European Journal of Biology and Medical Science Research

Vol.6, No.5, pp.48-63, December 2018

Published by European Centre for Research Training and Development UK (www.eajournals.org)

THE POTENTIAL APPLICATION OF BACTERIOPHAGES' PRODUCT THERAPY AS AN ALTERNATIVE TREATMENT FOR ANTIBIOTIC RESISTANCE PATHOGENIC BACTERIA

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ABSTRACT: *Bacteriophages or phages are viruses that invade only bacterial cells and, in the case of lytic phages, disrupt bacterial metabolism and cause the bacterium to lyse. They are naturally occurring predators of bacteria, ubiquitous in the environment, with high host specificity and capacity to evolve to overcome bacterial resistance which makes them an appealing option for the control of pathogens. Phage therapy involves the use of bacteriophages, viruses that only attack bacteria and are very host specific, to kill pathogenic microorganisms. Phages are self replicating agents that are able to multiply by taking over their host's DNA replication and protein synthesis machinery. They can possess two life cycles, lytic and lysogenic. Phages were predicted very early as therapeutic tools to fight pathogenic bacteria but the successful and generalized use of antibiotics to control bacterial infections and the difficulties in obtaining purified phage preparations, delayed the use of phages for therapy. Facing the fast emerging and widespread pathogenic bacteria that have acquired resistance to most or all available antibiotics, the World Health Organization warned that these multiple antibiotic resistant bacterial pathogens will very likely bring the world back to the pre-antibiotics era. The pressing public concern has triggered global efforts in developing novel alternative antibacterials, including bacteriophages and phage encoded lytic enzymes as two families of candidate antimicrobials. Therefore, phages and their products are currently believed to be a potential therapeutic option to treat bacterial infections that do not respond to conventional antibiotics.*

KEYWORDS: Antibiotic resistant bacteria, Bacteriophages, Phage encoded lytic enzymes, Phage therapy

INTRODUCTION

For more than half a century, physicians, Veterinarians and clinicians have been relying primarily on antibiotics to treat infectious diseases caused by pathogenic bacteria. However, the emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal or agricultural usage has made antibiotics less and less effective (Perisien *et al.*, 2008). These days' scientists are now facing the threat of superbugs, i.e. pathogenic bacteria resistant to most or all available antibiotics (Fischetti, 2006). During the last 30 years, no new classes of antibiotics have been found, even with the help of modern biotechnology such as genetic engineering. Pharmaceutical companies have mainly focused on the development of new products derived from the known classes of antibiotics, which is a cause of major concern. Thus, exploring alternative approaches to develop antibacterial products is also a worthwhile task, and re-examining the potential of promising older methods might be of value (Sulakvelidze *et al.*, 2001).

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

Phage therapy, or more precisely, therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections, is one approach that has great potential as a solution to the serious worldwide problem of drug-resistant bacteria (Vinodkumar *et al.,* 2008). Bacteriophages are bacteria specific viruses that invade only bacterial cells and, in the case of obligately lytic phages, destroy their host bacteria by disrupt bacterial metabolism and cause the bacterium to lyse. They are naturally occurring predators of bacteria, ubiquitous in the environment, with high host specificity and capacity to evolve to overcome bacterial resistance which makes them an appealing option for the control of pathogens. Phage therapy involves the use of bacteriophages, viruses that only attack bacteria and are very host specific, to kill pathogenic microorganisms. The art was first developed at the Pasteur Institute in Paris early in the twentieth century, but since the advent of chemical antibiotics in the 1940s, it has been little used in the West. Today, however, the increased prevalence of bacteria that are resistant to most or all available antibiotics is precipitating a major health crisis, as was again passionately stressed by the World Health Organization in their call to action on World Health Day (WHO, 2011).

Phage therapy involves the use of lytic phages for the treatment of bacterial infections, especially those caused by antibiotic resistant bacteria. In general, there are two major types of phages, lytic and lysogenic. Only the lytic phages (also known as virulent phages) are a good choice for developing therapeutic phage preparations (Borysowski and Gorski, 2008). The bactericidal ability of phages has been used to treat human and animals' infections for years as a complement or alternative to antibiotic therapy (Kysela & Turner, 2007). Bacteriophages not only help in the treatments of bacterial infections in animals and human beings but also used in birds, fishes, plants, food material and biofilm eradication. The use of phage or phage products in food production has recently become an option for the food industry as a novel method for biocontrol of unwanted pathogens, enhancing the safety of especially fresh and ready-to-eat food products (Curtin & Donlan, 2006). They can be used to combat pathogens in food at all stages of production in the classic 'farm-to-fork' continuum in the human food chain of animal origin (García *et al.*, 2008). Accordingly, in order to prevent transmission to humans, phages can be used:

i. In livestock to prevent diseases or reduce colonization;

ii. In food material (such as carcasses and other raw products) or in equipment and contact surfaces to reduce bacterial loads;

iii. in foods as natural preservatives to extend their shelf life.

Bacteriophages were discovered independently by Frederick Twort (1915) and Felix d'Hérelle (1917). Since their discovery, various studies have been undertaken on bacteriophages and at present more than 5500 bacteriophages have been discovered using the electron microscope (Ackermann, 2007).

Facing the fast emerging and widespread pathogenic bacteria that have acquired resistance to most or all available antibiotics, the World Health Organization (WHO) warned that these multiple antibiotic resistant bacterial pathogens will very likely bring the world back to the pre-antibiotics era. The pressing public concern has triggered global efforts in developing novel alternative antibacterials, including bacteriophages and phage encoded lytic enzymes as two families of candidate antimicrobials (Parisien *et al.*, 2008). Phages are currently being used

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therapeutically to treat bacterial infections that do not respond to conventional antibiotics (Thiel, 2004). Therefore, the objective of this critical manuscript is:

- To appraise the promising alternative therapies for bacterial infections that does not respond to conventional antibiotics.

HISTORICAL BACKGROUND OF BACTERIOPHAGGES

The history of bacteriophage discovery has been the subject of lengthy debates, including a controversy over claims for priority. Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity (against *Vibrio cholerae*) which he observed in the waters of the Ganges and Jumna rivers in India, and he suggested that an unidentified substance was responsible for this phenomenon and for limiting the spread of cholera epidemics (Hankin, 1896). Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis* (Samsygina and Boni, 1984), and the observations of several other investigators are also thought to have been related to the bacteriophage phenomenon (Twort, 1920). However, none of these investigators further explored their findings until Frederick Twort, a medically trained bacteriologist from England, reintroduced the subject almost 20 years after Hankin's observation by reporting a similar phenomenon and advancing the hypothesis that it may have been due to, among other possibilities, a virus. However, for various reasons including financial difficulties, Twort did not pursue this finding and it was another 2 years before bacteriophages were officially discovered by Felix d'Herelle, a French-Canadian microbiologist working at the Pasteur Institut in Paris (Twort, 1915).

The discovery or rediscovery of bacteriophages by d'Herelle is frequently associated with an outbreak of severe hemorrhagic dysentery among French troops in July-August 1915. Several soldiers were hospitalized, and d'Herelle was assigned to conduct an investigation of the outbreak. During these studies, he made bacterium-free filtrates of the patients' fecal samples and mixed and incubated them with Shigella strains isolated from the patients. A portion of the mixtures was inoculated into experimental animals and a portion was spread on agar medium in order to observe the growth of the bacteria. It was on these agar cultures that d'Herelle observed the appearance of small, clear areas, which he initially called *taches*, then *taches vierges*, and later '*plaques'*. D'Herelle's findings were presented during the September 1917 meeting of the Academy of Sciences, and they were subsequently published in the meeting's proceedings. In contrast to Hankin and Twort, d'Herelle had little doubt about the nature of the phenomenon, and he proposed that it was caused by a virus capable of parasitizing bacteria (Summers, 1999). The name bacteriophage was also proposed by d'Herelle. It was formed from bacteria and "phagein" (to eat or devour, in Greek), and was meant to imply that phages eat or devour bacteria (D'Herelle, 1917). D'Herelle, who considered himself to be the discoverer of bacteriophages, was made aware of the prior discovery of Twort but maintained that the phenomenon described by Twort was distinct from his discovery. In the meantime, in contrast to Twort, d'Herelle actively pursued studies of bacteriophages and strongly promoted the idea that phages were live viruses and not enzymes as many of his fellow researchers thought. The priority dispute come to an end eventually, and many scientists accepted the independent discovery of bacteriophages and simply referred to it as the "Twort-d'Herelle phenomenon" and, later, the "bacteriophage phenomenon" (Bordet, 1921).

Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

PHAGE BIOLOGY AND LIFE CYCLE

Phage Biology

Phages are basically a genome, mostly between 5 and 50 kilo base pairs (kbp), which can consist of ssRNA, dsRNA, ssDNA or dsDNA, encapsulated in a protein mantle, possibly with but most without lipid envelope. However, 96% of the more than 5500 phages that have been observed by electron microscopy belong to the dsDNA, non lipid enveloped tailed phages (Ackerman, 2003**)**. There are a variety of different morphological types of bacteriophages, although the majority exhibits the characteristics shown in Figure1. Except for the filamentous phages, all of the phage groups have a polyhedral capsid which contains the phage genome. The head (capsid) is a protein shell often in the shape of an icosahedron. It is usually joined to a tail, which is a helical protein structure required to penetrate the bacterial cell wall and inject the DNA or RNA into the host. The tail may or may not be a contractile structure and to this are connected usually six tail fibers containing receptors at their tips that recognize attachment sites on the bacterial cell surface. Not all phages possess tails or tail fibers and here other attachment mechanisms are in place (Ackermann, 2005). Phages, which are about 1/40th the size of most bacteria, are perhaps the simplest, most abundant organisms on earth, thriving wherever bacteria grow up. Like all viruses, they are only able to replicate inside their host. There are two major types of phages, virulent (lytic) and temperate. For therapeutic purposes, only the lytic phages are a good choice because they cause bacterial cell lysis and do not integrate in to host DNA. Phages are classified in to different groups by the International Committee on Taxonomy of Viruses (ICTV) according to their morphology and nucleic acid type. The ICTV presently recognizes one order, 13 families and 31 generas of phages (McAuliffe, 2007). The dsDNA tailed phages are most studied as reported in the scientific literature and possibly make up the majority of phages on the planet (Hatfull, 2008). They are classified under the order *Caudovirales* and divided in to three families based on their tail morphology. *Myoviridae* (has a contractile tail), *Syphoviridae* (has long, non-contractile tail) and *Podoviridae (*has short, non- contractile tail). In all of the three groups of phages, the signal that initiates genome ejection is passed through the tail. There are several other approaches for phage classification based on phage genome, most of which, however, categorize phages within the same group in which they are currently classified by ICTV (Aksyuk *et al*., 2009).

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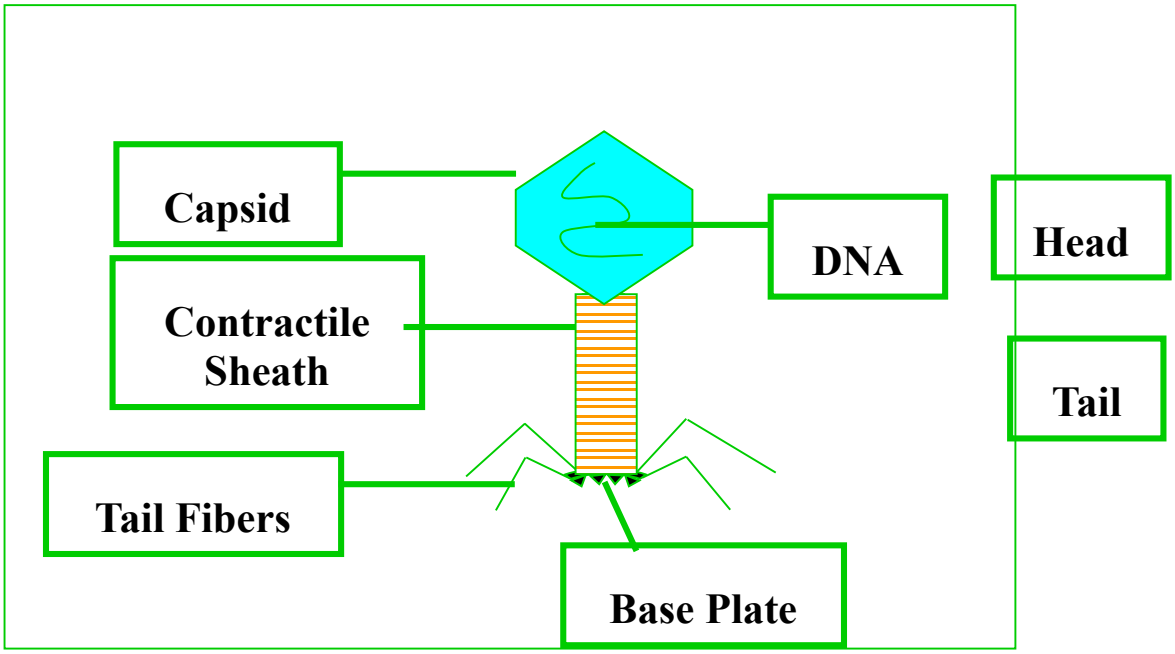

Figure1. Typical representation of a tailed bacteriophage.

Source: (Ackerman, 2003)

Phage Life Cycle

Bacteriophages bind and infect only bacterial cells. They specifically recognize different receptors on the bacteria that allow them to bind. Lytic and lysogenic cycles are the two possible life cycles phages can undergo (Sandeep, 2006; Skurnik and Strauch, 2006). Bacteriophages used for phage therapy replicate using a lytic cycle. In this form, the bacteriophage first attaches to a specific receptor site on a bacterial cell. It then injects its DNA or RNA into the host by releasing phage lysozyme through its tail, which degrades a portion of the cell wall. The tail sheath contracts, making the tail core drive through the cell wall and into the plasma membrane, where the nucleic acid is released. The nucleic acid is transported into the cell, whereas the capsid remains outside the cell (Ackermann, 2007). Biosynthesis of the viral nucleic acid then begins. The host DNA is degraded and protein synthesis is stopped as the phage DNA or RNA takes over the cell, using the host nucleotides and enzymes to replicate the phage's DNA/RNA. After this, the phage uses the machinery of the cell to make viral proteins, which happens exactly the same way as if the bacterial cell was making its own proteins, except viral RNA is now transcribed, not bacterial (Tortura *et al.*, 2010). The phage DNA/RNA and capsids assemble, lysozyme is synthesized within the cell to break down the cell wall again, and the new viruses are released. The bacteria cell is dead. From a single bacterial cell, in excess of 100 new virus particles may be liberated and each of these is able to go on to infect a new bacterial cell (Thomson *et al.*, 2004).

Temperate viruses are those that reproduce without killing their host cell. Typically they reproduce in two ways: through the lytic cycle and the lysogenic cycle. In the lysogenic cycle, the phage DNA integrates with the bacterial DNA as a stable prophage and is replicated with the bacterial DNA when the cells multiply; however, when the prophage senses that the

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bacterial host is under stress, it is induced to cut out from the bacterial chromosome and then enters the lytic cycle. At the end of the reproductive cycle, phage-induced factors contribute to lysis of the bacterial peptidoglycan so as to release the progeny (Wang *et al.*, 2000). The organization of the gene products, their function in the life cycle of the bacteriophage and also their application in biotechnology are indicated in Figure3 (McGrath *et al*., 2004). A recent review paper stated that the genome of most cultivable bacteria contains either full or defective prophages (Ackermann, 2007).

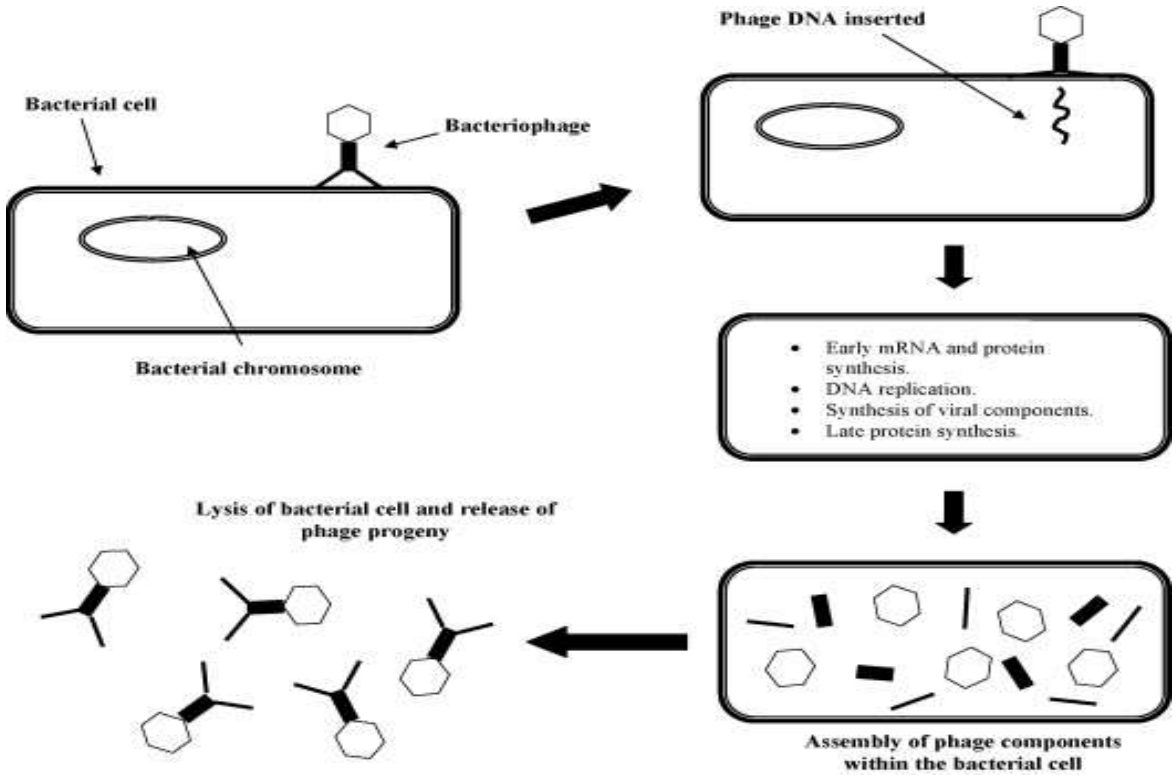

Figure2. Typical lytic cycle of a bacteriophage.

Source: (Hanlon, 2007)

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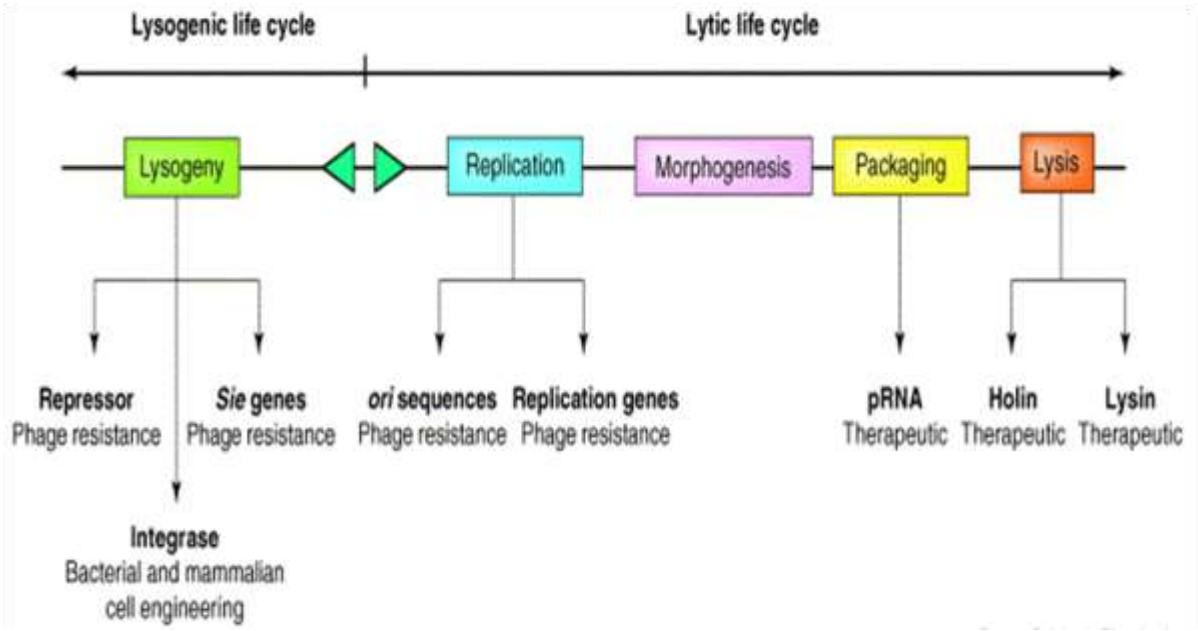

Figure3. Typical temperate phage gene organization and applications in biotechnology.

Source: (McGrath et al., 2004)

PHAGE AND PHAGE PRODUCTS THERAPY

Intact bacteriophages as a therapy

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. Phages were predicted very early as therapeutic tools to fight pathogenic bacteria. But the successful and generalized use of antibiotics to control bacterial infections and the difficulties in obtaining purified phage preparations, delayed the use of phages for therapy (McGrath *et al*., 2004). Recently interest in phage therapy has been revived. This is mainly to overcome the urgent problem of antibiotic resistance due to multidrug-resistant bacteria. The scale of antibiotic resistance now results in over five million people dying every year from infections not responding to antibiotics. According to recent World Health Organization (WHO) figures, in the US 14,000 people die each year from drug-resistant infections acquired in hospitals, and worldwide, 60% of such infections are drug-resistant. Bacteriophages are found in all bacteria, so it is hoped to be able to develop control therapies against pathogenic bacteria (Fischetti, 2001; Stone, 2002). Phage therapy is already applied in the agricultural, food-processing and fishery industries, and is used for the treatment of bacterial infections in Georgia and Eastern Europe. But there is a need for further carefully controlled empirical data on its efficacy and safety (Inal, 2003).

The therapy can be defined more broadly than just the application of phages to human and animal bodies to combat bacterial disease. Indeed, at its most inclusive phage therapy represents the application of specific pathogens (phages, which are pathogens of bacteria) to selectively reduce or eliminate pathogen-susceptible organisms from specific environments, including natural (e.g., forests, lakes, etc., as well as the bodies of humans and other animals)

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and artificial environments (e.g., farms, factories, offices, hospitals, etc.). In other words, phage therapy is simply another form of biological control. Now, it has become apparent that if some of the problems initially encountered with phage therapy can be overcome, it might have potential uses as an alternative or addition to antibiotic therapy (Kurtböke *et al.*, 1992).

Preparation of phages for therapy

Experimental phages are frequently isolated from environmental sources where their bacterial host is found and undergo minimal optimization procedures before use. However, to be successful clinically, bacteriophages must undergo rigorous selection and characterization (Carlson, 2005). A conventional phage propagation process involves cultivating host bacteria to high cell density and then infecting the bacterial culture to allow the explosive propagation of phages, followed by purification procedures via cesium chloride centrifugation designed to get rid of host bacteria, endotoxins and other bacterial debris and to elevate the Plaque-Forming Unit (PFU) of the phage preparation. The purified phage preparations, after checking the sterility, are then processed into final products in a variety of pharmaceutical forms that are able to be administered topically, orally, rectally, by inhalation or by injection. A large variety of required dosages for phage therapy have been suggested by different researchers. While some authors reported that the typical range is 10^6 to 10^{13} PFU/ kg body weight/day, some others suggested a much lower range of 10^5-10^{11} per dose (Sulakvelidze *et al.*, 2001). Researchers have developed a phage breeding protocol to isolate phages possessing increased virulence within a population. This process is based upon the use of an antiviral compound derived from pomegranate extract that destroyed bacteriophages free in suspension but not if bound to a host cell surface. When added to broth containing both phage and host bacteria, the process will select those phages that adsorb most rapidly, as the unbound virus will be killed. The progeny from that infection will be passed through repeated rounds of breeding to produce virus with enhanced virulence. The bacteriophage preparation may be a single clone active against one species of bacterium (monovalent) or in the form of cocktails of phage that are able to be used against a broad range of pathogens. This technology was employed to produce a bacteriophage that was specific for *Listeria monocytogenes* (Hibma *et al.*, 1997).

Phage pharmaco kinetics

Surprisingly little research has been performed on the fate of phages in animals and humans. The pharmacokinetics is complicated due to the self-replicating nature of phages. The *in vitro* growth data for a phage cannot be directly applied to the *in vivo* situation; in addition the *in vivo* data for one phage cannot be transferred to another phage. Critical parameters that affect phage therapy are the phage adsorption rate, burst size, latent period, initial phage dose and the clearance rate of the phage particles from the body fluids by the reticuloendothelial system (Payne and Jansen, 2003). Payne has developed mathematical models to predict the behavior of phages *in vivo* taking into account population dynamics. Two paradoxical observations should be mentioned here. It is certain that timing of the phage treatment is critical and that phage administered too early may result in clearing of the phage from the body before it reaches the replication threshold. Similarly, addition of antibiotics in parallel with phage may hamper the phage efficacy (Payne and Jansen, 2003).

The replication threshold and proliferation threshold are expressions used to describe the situation where a low concentration of host bacteria is present in the culture and it takes some time before the bacteria reach a density where a net increase in phage concentrations can be seen. Apparently, the threshold depends on the rare encounters of the phage with the relatively

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few host cells. *In vivo* experiments should demonstrate also whether the physical and chemical conditions encountered *in vivo* support the phage life cycle. In some body compartments bacteria may reside in an environment that does not provide phages with optimal conditions for infection (Kasman *et al.*, 2002). The initial dose increases exponentially as the virus multiplies within the susceptible bacterial host and is subsequently released. Often there is no need to carry out repeat dosing. There is evidence that phage can penetrate poorly vascularised tissues and can cross the blood–brain barrier (Alisky *et al.*, 1998).

Benefits of phage therapy over antibiotics

Phages appear to be better therapeutic agents as they have several advantages over traditional antibiotics as well as disadvantages (Matsuzaki *et al.*, 2005). Majority of them are summarized in the Table given below.

Table1. Comparison of phages and antibiotics regarding their prophylactic and therapeutic use.

Bacteriophages	**Antibiotics**
Phages are highly effective in killing their targeted bacteria i.e., their action is bactericidal	Some antibiotics are bacteriostatic, i.e., they inhibit the growth of bacteria, rather than killing them (e.g., chloramphenicol).
Production is simple and cheap.	Production is complex and expensive.
Phages are an 'intelligent' drug. They multiply at the site of the infection until there are no more bacteria. Then they are excreted.	They are metabolized and eliminated from the body and do not necessarily concentrate at the site of infection.
The pharmacokinetics of bacteriophage therapy is such that the initial dose increases exponentially if the susceptible bacterial host is available. In such cases, there is no need to administer the phages repeatedly.	Repeated doses of antibiotic are required to cure the bacterial disease.
The high selectivity/specificity of bacteriophages permits the targeting of specific pathogens, without affecting desirable bacterial flora which means that phages are unlikely to affect the "colonization pressure" of the patients	Antibiotics demonstrate bactericidal or bacteriostatic effects not only on the cause of bacterial disease, but on all microorganisms present in the body including the host normal microflora. Thus their non-selective action affects the patient's microbial balance, which may lead to various side effects.
Because of phages specificity, their use is not likely to select for phage resistance in other (non-target) bacterial species	The broad spectrum activity of antibiotics may select for resistant mutants of many pathogenic bacteria species.
Humans are exposed to phages throughout life, and well tolerate them. No serious side effects have been described.	Multiple side effects, including intestinal disorders, allergies, and secondary infections (e.g., yeast infections) have been reported.
Phage-resistant bacteria remain susceptible to other phages having a similar host range.	Resistance to antibiotics is not limited to targeted bacteria.
Phages are found throughout nature. This means that it is easy to find new phages when	Developing a new antibiotic (against antibiotic resistant bacteria) is a time

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bacteria become resistant to them. Selecting a new phage (e.g., against phageresistant bacteria) is a rapid process and frequently can be accomplished in days.	consuming process and may take several years to accomplish.
Phages may be considered as good alternative for patients allergic to antibiotics.	If patient is allergic to antibiotic, treatment is very difficult

Disadvantage of phage therapy

There are also some disadvantages with the phage therapy approach. These include:

- The problem which requires attention is the rapid clearance of phage by the spleen, liver and other filtering organs of reticuloendothelial system (Carlton, 1999). This can be taken care by doing serial passage in mice (Merril *et al.*, 1996) so as to obtain a phage mutant capable of evading the reticuloendothelial system and therefore capable of long circulation in the blood. The minor variations in their coat proteins enable some variants to be less easily recognized by the RES organs, allowing them in the circulation for longer periods than the "average" wild-type phage.
- This therapy cannot be used for intracellular bacteria as the host is not available for interaction.
- Theoretically development of neutralizing antibodies against phages could be an obstacle to the use phage therapy in recurrent infections. This needs to be confirmed experimentally. However, in the immunocompromised host where the immune system is depressed such as chronic infections, the phage therapy may work in this situation (Skurnik & Strauch, 2006).
- The shelf life of phages varies and needs to be tested and monitored.
- Phages are more difficult to administer than antibiotics. A physician needs special training in order to correctly prescribe and use phages.
- Most phages are highly immunogenic and promote a strong antibody response, raising fears that phage therapy might be applicable for *in vivo* use only once and for a relatively short time per patient.
- The *in vivo* growth of phages has the potential to release large quantities of endotoxin from lysed Gram-negative cells, with potential health concerns in humans and animals.

Phage products as a therapy

Phage endolysins

Endolysin is a generic term used to describe a range of bacteriophage-encoded peptidoglycan hydrolases, which are synthesized in phage-infected cells at the end of the multiplication cycle. These enzymes are also known as phage lysozymes, lysins, or muralytic/mureolytic enzymes. It is often composed of two structural domains, a C-terminal cell wall binding domain and one or two N-terminal catalytic domains. Their cell wall binding domains target the enzymes to their substrate, and the corresponding catalytic domains are able to cleave bonds in the peptidoglycan network. The main use of endolysin under natural condition is to degrade/lyse

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the peptidoglycan layer from inside to release newly assembled virions from the bacterial host during the lytic life cycle. The endolysin has no signal peptide. So, it cannot traverse the cell membrane. It needs the cooperative action of a small hydrophobic lysis protein, the holin. Holin pierces the cell membrane and paves the way for endolysin action on the cell wall resulting in lysis of the bacterial cell (Figure4a). Some phages have a filamentous morphology and these can escape from the host cell by extrusion through the cell wall without causing destruction of the host. These phages are of no relevance to therapy (Fischetti, 2005).

The killing capacity of endolysins has been known for a long time. In view of the prevalence of antibiotic resistant bacteria, these enzymes represent a truly novel and effective class of antimicrobials in the fight against bacterial infection. By recognizing unique receptors present in the bacterial cell wall, they exhibit highly specific targeting of their host cell while leaving the normal microflora intact (Nelson *et al.*, 2001). The use of endolysin as an attractive and complementary alternative to control bacterial infections has been studied both *in vitro* and *in vivo* for many bacterial infections. The enzymes have been proven as efficient antibacterial agents against major Gram-positive pathogens such as *Staph. aureus*, *B. anthracis*, *Strep. agalactiae* and *Strep. pneumonia*. Currently, an important limitation of endolysin therapy is the insensitivity of Gram-negative bacteria for the exogenous action of endolysins as the outer membrane shields the access of endolysins from the peptidoglycan. This action prevents the expansion of the range of effective endolysins to important Gram-negative pathogens (Cheng *et al.*, 2005).

There are two fundamentally distinct methods for endolysin production: the approach employing bacteria infected with corresponding phages and the method using recombinant bacteria hosting endolysin genes cloned from corresponding phages. A simple method to produce endolysin involves infecting high cell density bacterial culture with a specific phage suspension to allow bacteriolysis to occur. During the phage lytic cycle, endolysin are liberated into the culture. After the selection of the appropriate lytic enzyme against a specific bacterium, a carrier has to be chosen to deliver the enzyme therapy, which can consist of a single enzyme, a mix of lytic enzymes, shuffled, or chimericlytic enzymes. Lysins have a short half-life (15-20 min), but their action is so rapid that nanogram quantities of specific lysin can kill specific Gram-positive bacteria in seconds after contact (Fischetti, 2003).

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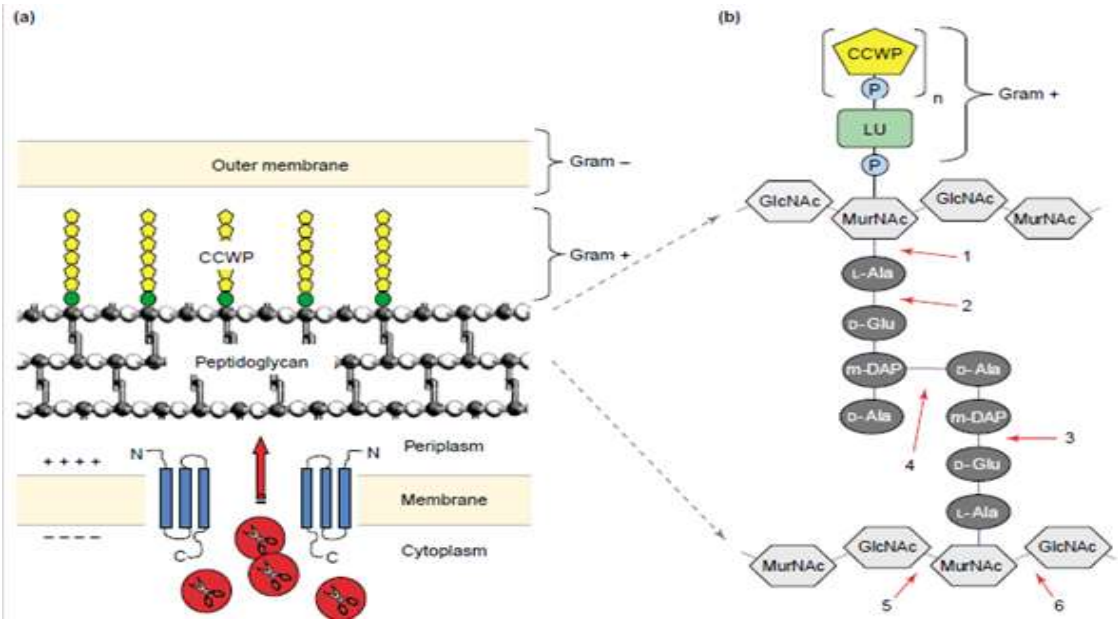

Source: (Loessner, 2005)

Figure4. A). Bacterial cell wall structure and endolysin targets.

B). The specific sites of cleavage by the major classes of endolysins of different enzymatic specificities are indicated by numbers: 1) *N*-acetylmuramoyl-L-alanine amidase; 2) L-alanoyl-D-glutamate endopeptidase; 3) D-glutamyl-m-DAP endopeptidase (note that this activity has not yet been identified in a phage endolysin); 4) interpeptide bridge-specific endopeptidases ;5) *N*-acetyl-β-D-glucosaminidase; and 6) *N*-acetyl-β-D-muramidase (also known as muramoylhydrolase and 'lysozyme') and lytic transglycosylase.

Phage tail-associated cell wall hydrolases

For the successful initiation of infection, the genome of the non-contractile and short tailed bacteriophage is injected in to the host cell by the help of cell wall hydrolase associated with the tail. For this purpose phage particles carry lytic enzymes that are capable of producing a local opening through the peptidoglycan layer. Lytic enzymes are different from endolysin in that, they are integral components of virion and used to digest cell wall from the outside (Borysowski *et al*., 2006). The infection initiation mechanism of phages in Gram-positive bacteria was hardly studied unlike in Gram-negative bacteria. There is one recent report on the bifunctional lytic activities of the phage φMR11 tail lysin against *S. aureus*. According to this report, the product of φMR11 gene 61 has two typical catalytic domains, the amidase and lysozyme domains in the N-terminal and C-terminal regions, respectively. These domains are located towards the end of the phage tail and both the amidase [amino acids (aa) 1-150] and the lysozyme (aa 401-624) domains but not the linker domain (aa 151-400) caused efficient lysis from without on *S. aureus* bacterial suspensions (Rashel *et al*., 2008) (Figure5a).

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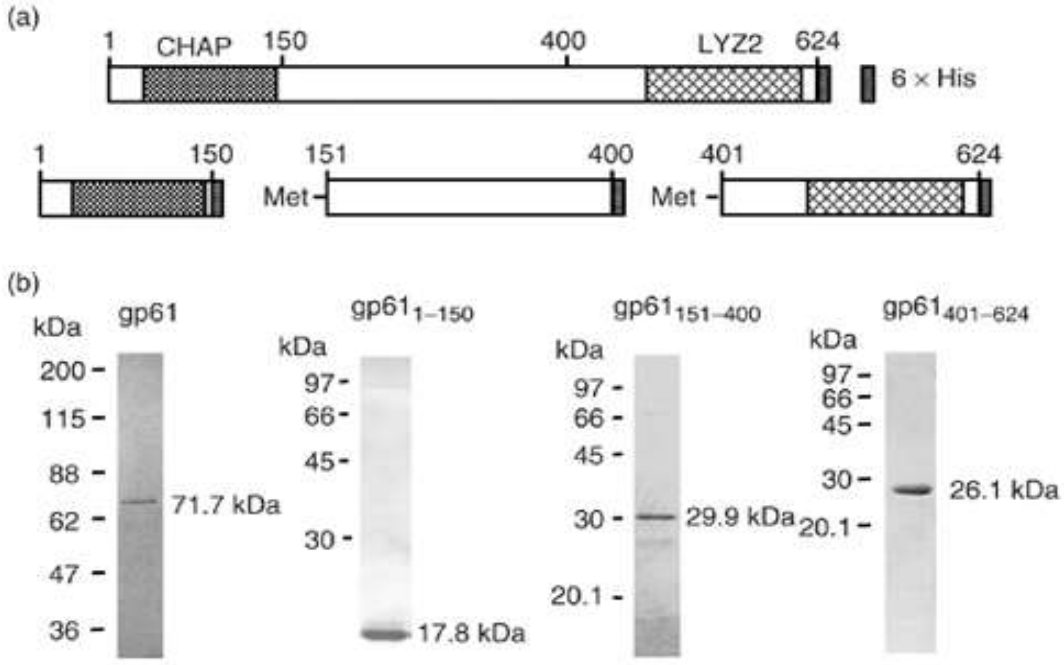

Figure5. Schematic representation of MR11 gp61 and its derivatives.

Source: (Rashel et al., 2008

(a) Full-length gp61, indicating the predicted N-terminal CHAP (aa 29–146) and C-terminal LYZ2 (aa 473–610) domains, with a His tag at the C-terminus. The CHAP region (aa 1–150) including the His tag at the C-terminus, the spacer region (aa 151–400) with the His-tag at the C-terminus, and the LYZ2 region (aa 401–624) with the His tag at the C-terminus are also shown.

(b) Purification of full-length and three individual regions of gp61.

CONCLUSIONS AND RECOMMENDATIONS

Phages were predicted very early as therapeutic tools to fight pathogenic bacteria but the successful and generalized use of antibiotics to control bacterial infections and the difficulties in obtaining purified phage preparations, delayed the use of phages for therapy. Over the years, antibiotics were successfully used to fight bacterial infections, but now more and more bacteria have developed resistance to these drugs. The pressing public concern has triggered global efforts in developing novel alternative antibacterials, including bacteriophages and phage encoded lytic enzymes as two families of candidate antimicrobials.

Based on the above conclusions, the following recommendations are forwarded:

- Phage and phage products therapy should be investigated well to counteract microorganisms that are multi-resistant to conventionally applied antibiotics.
- Numerous clinical trials and extensive investigations need to be performed to guarantee the safety and efficiency of phages and phage products therapy.

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- Phage therapy should be accepted by the public and the medical authorities and implemented to control antibiotic resistance bacteria.
- The interaction of simultaneous administration of phages and antibiotics should be studied.

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Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078