A Short Introduction to Bacteriophages

Ramin Mazaheri Nezhad Fard\textsuperscript{a,b,*}

\textsuperscript{a}Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
\textsuperscript{b}Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran, Iran.

\textbf{Introduction}

\textit{History}

Bacteriophages or phages are bacterial viruses that are able to invade prokaryotes (Mihara et al., 2016). The approximate population of phages is suggested to be $10^{31}$, which is 10 times greater than the bacterial population (Canchaya et al., 2004; Pedulla et al., 2003; Hendrix, 2002). Bacteriophages play a significant role in maintaining the bacterial population and are important for adopting pathogens to their hosts, which is termed as a ‘host-pathogen-phage interaction’ (Ventura et al., 2007; Boyd and Brussow, 2002). Furthermore, they can play roles in carbon cycling, bacterial pathogenicity, and bacterial evolution. Bacteriophages can be used to develop DNA vaccines, protein vaccines, novel antibiotics, novel antiviral drugs, gene therapy, and phage therapy (Turton et al., 2012; Serwer et al., 2007).

Bacteriophages were first discovered in the 19\textsuperscript{th} century. In 1896, Ernest Hankin, an English bacteriologist, inactivated \textit{Vibrio cholerae} using water filtrates from the
Ganges and Jamuna rivers. This was repeated in 1898 by Nikolay Gamaleya, a Russian bacteriologist, using Bacillus subtilis. In 1915, Frederick Twort, an English bacteriologist, proposed that the inactivator possibly was a bacterial virus. Later, in 1917, Felix d’Herelle, a French-Canadian microbiologist, reported the first bacterial lysis and named the lysis agent a bacteriophage. Since then, phage study has been continued by many researchers such as Clark and Clark (1927), Evans (1934), Rakieten and Tiffany (1938), Tiffany and Rakieten (1939), Evans (1941), Kjems (1955), Bleiweis and Zimmerman (1961), Brock et al. (1963), and Brock (1964).

**Taxonomy**

Currently, the International Committee on Taxonomy of Viruses (ICTV) categorizes phages in two major orders (Caudovirales and Ligamenvirales), five families, nine subfamilies, 145 genera, and 684 species. Unassigned families mostly belong to archaea (Table 1) (Krupovic et al., 2016; ICTV, 2015). Of the phage families, four main families are widespread in nature: *Siphoviridae*...
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**Morphology**

Bacteriophages are morphologically categorized in two major categories: tailed (head-tail) and PFP (polyhedral, filamentous or pleomorphic) phages. The classic structure of tailed phages represents an icosahedral head containing capsid and genome, a neck or collar, one tail or more, and a base plate (Figure 1) (Fokine and Rossmann, 2014; Yap and Rossmann, 2014). The phage tail includes a core covered by a helical sheath. The helical sheath ends at the base plate with long- and short-tail fibres or pins. The phage genome is composed of single- or double-strand DNA or RNA, and includes from a few to several hundred kilobases (Cokie et al., 2011; Frost et al., 2005). The bacteriophage genes include lysis, replication, regulation, packaging, structural, antimicrobial resistance, and housekeeping genes. These genes encode various proteins, such as head-tail-joining proteins, head-tail adaptor proteins, host specificity proteins, portal proteins, prohead protease, tail component, tail major proteins, tape measure proteins, large and small terminase subunits, amidase, holing, HNH endonuclease, and DNA primase (Son et al., 2010).

Morphologically, a majority (96%) of phages have tails; mostly a single tail. Other phages are PFP (Ackermann, 2001, 2007). More than half of all tailed phages have long, flexible, non-contractile tails, and they are classified in the *Siphoviridae* family, followed by the *Myoviridae* (long, non-flexible, contractile tails) and *Podoviridae* (long, non-flexible, non-contractile tails) families. Moreover, filamentous phages are classified into three families: *Inoviridae* (unassigned family), *Lipothrixviridae* (order: *Ligamenvirales*), and *Rudiviridae* (order: *Ligamenvirales*) (Ackermann, 2003) found in eubacteria (*Inoviridae*) (Campos et al., 2003) and archaea (*Lipothrixviridae* and *Rudiviridae*) (Geslin et al., 2003). *Inoviridae* are long-tube or short-rod phages with a total length of up to 2 μm, including a circular single-stranded DNA (ssDNA) genome (Klieve et al., 2004). They are broadly distributed in nature, isolated from a variety of bacteria, such as enterobacteria, *Vibrio* spp., *Pseudomonas* spp., *Xanthomonas* spp., *Acholeplasma* spp., *Spiroplasma* spp., and *Enterococcus* spp. (*Fauquet and Pringle, 2000*). The *Leviviridae* family includes polyhedral phages and contains a linear single-stranded RNA (ssRNA) genome (Vinje et al., 2004). Usually, ssRNA phages are found in mammalian bacteria but not in avian bacteria (Bollback and Huelsenbeck, 2001). The *Leviviridae* family members are small phages with a size of 23–30 nm (*Love et al., 2008; Klovins et al., 2002*). Another PFP phage, the *Guttaviridae* family, is a droplet-shaped phage. It was introduced by Zillig et al. (1998) and Arnold et al. (2000) in archaea, and later by Mazaheri Nezhad Fard et al. (2010) in eubacteria (*Liu et al., 2009; Lips, 2006; Vestergaard et al., 2005*). Morphology of the phage shows long thin filaments at one end and a teardrop cell containing a highly methylated circular double-stranded DNA (dsDNA) genome with an approximate size of 20 kb (*Sau and Deb, 2008; Prangishvili and Garrett, 2004;*).

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**Figure 1.** Graphic of a tailed phage (from Wikimedia Commons).
The *Fuselloviridae* family contains a lemon-shaped phage with peripheral projections and short fibres attached to one pole and a circular, supercoiled dsDNA genome (Kraft et al., 2004; Wiedenheft et al., 2004; Prangishvili et al., 2001). *Fuselloviridae* phages have mostly been isolated from archaea but were first reported in eubacteria by Mazaheri Nezhad Fard et al. in 2010 (Xiang et al., 2005; Rice et al., 2004; Geslin et al., 2003; Stedman et al., 2003).

### Ecology

Bacteriophages are important agents in transferring mobile genetic elements (MGEs) to bacteria by transduction. Transduction occurs in three main steps: 1) phage binding to bacterial specific receptors such as surface protein, carbohydrate, or lipopolysaccharide molecule; 2) genome injecting into the bacterial cell; and 3) phage protein synthesis, using bacterial replication machinery (Heinemann and Bull, 2007). Bacteria-specific receptors can be located in the outer membrane of Gram-negative bacteria, the cell wall of Gram-positive bacteria, the capsule or slime layer, and in association with flagella or pili (Lindberg, 1973). Blocking each of these steps may result in bacterial resistance to phages (Clokie et al., 2011). Another resistance mechanism includes clustered, regularly interspaced, short palindromic repeats or CRISPR within the bacterial genome (Hegstad et al., 2010). Generally, transduction contributes greatly to indirect horizontal gene transfer (HGT) (Weinbauer, 2004; Weinbauer and Rassoulzadegan, 2004). Furthermore, transduction may help transfer R plasmids between Gram-positive bacteria. Transduction has widely been used in studies on Gram-positive bacteria such as enterococci, streptococci, staphylococci, *Listeria* spp., *Bacillus* spp., and *Lactobacillus* spp. This includes studies in the fields of mutagenesis, packaging, and replicating (Mazaheri Nezhad Fard et al., 2011; Lindsay and Holden, 2006; Chandry et al., 2002; Hodgson, 2000).

Two functional groups of lysogenic and lytic phages have already been described (Drulis-Kawa et al., 2015; Canchaya et al., 2003). Lysogenic phages (also known as temperate or mild phages) infect bacteria and integrate their genome into the host genome, which is called a prophage (Lindsay et al., 2009). Prophages can be found in gammaproteobacteria and low-GC Gram-positive bacteria, and they shift to a lytic phase (Weaver et al., 2009; Brussow et al., 2004). This process or lysogenic induction is mediated by ultraviolet (UVC) or some antibiotics, such as mitomycin C and norfloxacin (Rokney et al., 2008; Aertsen et al., 2005). In bacteria, prophages encode outer membrane proteins (OMPs), pathogenicity islands, and virulence factors such as toxins (e.g. cytotoxic, enterotoxic, and neurotoxic), leukocidins, and superantigens (Stevens et al., 2009; Maiques et al., 2007; Ubeda et al., 2007; Baba et al., 2002; Ruzin et al., 2001). Examples of the common toxins include diphtheria, botulism, pertussis or whooping cough, yersiniosis, spirochetosis, scarlet fever, food poisoning, and veroxotoxins (Frank et al., 2013; Novick, 2003; Krylov, 2003; Boyd and Brussow, 2002). Examples of other proteins and virulence factors include auxiliary metabolic enzymes, ADP-ribosyltransferase toxins, LPS-modifying enzymes, type III effector proteins, detoxifying enzymes, hydrolytic enzymes, pore-forming lysins, serum resistance-associated proteins, and antibiotic resistance genes.

### Applications

Nowadays, many global studies are being carried out on phages by international researchers and there are regional studies by Iranian researchers as well (Sabouri Ghannad et al., 2012; Khajeh Karamoddini et al., 2011a, b). In addition to the broad genetic research on phages, one of the most interesting areas of phage research can be its treatment application. The potential of phage treatment was first suggested by d’Herelle at the Pasteur Institute in Paris (Wittebole et al., 2014). However, the idea of a phage therapy centre was first expressed by the Georgian physician and bacteriologist, George Eliava, who founded the Eliava Institute in Tbilisi in 1923 (www.eliava-institute.org). This was later followed by the foundation of the Institute of Immunology and Experimental Therapy by the Polish immunologist and microbiologist, Ludwik Hirszfeld, in Wroclaw in 1952 (www.iitd.pan.wroc.pl) and Phage Therapy Center in Tbilisi (www.phagetherapycenter.com). In general, phages are used to treat different infections, such as dysentery, salmonellosis, and gastroenteritis, most commonly in East European countries and countries of the former Soviet Union (Sarkar, 2002; Sulakvelidze et al., 2001). They are used to treat skin, mucosa, and wound infections (Taylor et al., 2002; Sulakvelidze et al., 2001). However, phage therapy may include some undesirable disadvantages alongside its life-saving advantages. Some of the advantages include effectiveness against multiple drug resistant (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. Some of the undesirable disadvantages include low public acceptance, limited accessibility, serum phage neutralization, and bacterial phage resistance. In conclusion, phages are important entities on earth due to their multiple roles in bacterial metabolism and surveillance in nature. However, further studies are needed to explore unknown features of the phages that can improve human lifestyle.
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Competing Interests

The authors declare no competing interest.

References

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