed to increase the absorption and endosomal release of phages [126]. The absorption and nuclear targeting of modified phages like lambda have also been improved by the protein transduction domain of the human immunodeficiency virus (HIV) tat protein and the nuclear localization signal of the simian virus 40 (SV40) T antigen [127].

Other displayed peptides that can facilitate gene delivery via phages include integrin binding peptides that enhance binding and uptake [124] and DNA degradation reducing DNase II inhibitors [125]. To screen the ability of phages to target specific cells and tissues, phage display libraries have been used in mice many times and every time phages were found in specific tissues [128]. For instance, to isolate phages that target the liver, mice were inoculated with phage display libraries and phages were isolated after extracting the livers [116]. A similar in vitro strategy is used for the isolation of phage-displayed peptides that enhance cytoplasmic uptake into mammalian cells [129]. Therefore, phages once more demonstrated their versatility by making it possible to target particular tissues either by randomly scanning phage display libraries or by rational design [116].

Conclusion and recommendations

Generally, phages are a type of virus extensively disseminated in nature whose progression is firmly connected with the bacterial cell. They have been used for various types of applications starting from their discovery time and are still playing significant roles in modern biotechnology since their genomes are easily manipulated. Either lytic or lysogenic bacteriophages are estimated to be found everywhere the host bacteria live. Hence, it's possible to get and isolate these viruses from any type of sample from which the hosts can be obtained. Phages have increased potential application in different parts of the world, especially in western countries, as therapeutic agents in different hospitals, clinics and food industries due to the rise in antimicrobial resistance strains of different bacteria pathogens. However, in developing countries, these phage futures are not well pursued or investigated. Bacteriophages look to be a great solution as an alternative treatment against bacterial diseases, but they are also used as interesting tools in vaccine delivery vehicles, modulating bacterial populations and assisting in diagnosis. Therefore, based on

the above conclusion, the following points are forwarded as recommendations.

- It is recommended that bacteriophage therapy be expanded in various hospitals, clinics, and food industries to alleviate the burden of multi-drug-resistant bacterial pathogens.
- It is good if the building different phage companies are used for the production of different phage cocktails that have treatment effects will be constructed.
- It is recommended that more awareness be raised about bacteriophage research in order to develop monitoring and safety procedures that will lead to the widespread use of bacteriophages in various fields, including clinics.

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