Iranian Journal of Virology 2018;12(2): 41-56
©2018, Iranian Society of Virology

Review Article

Bacteriophages: Ecology and Applications

Ramin Mazaheri Nezhad Fard [1,2*]

1. Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2. Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Table of Contents

Bacteriophages: Ecology and Applications……...1

Abstract 1

Introduction 2

Background 2

Ecology 2

Genomics 6

Collection 7

Applications 8

Conclusions 11

Acknowledgments 11

References 11

Abstract

Bacteriophages are prokaryotic viruses, which multiply in bacteria and archaea. These viruses are important in transferring mobile genetic elements such as virulence and antimicrobial resistance genes to bacteria under a process called transduction. Although bacteriophages have long been addressed with their various medical applications, an exciting application of these viruses is linked to their infection treatment potential. Since antimicrobial resistance is rapidly extending in bacterial populations and no novel antibiotics have been introduced to the market in decades, alternative treatment protocols such as phage therapy must be further supported to secure the future of infection treatments. The present review molecularly introduces bacteriophages and their major applications.

Keywords: Bacteriophage, ecology, transduction, infection, alternative treatment

Introduction

Background

However viruses are not biologically categorized as living cells, they are enlisted in literatures as microbes. One of these non-living microbes, bacteriophages, play great roles in bacterial pathogenicity and coevolution. Practically, they can be used for the development of novel DNA and protein vaccines, discovery of new antibiotics and antiviral drugs, introduction of modern gene and phage therapies, and studies of bioremediations (1, 2). Apparently, phage therapy is the most interestingly futuristic application of the bacteriophages since the bacterial antimicrobial resistance is a major complexity in infection treatment and possibly becoming a catastrophic issue in future. This review explains some of the major current and future applications of the bacteriophages after a brief introduction on the phage ecology.

Ecology

Although some may like to use "physiopathology" for the bacteriophage life and activities, "ecology" seems a further appropriate descriptive word for their life cycle as bacteriophages and viruses, in general, do not have any live metabolism or physiology (that is why viruses are categorized in none of the two main domains of life). However, like other viruses, bacteriophages include a precisely intelligent life strategy to multiply in bacteria. Bacteriophages infect bacterial hosts by a process called transduction. Transduction can be a part of the viral life cycle in bacteria. Bacteriophages life cycle includes infection of bacteria and multiply in them in five major steps: 1) Binding or attachment (commonly adsorption in viruses) of the bacteriophage to a bacterial specific receptor such as surface protein, carbohydrate or lipopolysaccharide molecule; 2) Injection of the bacteriophage genome into the bacterial cell (rather cell penetration in eukaryotic viruses); 3) Synthesis of the bacteriophage genome and proteins using bacterial replication machinery; often as fast as half an hour for lytic bacteriophages. For the lysogenic bacteriophages, genome is inserted in the host genome without multiplication); 4) Assembly of the bacteriophages; and 5) Release of the bacterio-phages; mostly by lysis or extrusion but in a few cases by budding (3). The bacterial phage receptors can be located in the outer membrane of Gram-negative bacteria, in the cell wall of Gram-positive bacteria, in the capsular or slime layer, and in association with flagella or pili (4).

Bacteria can resist bacteriophage infections by blocking each of above steps (5). This pressure has resulted in an "evolutionary arms race" (in some extend resembling "Red Queen hypothesis") between the bacterio-phages and their host bacteria through the time; in which, adaptive variations and selections occur in the host as well as its parasites (6).

Furthermore, some bacterial genera resist bacteriophage infections using clustered, regularly interspaced, short palindromic repeats (CRISPR) within their genomes, which contain sequences similar to those of bacteriophages (7). The CRISPR/Cas system acts as a prokaryotic immune system against foreign genetic elements such as bacteriophages and plasmids and is seen in nearly 40% of bacterial and 90% of archaeal tested genomes (8).

Further bacterial resistance mechanism against bacteriophages includes restriction-modification (RM) system seen in nearly 90% of prokaryotic genomes; by which, restriction enzymes cleave foreign DNA and modification enzymes protect host DNA from changes by predators (9).

Several other antiviral resistance mechanisms have been described in bacteria such as abortive (Abi) mediated resistance (mostly in gamma-proteobacteria and Firmicutes) which results in the death (suicide) of host cells (10). Generally, transduction is one of the most

***Corresponding author**:
Dr. Ramin Mazaheri Nezhad Fard
Assistant Professor in Medical Bacteriology
Email: r-mazaherinf@sina.tums.ac.ir
Office: +98-21-42933208
Mobile: +98-9129303788
Address: Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Pour Sina St., Keshavarz Blvd., Tehran, Iran.

widespread forms of indirect horizontal/lateral gene transfer (HGT/LGT) and is reported as a significant mechanism in microbial ecology (11, 12).

Sometimes, bacteriophage transduction results in the transfer of multiple-resistance R plasmids between Gram-positive bacteria (e.g. Staphylococcus. aureus and S. pyogenes); however, cellular conjugation is recognized as the major route of transferring mobile genetic elements (MGEs) within bacteria (13).

Transduction has been studied broadly in Gram-positive bacteria such as enterococci, streptococci, staphylococci, Listeria spp., Bacillus spp. and Lactobacillus spp. (14–18).

These studies of transduction included different functions such as mutagenesis, packaging and replicating activities of lytic and temperate bacterio-phages. For example, transduction is supposed to be the most common HGT mechanism in S. aureus (15). In summary, transduction has been demonstrated to have a role in transfer of antimicrobial resistance genes in a variety of bacteria.

Bacteriophages are functionally categorized into two major groups: lysogenic and lytic bacteriophages (19, 20). The former group (alternatively called temperate or mild bacteriophages) infects bacteria and resides inside them without any disruption in the bacterial cell, while the latter (also known as virulent or killer bacteriophages) multiplies at the expense of bacterial life (Figure 1). Further bacteriophage functions in the bacterial host include chronic and persistent infections. In chronic infections, the bacteriophage progeny is released from the host cell by budding with no lysis occurs. In persistent infections (also known as pseudolysogeny or phage-carrier state), bacteriophages multiply in a part of the bacterial population (11). In lysogeny, in which the viral genome is integrated into the bacterial DNA and known as a "prophage", the bacteriophage uses its host for replication of its genome and expression of some proteins (21, 22). Prophages are found in two-thirds of all gammaproteobacteria and low-GC Gram-positive bacteria mostly share in conjugative plasmids and related proteins (22, 23). Lysogenic bacteriophages can shift to a lytic phase (lysogenic induction) by sublethal doses of ultraviolet (UVC) irradiation or subtherapeutic concentrations of some antibiotics such as mitomycin C and norfloxacin (24–27). This results in replication and production of progeny bacteriophages. Furthermore, other antimicrobials such as some animal growth-promoting antibiotics are known to contribute to the HGT of antimicrobial resistance between bacteria by means of bacteriophages but with different mechanisms (27). A possible explanation is the release of bacteriophages from the dead bacteria directly into the intestines due to the action of the antibiotics. Olaquindox and carbadox are two of these antibiotics, which contribute to spread Shiga toxin-encoding bacteriophages in animal intestines (28). In contrast, some other antibiotics such as tylosin and monensin have an inhibitory effect on bacteriophage induction (27). However, bacteriophages can be inactivated by ultraviolet (UVB) irradiation; some are sensitive to chemicals such as ether and chloroform (29, 30). In both Gram-positive and Gram-negative bacterial pathogens, inserted bacteriophage genomes—prophages—can encode and express virulence factors such as toxins (e.g. Shiga toxins of Escherichia coli, leukocidins, and superantigens of S. aureus and S. pyogenes) (31–35).

Lysogeny can rapidly drive genetic changes and evolution by processes such as gene duplication and mutation in contrast to vertical gene transfer. In fact, HGT replaces a sexual life cycle in bacteria. The DNA transferred horizontally ranges in size from less than 1 kb to more than 100 kb (20) and can encode metabolic pathways, surface structures, toxins and mobile pathogenicity islands (22, 31, 35). For example, toxins of Corynebacterium diphtheria (diphtheria), Clostridium botulinum (botulism), Bordetella pertussis (pertussis or whooping cough), Yersinia enterocolitica (yersiniosis), spirochetes (spirochetosis), S. pyogenes (scarlet fever), S. aureus (food poisoning) and E. coli (Shiga toxins) are all bacteriophage encoded (36–39). Furthermore, some outer membrane proteins (OMPs) of E. coli and some antibiotic resistance genes (e.g. van genes) are encoded by genes carried by

bacteriophages. Examples of other proteins that are encoded by the genes carried by the bacteriophages include ADP-ribosyl transferase toxins, superantigens, LPS-modifying enzymes, type III effector proteins, detoxifying enzymes, hydrolytic enzymes and serum resistance proteins. Moreover, bacteriophages may carry metabolic genes acquired from the host genome; named auxiliary metabolic genes (AMGs) (36). Some AMGs which are not essential for the bacteriophage life cycle but help during lytic replication include those functioning in photosynthesis (40), the pentose phosphate pathway (41), phosphate acquisition (42, 43), nucleotide metabolism (44–47) and cyto-skeletal construction (48). Many prophage genes linked to potential virulence factors have been identified in bacterial pathogens, but their role in bacterial pathogenicity is still unknown (22). However, the products of these genes may have significant effects on the host bacteria, which can have its phenotype modified by expression of genes encoded by the prophage. These changes range from protection against further bacteriophage infection to increasing the virulence of a pathogenic host. For example, in some cases, regulatory proteins expressed by prophages are known to alter the pathogenesis of the host bacteria (49). However, prophages may not be necessary for the evolution of every pathogen. Moreover, acquisition of virulence genes is not a unique evolutionary mechanism in bacteria. Pathogenic bacteria also develop from commensal bacteria by loss of genes, which may accidentally occur by excision of temperate bacteriophages. In contrast to temperate bacteriophages, the mechanism of lytic bacteriophages is quite different. In the lytic phase, the bacteria are destroyed by the viriome as a natural consequence of multiplication (50, 51). Bacteriophages (usually dsDNA bacteriophages) use murein-degrading enzymes (endolysins or lysins) and small hydrophobic pore-forming proteins (holins), which assist the endolysin, in the destruction of bacterial cell wall and release of newly synthesized bacteriophages (52, 53). These enzymes work synergically with each other and with antibiotics as well. Up to 10−100 virions/cell can be released. The relationship between the activity of lytic bacteriophages and the decrease in number of enteropathogens has been studied (54). Lysogenic bacteriophages, however, only undergo the lytic phase under special usually stressful conditions (55, 56).

Bacteriophages can play a role in transferring genes, such as those responsible for antibiotic resistance, via transduction (57). Gene transfer via transduction occurs when bacteriophages package host bacterial DNA rather than bacteriophage DNA during bacteriophage replication. Transduction is divided into two major types, generalized and specialized, based on the number and location of genes transferred (58). In generalized transduction, potentially any bacterial gene can be transferred into the recipient cell. The bacterial host DNA is broken down into smaller fragments and randomly packed into the replicating bacteriophage (lytic) particles (22). The size of packed DNA is small enough to fit into a bacteriophage head (20). This genome transfer may result in recombination.

In specialized transduction, in contrast, only certain genes can be transferred from the donor bacteria to the recipient cell, meaning that only the host DNA on either side of the insertion site of prophage can be transferred (59, 60). The release of inserted prophages is controlled by several factors as environmental (e.g. radiation, humidity, temperature), nutritional and chemical (e.g. antibiotics) conditions (61). Possible recombination between the donor and recipient genes may occur. Lysogenic conversion is naturally seen in both Gram-positive and Gram-negative bacteria (62). This

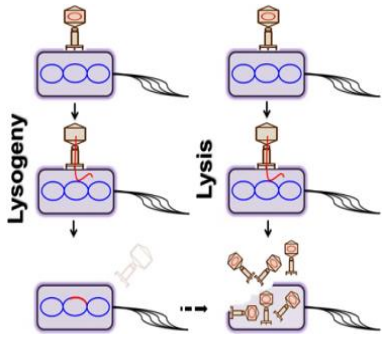


Fig. 1. Schematic of lysogeny and lysis phases (courtesy of R. Mazaheri Nezhad Fard)

process plays an important role in bacterial evolution as many virulence factors can be transferred between different bacteria (20).

This is important because some changes in bacterial virulence and fitness such as acquisition of antimicrobial resistance can also occur (22). Examples of virulence genes transferred between Gram-positive bacteria by lysogenic conversion include neurotoxin genes in C. botulinum (63, 64), enterotoxin, staphylokinase and toxic shock syndrome toxin-1 genes in S. aureus (65–68), erythrogenic toxin genes in S. pyogenes (69, 70) and diphtheria toxin genes in C. diphtheriae (71). Examples of virulence genes transferred between Gram-negative bacteria by lysogenic conversion include Shiga toxins, enterohemolysin, serum resistance and host cell envelope protein genes in E. coli O157:H7 (72–75), O-antigen acetylase and glucosyl transferase genes in Shigella flexneri (76–80), type III effector, superoxide dismutase, neuraminidase, insertion element and glucosylation genes in Salmonella enterica (81—85), cholera toxin, G-protein like and TCP pilin genes in V. cholera (86–88) and cytotoxin genes in Pseudomonas aeruginosa (89). In contrast, most of the antimicrobial resistance genes are transferred by the mechanisms other than transduction such as the conjugation. Conjugation is the major mechanism of acquired gene (e.g. antibiotic resistance and virulence) transfer in Gram-positive bacteria (90). This finding supports the hypothesis proposing the genetic exchange between low-GC bacteria (91). It is estimated that most of the myoviruses which infect the different genera of Listeria, Staphylococcus, Bacillus and Enterococcus belong to broad-host-ranges, virulent SPO1-like bacteriophages such as A511 (92). Furthermore, interspecies transduction has been confirmed in Gram-negative bacteria as well (93). Affinity of bacteriophages to mammalian cells was shown in the late 90s (94). However, the importance of bacteriophages in gene transfer is underestimated, compared to other transfer routes such as transformation and conjugation.

Contrary to this heterospecific transfer evidence, literature reviews commonly mention that most of the bacteriophages are highly species specific, having a strong affinity to a particular group of bacteria (95–99). This specific host range—which is used in the typing of bacteria such as Escherichia, Salmonella and Shigella—is possibly due to the production of specific lysing enzymes by the bacteriophages (52). However, these enzymes sometimes act more flexibly and lyse a broader spectrum of bacterial genera, such as the effect of an enterococcal bacteriophage lysin on other Gram-positive pathogens such as S. pyogenes, Group B streptococci, and S. aureus (100). O'Flaherty et al. (2005) cloned and expressed a staphylococcal lysin (LysK) in L. lactis (101). They found LysK had an antimicrobial potency against staphylococci. Moreover, some researchers argue that the bacteriophage infection mechanism is established on a strain-specific basis (102). For example, vibriophages have successfully been used for typing of 1000 Vibrio strains (103).

Genomics

Recently, comparative genomic hybridization (CGH) studies have shown a significant variation in genomes of different bacteria (104). A variety of MGEs such as bacteriophages are presumed to contribute greatly to this variation. Nowadays, hundreds of bacteriophage genomes have been sequenced; however, it is considerably lower than the number of bacterial genomes sequenced despite usually having a genome 100 times larger (105). Fiers from the University of Ghent in Belgium was the first to complete sequence of a gene (1972) and the genome of bacteriophage MS2 (1976) (106).

Although some identities are seen within the genomes of different bacteriophages of the same bacterial host, there is a significant diversity between the different bacteriophage clusters. The dsDNA tailed bacteriophages constitute a substantial proportion of bacteriophage population; some are completely sequenced (107–109). Most of these sequenced bacteriophages infect enteric and dairy bacteria as well as Mycobacterium spp., Pseudomomas spp., Staphylococcus spp. and marine bacteria.

A variety of techniques is used for preparing microbial genomes for sequencing in prokaryotes. Degenerate oligonucleotide primed amplification (DOP-PCR) is an example of these techniques used for the whole genome amplification (WGA) of microbial DNA. Other techniques include multiple displacement amplification (MDA), primer extension preamplification (PEP), improved primer extension preamplification (iPEP) and long products from low DNA quantities degenerate-oligonucleotide-primed PCR (LL-DOP-PCR).

These methods, capable of generating high-quality amplicons from the entire genome (110), were first described by Telenius et al. in 1992 (111).

The species-independent method, DOP-PCR, is applied for fingerprinting and genome mapping of microorganisms even with low quantity or poor quality genomic DNA template (112). In general, the whole genome amplification of genomic DNA is carried out using different methods such as Sanger method and "next generation" sequencing method. The Sanger method, introduced by Sanger et al. in 1977, is the premier method for de novo whole genome sequencing (113). This technique includes synthesis of a complementary DNA template using natural 2-deoxynucleotides (dNTPs) and termination of synthesis using 2,3-dideoxynucleotides (ddNTPs) by DNA polymerase (114). However, this method is relatively expensive and time consuming (113). Other methods for high-throughput DNA sequencing have recently been developed that are relatively cheaper and much faster. Some of these methods include sequencing by hybridization (SBH), nanopore sequencing and sequencing by synthesis (SBS) (115).

For examples, the Illumina Solexa Genome Analyzer (Illumina, USA) (www.illumina .com), which has been introduced in early 2000s and uses a "sequencing by synthesis" technology, sequences millions of short fragments or reads (generally 25–50 bp) in parallel (113). Another powerful sequencing machine, Roche 454 GS FLX (Roche, Switzerland) (www.roche.com), has been introduced to the market in 2005 using pyrosequencing technology. These technologies are not only very accurate, but also do not require extra cost or incurring steps such as cloning. These novel technologies also allow single nucleotide polymorphisms (SNPs) detection, cancer genome analysis and large high-GC genome sequencing (116).

Collection

Bacteriophages can be detected, isolated, identified and characterized using a variety of phenotypic and genotypic methods. However, a precise identification of bacteriophages needs both methods. Briefly, phenotypic methods include plaque assay and purification methods, bacteriophage induction and the EM.

Bacteriophages can be detected using "Cross Test" plaque assay method (117, 118). The method uses a bilayer agar including the indicator bacteria. Solid purification methods (sometimes up to five times of repetition) are mostly used to make a "pure line" of the bacteriophages (119). Bacteriophage induction is used to identify lysogenic bacteriophages through shifting to lytic ones using UVC irradiation or some antibiotics. After these methods used to detect and isolate the bacteriophages, transmission EM or scanning EM (SEM, TEM, CTEM, STEM) is used for the bacteriophage morphology studies.

Preparation of bacteriophages for the EM study includes concentration, stabilization and heavy-metal staining (mostly positive rather than negative staining) (120). Genotypic methods include PCR, genome typing, genome walking, whole/complete genome sequencing and finger printing techniques such as amplified fragment length polymorphism (AFLP), multilocus sequence typing (MLST), multiple-locus variable number tandem repeat analysis (MLVA), pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) (121–124). In general, PCR of bacteriophage partial genes or genome may not be very helpful since the bacteriophage genome is relatively much smaller (sometimes only a few thousand bases) than the bacterial genome. Therefore, differentiation between closely related bacteriophages

may not be possible. This prevents the accurate identification and characterization of the bacteriophages. Similarly, genome-typing methods, in which restriction endonuclease enzymes are used, only help to categorize bacteriophages in Baltimore method. Genome walking helps when the multi-locus amplification methods fail to produce the complete genome and genome gaps are needed to be filled. Thus, further comprehensive molecular methods such as sequencing are recommended. For the whole genome sequencing (WGS) of a bacteriophage, first the amplified genome is cloned to an appropriate competent host (commonly E. coli) using a plasmid vector. Genome amplification can be carried out by means of various PCR techniques such as DOP-PCR, Semi DOP-PCR and Long-range PCR. The cloned genome is amplified using Colony-PCR and then will be sequenced. For the old-fashioned Sanger sequencing method, amplified PCR products first are used in post cycle-sequencing reaction (dye terminator) purification. The products of the cycle sequencing reaction are then injected electrokinetically into capillaries filled with polymer. The negatively charged DNA fragments move through the polymer in the capillaries toward the positive electrode due to applying high voltage. Capillary electrophoresis resolves DNA molecules by molecular weight. These separated fluorescent-labeled DNA fragments (dye terminated) move through the path of a laser beam and are detected by an optical detection device. The data collection software converts the fluorescence signal to digital data then records these data. After electrophoresis, data are analyzed by collection software (primary analysis) and downstream software (secondary analysis). Sequencing results are analyzed using Software such as DNASTAR Lasergene (DNASTAR, USA; www.dnastar.com) and CLC Workbench (QIAGEN, Germany; www.qiagen.com) and bioinformatics database such as GenBank and ExPASy. In addition to the mentioned technique, commercial shotgun sequencing methods are used to sequence the bacteriophage genome, especially when large genomes are targeted. Usually, bacterial artificial chromosomes (BACs) are constructed from the bacteriophage genome and then constructed genomes are passed through a mesh using the pressure of an air gun to produce millions of short sequences, namely reads. These short reads can be assembled using a closely related genome sequence assembly (template assembly using previously sequenced genomes as backbone), de novo (blind) assembly or a combination of the two methods. Contrary to top-down sequencing methods, in which a large source clone is firstly mapped by a BAC to BAC approach and then is broken up into smaller sub-clones, shotgun sequencing methods or the whole-genome shotgun method produce random mixtures and sub-clones instead of a physical map (125). Therefore, this method is much faster and cheaper but assembly of random sequenced reads is more difficult and more likely to fail to produce the complete genome. However, whole genome shotgun approach using DNA library is reported as the most efficient method to sequence the genome of bacteria and DNA bacteriophages (126). However, shotgun-sequencing methods are relatively more expensive than in house sequencing methods.

Applications

Recent public interests in bacteriophages are briefly linked to genome and evolution research and infection therapy (127).

Bacteriophages have played a long act in modern research. In 1952, two American geneticists, Alfred Hershey and Martha Chase, used T2 bacteriophages in their famous experiments so called “the Hershey-Chase blender experiments” to investigate whether DNA or protein was the genetic material of life (Figure 2) (128). They were awarded the Nobel Prize for Physiology or Medicine in 1969.

More, bacteriophage phiX174 was the first organism that its genome was completely sequenced by Sanger et al. in 1977 (Figure 3) (114). Giving further examples for the role of bacteriophages in research, phage display, SEPTIC method, phage-ligand technology and model organisms can be listed. In phage

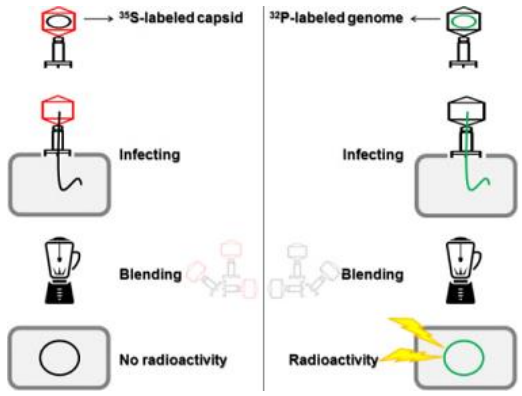


Fig. 2. Schematic of the Hershey-Chase blender experiment (courtesy of R. Mazaheri Nezhad Fard).

display, protein-protein, protein-peptide and protein-DNA interactions are studied using bacteriophages (in vitro selection). In this 1985-introduced laboratory technique, a protein-encoding gene is inserted into a bacteriophage (e.g. lambda, M13, T4, T7) gene encoding coat protein, resulting in display of the interest protein on the surface of the bacteriophage (Figure 4) (129–131). Various bacteriophages, mostly filamentous ones, are used in phage display. Phage display is a favorably precise technique, which can be used especially in Ab-Ag interaction studies. Nowadays, phage display is preferably used to express and study proteins rather than other techniques such as bacterial display, yeast display, ribosome display and mRNA display. Various databases and online computational tools are available for the analysis of the phage display generated data (132). Another technique, the SEPTIC bacterial sensing and identification method, is based on the ion emission during the bacteriophage infection. This is a highly specific and rapid method for the bacteriophage detection and identification (133). In 2011, the US Food and Drug Administration (FDA) approved the first in vitro diagnostic product using cocktail bacteriophages with the commercial name of KeyPath MRSA/MSSA Blood Culture Test (MicroPhage, USA). This rapid test detects methicillin-resistant S. aureus (MRSA) as well as methicillin-susceptible S. aureus (MSSA) in blood cultures. Phage-ligand technology uses recombinantly expressed bacteriophage proteins for various purposes such as binding of bacteria and their components and lysis of bacteria. Furthermore, bacteriophages are well known as model organisms in research.

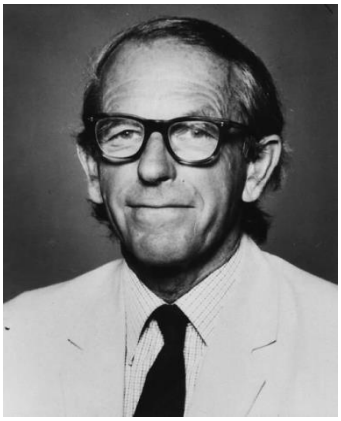


Fig. 3. Frederick Sanger (from National Library of Medicine).

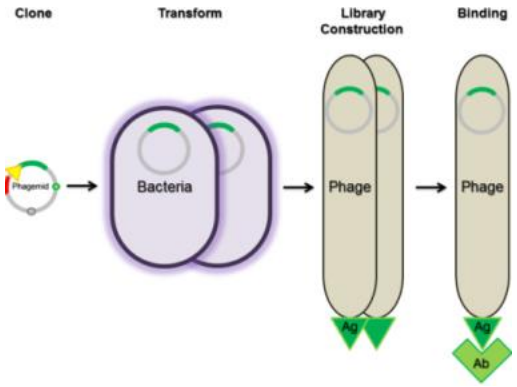


Fig. 4. Schematic of the phage display (courtesy of R. Mazaheri Nezhad Fard).

In addition to broad genetic research targets of bacteriophages; however, the most interesting area of the bacteriophage research seemingly belongs to medical treatments. Treatment potency of the bacteriophages was first suggested by the discoverer of bacteriophages, the famous French-Canadian scientist Felix d'Herelle, at the Institute Pasteur in Paris, 1919 (134). This was followed by the first report on the application of bacteriophages in treatment of staphylococcal skin infections in 1921 by Richard Bruynoghe and Joseph Maisin. Later, Newton Larkum, an American pathologist, published a series of papers on bacteriophages and phage therapy in 1926, 1929, 1930 and 1933; some were part of his PhD thesis in Yale University, USA (135–139). However, the idea of establishing a phage therapy center is coined to the Georgian physician, bacteriologist and

Table 1. Comparison of bacteriophages with antimicrobials in treatment of infections

Bacteriophages	Antimicrobials	Comments
Mostly highly specific; secondary infections mostly rare	Target pathogens & microflora. Changes in microbial balance may lead to secondary infections	High specificity may considered as a disadvantage because pathogens must be identified before treatment. Antimicrobials may be more effective than phages when treatments are blind
Multiply in infection sites	Generally distributed over the body not necessarily concentrate in infection sites	Phages may prescribed in less doses to achieve the optimal therapeutic effects
No serious side effects	Common side effects; e.g. allergies, secondary infections	Minor side effects reported in phage therapy
Pathogens resistant to a phage not necessarily resistant to other phages	Multiple resistances seen often	Antimicrobials select for resistant bacteria not just for resistant mutants due to their broader-spectrum activities
Finding novel phages relatively rapid & cheap	Developing novel antimicrobials very time & money consuming	Phages can be selected against antimicrobial or phage-resistant bacteria by natural selection

Modified from Sulakvelidze et al., 2001

bacteriophage researcher Professor George Eliava, who founded the Eliava Institute in Tbilisi in 1923 (www.eliava-institute.org). Moreover, the institute was developmentally contributed by Felix d'Herelle to establish the World Centre of Phage Research and Phage Therapy. This was later followed by the foundation of Institute of Immunology and Experimental Therapy by the Polish Academy of Sciences and Professor Ludwik Hirszfeld, famous Polish immunologist and microbiologist, in Wroclaw, Poland, in 1952 (www.iitd.pan.wroc.pl). Nowadays, other phage therapy centers serve patients, including Phage Therapy Center in Tbilisi, Georgia, owned by the Phage International (www.phagetherapycenter.com). In general, bacteriophages have medically been used to treat different infections such as dysentery, salmonellosis and gastroenteritis, mostly in East European countries (103, 140). Additionally, they are used to treat skin, mucosa and wound infections (140). Some commercially made pharmaceutical bacteriophage products include Stafal®, Staphylon®, Intestibacteriophagum Liquidum® Pyobacteriophagum® and Pyobacteriophagum Poly-valentum® (Figure 5).

For example, Pyobact-eriophagum Polyvalentum® is claimed to effectively lyse various eubacterial genera, including Proteus spp., Streptococcus spp., Staphylococcus spp., Klebsiella pneumonia, P. aeruginosa and E. coli. In addition to successful stories from human medicine trials, clinical trials in veterinary medicine have demonstrated some hoping results. Examples include treatments of pet dogs with bacterial otitis (141).



Fig. 5. Pyobacteriophagum™ ampules including bacteriophages produced by Eliava BioPreparations in Eliava Institute, Tbilisi, Georgia (modified from Eliava Institute website).

Nowadays, governments are becoming more and more interested in phage therapy and support related studies by funding them. This has been accelerated as fast developing antimicrobial resistance threatens public health and urges both public and private medical sections to shift to antibiotic replacements (142). The so-called tsunami of microbial resistance alerts decision makers to bank more money in alternative methods of treating infections, especially phage therapy. The question is that why they have not done this yet as the mission must had been completed years ago. A possible answer could be found when comparing the small effort expended to substitute antimicrobials and those expended to

replace oil-derived energy production! It sounds familiar, as everywhere-accessible resources do not seem fascinating enough to fuel new engines! Fortunately, limited-action antimicrobials have emerged decision makers and authorities to start new engines. For example, a new project on phage therapy is running by the Phagoburn, a European R&D project funded by the European Commission (www.phagoburn.eu). The major purpose of the project is assessment of phage therapy for the treatment of burn wounds infected with E. coli and P. aeruginosa. However, similar to most other treatment protocols, phage therapy includes advantages as well as undesirable disadvantages (Table 1).

Some of the advantages of phage therapy include effectiveness against MDR bacteria, high specificity for target bacteria, no selective resistance, rapid response to resistant mutants, no chemical residues, uncommon side effects (e.g. Jarisch-Herxheimer reaction, toxic shock syndrome) and cheaper development costs.

Of possible phage therapy disadvantages, the following issues can be concerned: public acceptance, treatment accessibility, phage neutralization, lysis-lysogeny intershift and possible bacterial resistance. However, the helpfulness of bacteriophages is not limited to in vivo bacterial eliminations since an interesting application of bacteriophages is linked to their use as in vitro neutralizers of pathogenic bacteria. An ongoing hypothetical idea includes neutralizing bacterial bioweapons such as anthrax and botulism using bacteriophages. Other limited currently available ideas include spray of bacteriophages in horticulture for the prevention of plant decays and diseases caused by the bacteria and use of bacteriophages as biocides on peripheral hard surfaces such as surfaces in clinics and hospitals and surfaces of medical devices such as catheters as well as soft surfaces such as uniforms and curtains. Since 2006, FDA and US Department of Agriculture (USDA) have approved bacteriophage products such as those used in treatment of ready-to-eat (RTE) meat products, on cheese to clear L. monocytogenes and on almost all food products.

Therefore, bacteriophages are given credits as "generally recognized as safe and effective" or GRASE (GRAS/GRAE or GRAS/E) by the admini-strator organizations (143).

Conclusions

In conclusion, bacteriophages provide novel research and treatment solutions for the current scientific and medical problems. Major examples include phage display, genetic study, DNA and recombinant vaccination, antimicrobial alternation and phage therapy. Further studies on the bacteriophage ecology and genomics are necessary to decode additional applications of these useful viruses.

Acknowledgments

The author wishes to thank Prof. M. Barton from University of South Australia and A/Prof. M. Heuzenroeder from University of Adelaide, Australia.

References

1. Turton JF, Perry C, Hannah MJ. Isolation of bacteriophage against currently circulating strains of Acinetobacter baumannii. J Med Microb Diagn. 2012;1:109–11.
2. Serwer P, Hayes SJ, Thomas JA, Griess GA, Hardies SC. Rapid determination of genomic DNA length for new bacteriophages. Electrophoresis. 2007;28:1896-902.
3. Heineman RH, Bull JJ. Testing optimality with experimental evolution: Lysis time in a bacteriophage. Evolution. 2007;61:1695–709.
4. Lindberg AA. Bacteriophage receptors. Annu Rev Microbiol. 1973;27:205–41.
5. Clokie MRJ, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage. 2011;1:31–45.
6. Stern A, Sorek R. The phage-host arms race: Shaping the evolution of microbes. BioEssays. 2011;33:43–51.
7. Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and Enterococcus faecium. Clin Microbiol Infect. 2010;16:541–54.

8. Sorek R, Kunin V, Hugenholtz P. CRISPR— a widespread system that provides acquired resistance against phages in bacteria and archaea. Nat Rev Microbiol. 2008;6:181–6.
9. Roberts RJ, Vincze T, Posfai J, Macelis D. REBASE—a database for DNA restriction and modification: enzymes, genes and genomes. Nucleic Acids Res. 2010;38:D234–6.
10. Chopin, MC, Chopin A, Bidnenko E. Phage abortive infection in lactococci: variations on a theme. Curr Opin Microbiol. 2005;8:473–9.
11. Weinbauer MG. Ecology of prokaryotic viruses. FEMS Microbiol Rev. 2004;28:127–81.
12. Weinbauer MG, Rassoulzadegan F. Are viruses driving microbial diversification and diversity? Environ Microbiol. 2004;6:1–11.
13. Mathur S, Singh R. Antibiotic resistance in food lactic acid bacteria-a review. Int J Food Microbiol. 2005;105:281– 95.
14. Mazaheri Nezhad Fard R, Barton MD, Heuzenroeder MW. Bacteriophage-mediated transduction of antibiotic resistance in enterococci. Lett Appl Microbiol. 2011a,52:559–64.
15. Lindsay JA, Holden MTG. Understanding the rise of the superbug: investigation of the evolution and genomic variation of Staphylococcus aureus. Funct Integr Genomics. 2006;6:186–201.
16. Chandry PS, Moore SC, Davidson BE, Hillier AJ. Transduction of concatemeric plasmids containing the cos site of Lactococcus lactis bacteriophage sk1. FEMS Microbiol Lett. 2002;216: 85−90.
17. Hodgson DA. Generalized transduction of serotype 1/2 and serotype 4b strains of Listeria monocytogenes. Mol Microbiol. 2000;35:312−23.
18. Storrs MJ, Courvalin P, Foster TJ. Genetic analysis of gentamicin resistance in methicillin- and gentamicin-resistant strains of Staphylococcus aureus isolated in Dublin hospitals. Antimicrob Agents Chemother. 1988;32:1174−81.
19. Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B. Bacteriophages and phage-derived proteins – application approaches. Curr Med Chem. 2015;22:1757–73.
20. Canchaya C, Fournous G, Chibani-Chennoufi S, Dillmann M, Brussow H. Phage as agents of lateral gene transfer. Curr Opin Microbiol. 2003; 6:417–24.
21. Lindsay AM, Zhang M, Mitchell Z, Holden MT, Waller AS, Sutcliffe IC, et al. The Streptococcus equi prophage-encoded protein SEQ2045 is a hyaluronan-specific hyaluronate lyase that is produced during equine infection. Microbiology. 2009;155:443–9.
22. Brussow H, Canchaya C, Hardt W. Phages and the evolution of bacterial pathogens: from genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev. 2004;68:560–602.
23. Weaver KE, Kwong SM, Firth N, Francia MV. The RepA N replicons of Gram-positive bacteria: A family of broadly distributed but narrow host range plasmids. Plasmid. 2009;61:94–109.
24. Rokney A, Kobiler O, Amir A, Court DL, Stavans J, Adhya S, et al. Host responses influence on the induction of lambda prophage. Mol Microbiol. 2008;68:29–36.
25. Aertsen A, Faster D, Michiels CW. Induction of Shiga toxin-converting prophage in Escherichia coli by high hydrostatic pressure. Appl Environ Microbiol. 2005;71:1155–62.
26. Yang HY, Kim YW, Chang HI. Construction of an integration-proficient vector based on the site-specific recombination mechanism of enterococcal temperate phage ΦFC1. J Bacteriol. 2002;184: 1859–64.
27. Kohler B, Karch H, Schmidt H. Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga-toxin-2-converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. Microbiology. 2000;146: 1085–90.
28. Schmidt H. Shiga-toxin-converting bacteriophages. Res Microbiol. 2001;152:687–695.
29. Clark EM, Wright H, Lennon KA, Craik VA, Clark JR, March JB. Inactivation of recombinant bacteriophage lambda by use of chemical agents UV radiation. Appl Environ Microbiol. 2012;78: 3033–6.
30. Liao W, Song S, Sun F, Jia Y, Zeng W, Pang Y. Isolation, characterization and genome sequencing of phage MZTP02 from Bacillus thuringiensis MZ1. Arch Virol. 2008;153:1855–65.
31. Stevens RH, Porras OD, Delisle AL. Bacteriophages induced from lysogenic root canal isolates of Enterococcus faecalis. Oral Microbiol Immunol. 2009;24:278–84.
32. Maiques E, Ubeda C, Tormo MA, Ferrer MD, Lasa I, Novick RP, et al. Role of staphylococcal phage and sapi integrase in intra- and interspecies sapi transfer. J Bacteriol. 2007;189:5608–16.
33. Ubeda C, Barry P, Penades JR, Novick RP. A pathogenicity island replicon in Staphylococcus aureus replicates as an unstable plasmid. Proc Natl Acad Sci USA. 2007;104:14182–8.
34. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, et al. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet. 2002;359:1819–27.

35. Ruzin A, Lindsay J, Novick RP. Molecular genetics of SaPI1 – a mobile pathogenicity island in Staphylococcus aureus. Mol Microbiol. 2001;41: 365–77.
36. Frank JA, Lorimer D, Youle M, Witte P, Craig T, Abendroth J, et al. Structure and function of a cyanophage-encoded peptide deformylase. ISME J. 2013;7:1150−60.
37. Novick RP. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of Staphylococcus aureus. Plasmid. 2003;49 93–105.
38. Krylov VN. The role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria. Russ J Genetics. 2003;39:483–504.
39. Boyd EF, Brussow H. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. Trends Microbiol. 2002;10:521−9.
40. Mann NH, Cook A, Millard A, Bailey S, Clokie M. Marine ecosystems: bacterial photosynthesis genes in a virus. Nature. 2003;424:741.
41. Thompson LR, Zeng Q, Kelly L, Huang KH, Singer AU, Stubbe J, et al. Phage auxiliary metabolic genes and the redirection of cyano-bacterial host carbon metabolism. Proc Natl Acad Sci USA. 2011;108:E757–64.
42. Zeng Q, Chisholm SW. Marine viruses exploit their host's two-component regulatory system in response to resource limitation. Curr Biol. 2012; 22:124–8.
43. Goldsmith DB, Crosti G, Dwivedi B, McDaniel LD, Varsani A, Suttle CA, et al. Development of phoH as a novel signature gene for assessing marine phage diversity. Appl Environ Microbiol. 2011;77:7730–9.
44. Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM, et al. Functional metagenomic profiling of nine biomes. Nature. 2008; 452:629–32.
45. Mann NH, Clokie MRJ, Millard A, Cook A, Wilson WH, Wheatley PJ, et al. The genome of S-PM2, a 'photosynthetic' T4-type bacteriophage that infects marine Synechococcus strains, J Bacteriol. 2005;187:3188–200.
46. Sullivan MB, Coleman ML, Weigele P, Rohwer F, Chisholm SW. Three Prochlorococcus cyanophage genomes: signature features and ecological interpretations. PLoS Biol. 2005;3:e144.
47. Rohwer F, Segall A, Steward G, Seguritan V, Breitbart M, Wolven F, et al. The complete genomic sequence of the marine phage roseophage SIO1 shares homology with nonmarine phages. Limnol Oceanogr. 2000;45:408–18.
48. Kraemer JA, Erb ML, Waddling CA, Montabana EA, Zehr EA, Wang H, et al. A phage tubulin assembles dynamic filaments by an atypical mechanism to center viral DNA within the host cell. Cell. 2012;149:1488–99.
49. Serra-Moreno R, Acosta S, Hernalsteens JP, Jofre J, Muniesa M. Use of the lambda Red recombinase system to produce recombinant prophages carrying antibiotic resistance genes. BMC Mol Biol. 2006;7:31−42.
50. Deresinski S. Bacteriophage therapy: exploiting smaller fleas. Clin Infect Dis. 2009;48:1096–101.
51. Shao Y, Wang I. Bacteriophage adsorption rate and optimal lysis time, Genetics. 2008;180:471–82.
52. Fischetti VA. Bacteriophage lysins as effective antibacterials. Curr Opin Microbiol. 2008;11:393–400.
53. Wang I. Lysis timing and bacteriophage fitness. Genetics. 2006;172:17–26.
54. Mizoguchi K, Morita M, Fischer CR, Yoichi M, Tanji Y, Unno H. Coevolution of bacteriophage PP01 and Escherichia coli O157:H7 in continuous culture. Appl Environ Microbiol. 2003;69:170–6.
55. Shkilnyj PG, Koudelka B. Effect of salt shock on stability of λimm434 lysogens. J Bacteriol. 2007;189:3115–23.
56. McCabe BC, Pawlowski DR, Koudelka GB. The bacteriophage 434 repressor dimer preferentially undergoes autoproteolysis by an intramolecular mechanism. J Bacteriol. 2005;187:5624–30.
57. Ortmann AC, Lawrence JE, Sutlle CA. Lysogeny and lytic viral production during a bloom of the Cyanobacterium synechococcus spp. Microb Ecol. 2002;43:225−31.
58. Kelly BG, Vespermann A, Bolton DJ. Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens. Food Chem Toxicol. 2009;47:969–77.
59. Tanyashin VI, Zimin AA, Shlyapnikov MG, Boronin AM. Transduction of plasmid antibiotic resistance determinants with pseudo T-Even bacteriophages. Russ J Genetics 2003;39:761–772, translated from Genetika (2003). 39:914–26.
60. Mazodier P, Davies J. Gene transfer between distantly related bacteria. Annu Rev Genet. 1991; 25:147−71.
61. Morrison M. Do ruminal bacteria exchange genetic material? J Dairy Sci. 1996;79:1476–86.
62. Boyd EF, Davis BM, Hochhut B. Bacteriophage–bacteriophage interactions in the evolution of pathogenic bacteria. Trends Microbiol. 2001;9: 137−44.
63. Hauser D, Eklund MW, Boquet P, Popoff MR. Organization of the botulinum neurotoxin C1 gene

and its associated non-toxic protein genes in Clostridium botulinum C 468. Mol Gen Genet. 1994;243:631–40.
64. Barksdale L, Arden SB. Persisting bacteriophage infections, lysogeny, and phage conversions. Annu Rev Microbiol. 1974;28:265–99.
65. Fitzgerald, JR, Monday SR, Foster TJ, Bohach GA, Hartigan PJ, Meaney WJ, et al. Characterization of a putative pathogenicity island from bovine Staphylococcus aureus encoding multiple superantigens. J Bacteriol. 2001;183:63–70.
66. Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in Staphylococcus aureus. Mol Microbiol. 1998;29: 527–43.
67. Coleman DC, Sullivan DJ, Russell RJ, Arbuthnott JP, Carey BF, Pomeroy HM. Staphylococcus aureus bacteriophages mediating the simultaneous lysogenic conversion of beta-lysin, staphylokinase and enterotoxin A: molecular mechanism of triple conversion. J Gen Microbiol. 1989;135:1679–97.
68. Betley MJ, Mekalanos JJ. Staphylococcal enterotoxin A is encoded by phage. Science. 1985; 229:185–7.
69. McShan WM, Ferretti JJ. Genetic diversity in temperate bacteriophages of Streptococcus pyogenes: identification of a second attachment site for phages carrying the erythrogenic toxin A gene. J Bacteriol. 1997;179:6509–11.
70. Weeks CR, Ferretti JJ. The gene for type A streptococcal exotoxin (erythrogenic toxin) is located in bacteriophage T12. Infect Immun. 1984; 46:531–6.
71. Freeman VJ. Studies on the virulence of bacteriophage-infected strains of Corynebacterium diphtheria. J Bacteriol. 1951;61:675–88.
72. Neely MN, Friedman DI. Functional and genetic analysis of regulatory regions of coliphage H-19B: location of shiga-like toxin and lysis genes suggest a role for phage functions in toxin release. Mol Microbiol. 1998;28:1255–67.
73. Beutin L, Stroeher UH, Manning PA. Isolation of enterohemolysin (Ehly2)-associated sequences encoded on temperate phages of Escherichia coli. Gene. 1993;132:95–9.
74. Barondess JJ, Beckwith J. A bacterial virulence determinant encoded by lysogenic coliphage λ. Nature. 1990;346:871–4.
75. Newland JW, Strockbine NA, Miller SF, O'Brien AD, Holmes RK. Cloning of Shiga-like toxin structural genes from a toxin converting phage of Escherichia coli. Science. 1985;230:179–81.
76. Mavris M, Manning PA, Morona R. Mechanism of bacteriophage SfII-mediated serotype conversion in Shigella flexneri. Mol Microbiol. 1997;26:939–50.
77. Huan PT, Bastin DA, Whittle BL, Lindberg AA, Verma NK. Molecular characterization of the genes involved in O-antigen modification, attachment, integration and excision in Shigella flexneri bacteriophage SfV. Gene. 1997a,195:217–27.
78. Huan PT, Whittle BL, Bastin DA, Lindberg AA, Verma NK. Shigella flexneri typespecific antigen V: cloning, sequencing and characterization of the glucosyl transferase gene of temperate bacteriophage SfV. Gene. 1997b,195:207–16.
79. Clark CA, Beltrame J, Manning PA. The oac gene encoding a lipopolysaccharide O-antigen acetylase maps adjacent to the integrase-encoding gene on the genome of Shigella flexneri bacteriophage Sf6. Gene. 1991;107:43–52.
80. Verma NK, Brandt JM, Verma DJ, Lindberg AA. Molecular characterization of the O-acetyl transferase gene of converting bacteriophage SF6 that adds group antigen 6 to Shigella flexneri. Mol Microbiol. 1991;5:71–5.
81. Stanley TL, Ellermeier CD, Slauch JM. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects Salmonella enterica serovar Typhimurium survival in Peyer's patches. J Bacteriol. 2000;182:4406–13.
82. Mirold S, Rabsch W, Rohde M, Stender S, Tschape H, Russmann H, et al. Isolation of a temperate bacteriophage encoding the type III effector protein SopE from an epidemic Salmonella typhimurium strain. Proc Natl Acad Sci USA. 1999;96:9845-50.
83. Figueroa-Bossi N, Bossi L. Inducible prophages contribute to Salmonella virulence in mice. Mol Microbiol. 1999;33:167–76.
84. Hardt WD, Urlaub H, Galan JE. A substrate of the centisome 63 type III protein secretion system of Salmonella typhimurium is encoded by a cryptic bacteriophage. Proc Natl Acad Sci USA. 1998;95: 2574–9.
85. Wright A. Mechanism of conversion of the Salmonella O antigen by bacteriophage ε34. J Bacteriol. 1971;105:927–36.
86. Karaolis DK, Somara S, Maneval Jr DR, Johnson JA, Kaper JB. A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. Nature. 1999;399:375–9.
87. Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science. 1996;272:1910–14.

88. Reidl J, Mekalanos JJ. Characterization of Vibrio cholerae bacteriophage K139 and use of a novel mini-transposon to identify a phage-encoded virulence factor. Mol Microbiol. 1995;18:685–701.
89. Hayashi T, Matsumoto H, Ohnishi M, Terawaki Y. Molecular analysis of a cytotoxin-converting phage, ΦCTX, of Pseudomonas aeruginosa: structure of the attP-cos-ctx region and integration into the serine tRNA gene. Mol Microbiol. 1993;7: 657–67.
90. Dunny GM, Leonard BAB, Hedberg PJ. Pheromone-inducible conjugation in Enterococcus faecalis: Interbacterial and host-parasite chemical communication. J Bacteriol. 1995;177:871–6.
91. Lepage E, Brinster S, Caron C, Ducroix-Crepy C, Rigottier-Gois L, Dunny G, et al. Comparative genomic hybridization analysis of Enterococcus faecalis: identification of genes absent from food strains. J Bacteriol. 2006;188:6858–68.
92. Klumpp J, Dorscht J, Lurz R, Bielmann R, Wieland M, Zimmer M, et al. The terminally redundant, nonpermuted genome of Listeria bacteriophage A511: a model for the SPO1-like myoviruses of Gram-positive bacteria. J Bacteriol. 2008;190:5753–65.
93. Brabban AD, Hite E, Callaway TR. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog Dis. 2005;2:287−303.
94. Larocca D, Witte A, Johnson W, Pierce GF, Baird A. Targeting bacteriophage to mammalian cell surface receptors for gene delivery. Hum Gene Ther. 1998;9:2393−9.
95. Parisien BA, Zhang J, Mandeville R, Lan CQ. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J Appl Microbiol. 2008;104:1−13.
96. Harcombe WR, Bull JJ. Impact of phages on two-species bacterial communities. Appl Environ Microbiol. 2005;71:5254–9.
97. Chibani-Chennoufi S, Bruttin A, Dillmann M, Brussow H. Phage-host interaction: an ecological perspective. J Bacteriol. 2004;186:3677–86.
98. Langley R, Kenna DT, Vandamme P, Ure R, Govan JRW. Lysogeny and bacteriophage host range within the Burkholderia cepacia complex. J Med Microbiol. 2003;52:483–90.
99. Weber-Dabrowska B, Mulczyk M, Gorski A. Bacteriophage therapy of bacterial infections: an update of our institute`s experience. Arch Immunol Ther Exp. 2000;48:547−51.
Taylor PW, Stapleton PD, Luzio JP. New ways to treat bacterial infections. DDT. 2002;7:1086–91.
100. Yoong P, Schuch R, Nelson D, Fischetti VA. Identification of a broadly active phage lytic enzyme with lethal activity against antibiotic-resistant Enterococcus faecalis and Enterococcus faecium. J Bacteriol. 2004;186:4808−12.
101. O'Flaherty S, Coffey A, Meaney W, Fitzgerald GF, Ross RP. The recombinant phage lysin LysK has a broad spectrum of lytic activity against clinically relevant staphylococci, including methicillin-resistant Staphylococcus aureus. J Bacteriol. 2005;187:7161–4.
102. Bradbury J. "My enemy's enemy is my friend": using phages to fight bacteria. Lancet. 2004;363:624–5.
103. Sarkar BL. Cholera bacteriophages revisited. ICMR Bull. 2002;32:33−7.
104. van Schaik W, Willems RJL. Genome-based insights into the evolution of enterococci. Clin Microbiol Infect. 2010;16:527−32.
105. Hatfull GF. Bacteriophages: nature's most successful experiment. Microbiol Today. 2008;35: 188−91.
106. Fiers W, Contreras R, Duerinck F, Haegeman G, Iserentant D, Merregaert J, et al. Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene. Nature. 1976;260:500–7.
107. Mazaheri Nezhad Fard R, Barton MD, Arthur JL, Heuzenroeder MW. Whole genome sequencing and gene mapping of a newly isolated lytic enterococcal bacteriophage EFRM31. Arch Virol. 2010a,155:1887–91.
108. Mazaheri Nezhad Fard R, Barton MD, Heuzenroeder MW. Novel bacteriophages in Enterococcus spp. Curr Microbiol. 2010b,60:400–6.
109. Hatfull GF, Pedulla ML, Jacobs-Sera D, Cichon PM, Foley A, Ford ME, et al. Exploring the mycobacteriophage metaproteome: Phage genomics as an educational platform. PLoS Genet. 2006;2:835−47.
110. Lasken RS, Egholm M. Whole genome amplification: abundant supplies of DNA from precious samples or clinical specimens. Trends Biotechnol. 2003;21:531−5.
111. Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BAJ, Tunnacliffe A. Degenerate oligonucleotide-primed PCR: General amplification of target DNA by a single degenerate primer. Genomics. 1992;13:718−25.
112. Barbaux S, Poirier O, Cambien F. Use of degenerate oligonucleotide primed PCR (DOP-PCR) for the genotyping of low-concentration DNA samples. J Mol Med. 2001;79:329–32.
113. Amaral AJ, Megens HJ, Kerstens HHD, Heuven HCM, Dibbits B, Crooijmans RPMA, et al. Application of massive parallel sequencing to

whole genome SNP discovery in the porcine genome. BMC Genomics. 2009;10:374–83.
114. Sanger F, Air GM, Barrell BG, Brown NL, Coulson AR, Fiddes JC, Hutchison CA, Slocombe PM, Smith M. Nucleotide sequence of bacteriophage ΦX174 DNA. Nature. 1977;265:687–95.
115. Metzker ML. Emerging technologies in DNA sequencing. Genome Res. 2005;15:1767–76.
116. Liu L, Li Y, Li S, Hu N, He Y, Pong R, et al. Comparison of next-generation sequencing systems. J Biomed Biotech. 2012;2012:251364.
117. Hansen VM, Rosenquist H, Baggesen DL, Brown S, Christensen BB. Characterization of Campylobacter phages including analysis of host range by selected Campylobacter penner serotypes. BMC Microbiol. 2007;1:90–98.
118. Rakieten ML. Studies with Staphylococcus bacteriophage I. The preparation of polyvalent Staphylococcus bacteriophage. Yale J Biol Med. 1932;4:807–818.
119. Rakieten ML, Rakieten TL. The inactivation of "pure line" phages by bacterial extracts and the loss of phage types in vivo. Yale J Biol Med. 1937;10:191–208.
120. Deveau H, Labrie SJ, Chopin MC, Moineau S. Biodiversity and classification of lactococcal phages, Appl Environ Microbiol. 2006;72:4338–4346.
121. Li W, Raoult D, Fournier PE. Bacterial strain typing in the genomic era. FEMS Microbiol Rev. 2009;33:892–916.
122. McBride SM, Coburn PS, Baghdayan AS, Willems RJL, Grande MJ, Shankar N, et al. Genetic variation and evolution of the pathogenicity island of Enterococcus faecalis. J Bacteriol. 2009;191:3392–402.
123. Ogier JC, Serror P. Safety assessment of dairy microorganisms: The Enterococcus genus. Int J Food Microbiol. 2008;126:291–301.
124. Werner G, Klare I, Witte W. The current MLVA typing scheme for Enterococcus faecium is less discriminatory than MLST and PFGE for epidemic-virulent, hospital-adapted clonal types. BMC Microbiol. 2007;7:28–37.
125. Kae H. Genome projects: uncovering the blueprints of biology. in: The Science Creative Quarterly (2009). 2003;4.
126. Kaiser O, Bartels D, Bekel T, Goesmann A, Kespohl S, Puhler A, et al. Whole genome shotgun sequencing guided by bioinformatics pipelines—an optimized approach for an established technique. J Biotechnol. 2003;106:121–33.
127. Kiljunen S, Hakala K, Pinta E, Huttunen S, Pluta P, Gador A, et al. Yersiniophage wR1-37 is a tailed bacteriophage having a 270 kb DNA genome with thymidine replaced by deoxyuridine. Microbiology. 2005;151: 4093–102.
128. Hershey A, Chase M. Independent functions of viral protein and nucleic acid in growth of bacteriophage. J Gen Physiol. 1952;36:39–56.
129. Løset G, Roos N, Bogen B, Sandlie I. Expanding the versatility of phage display II: improved affinity selection of folded domains on protein VII and IX of the filamentous phage. PLoS ONE. 2011;6:e17433.
130. Smith GP, Petrenko VA. Phage Display. Chem Rev. 1997;97:391–410.
131. Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science. 1985;228:1315–7.
132. Huang J, Ru B, Dai P. Bioinformatics resources and tools for phage display. Molecules. 2011;16:694–709
133. Dobozi-King M, Seo S, Kim JU, Young R, Cheng M, Kish LB. Rapid detection and identification of bacteria: Sensing of phage-triggered ion cascade (SEPTIC). J Biol Phys Chem. 2005;5:3–7.
134. Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence. 2014;5:226–35.
135. Bruynoghe R, Maisin J. Essais de therapeutique au moyen du bacteriophage. C R Soc Biol. 1921;85:1120–1.
136. Larkum NW. Bacteriophagy in urinary infection part II. Bacteriophagy in the bladder. J Bacteriol. 1926;12:225–42.
137. Larkum NW. Bacteriophage from a public health standpoint. Am J Public Health Nations Health. 1929;19:31–6.
138. Larkum NW. Production of antitoxins by means of bacteriophage. Am J Public Health Nations Health. 1933;23:1155–8.
139. Larkum NW, Semmes MF. Filtration of bacteriophage. J Bacteriol. 1930;19:213–22.
140. Sulakvelidze A, Alavidze Z, Morris Jr JG. Bacteriophage therapy. Antimicrob Agents Chemother. 2001;45:649–59.
141. Wright A, Hawkins C, Anggard E, Harper D. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. Clin Otolaryngol. 2009;34:349–357.
142. Mazaheri Nezhad Fard R, Heuzenroeder MW, Barton MD. Antimicrobial and heavy metal resistance in commensal enterococci isolated from pigs. Vet Microbiol. 2011b,148:276–282.

143. Tiwari R, Chakraborty S, Dhama K, Wani MY, Sanjay Kapoor AK. Wonder world of phages: potential biocontrol agents safeguarding biosphere and health of animals and humans- current scenario and perspectives. Pak J Biol Sci. 2014;7:316–28.