My enemy's enemy is my friend: Bacteriophage therapy

Carlota Ruiz Martinez,
Universitat Autònoma de Barcelona, Grau en Biologia 2012 - 2013

Introduction

1. Biology of bacteriophages:

Bacteriophages (phage) are viruses that only infect bacteria and typically attack only a single bacterial strain (Fig.1). This specificity, together with the killing capacity makes them the "natural enemies" of bacteria. [1]

2. Life cycle of phages: Phages can be divided into two groups

Lytic phage

- Repeats a cycle in which self-proliferation is synchronous with destruction of bacteria (lytic cycle)

Lysogenic phage

- Has a lysogenic cycle (the phage genome multiplies cooperatively with the host without destroying it) in addition to a lytic cycle

Figure 2 illustrates the general mechanism of baceteriolysis by tailed phages.

3. Objective:

The aim of this project is to study in detail bacteriophage therapy and its particularities, breaking this into different types of therapy and analyze the problems to be overcome and the potential solutions that are possible.

Figure 1. Phage T4. From Science Source

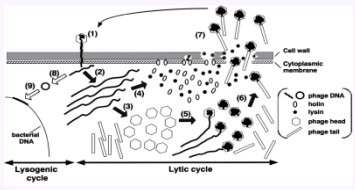

Figure 2. Bacteriolysis. (1) Adsorption and DNA injection; (2) DNA replication; (3) production of head and tail; (4) synthesis of lysin; (5) DNA packaging; (6) completion of phage; (7) disruption of the cell wall and release the progeny; (8) circularization of DNA; (9) integration of the phage DNA into the host genome. *From* [1]

Results

1. Types of bacteriophage therapy:

Phage therapy involves using phages or their products as bioagents for the treatment or prophylaxis of bacterial infectious diseases. There are different alternatives:

1. **Therapy using living phages:** is the "classical" version in which the phage infect the bacteria near the surface and kill them by internal replication and continues multiplying for as long as there are bacteria to infect.

2. **Phage therapy using non-replicating genetically modified phages:** Hagens *et al.* constructed a recombinant phage Pf3R, in which the export protein gene of the genome was replaced with a restriction endonuclease gene (Fig. 3). The endonuclease digested the host genomic DNA and consequentially killed the bacteria with minimal release of endotoxin *in vitro*. [2]

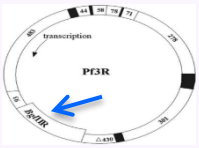

Figure 3. Schematic representations of the genetic organization of the modified phage Pf3R. *From* [2]

3. **Utilization of phage lysin:** most tailed phages produce peptidoglycan hydrolase (lysin) to release their progeny at the final stage of multiplication.

Lysin (Fig.4) destroys the peptidoglycan directly exerting a bacteriolytic effect. It can also destroy the cell walls of non-growing bacteria. [1]

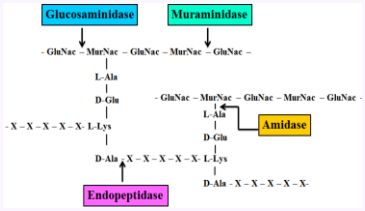

Figure 4. Attack points of phage encoded lysins on the peptidoglycan of gram-positive bacteria. Amidase, endopeptidase, muramidase or glucosaminidase may be released, depending on the cutting site. *From* [1]

4. **Protein antibiotics:** some small phages do not have the genes for holin or lysin proteins. Instead, they produce a protein that inhibits a step in murein monomer synthesis. Their inhibitory gene products are known as "protein antibiotics". [1, 3]

2. Problems to overcome

Accessibility	Restriction / Modification	Immunogenicity	Resistance
Phages won't be successful where they cannot make contact with the target bacteria, or do so only in a very limited way (Ex. Intracellular bacteria)	In order to protect themselves many types of bacteria have developed a method to chop up any foreign DNA → endonuclease that restrict the infection of phages.	In prolonged treatments has been observed a development of antibodies against phages.	In continued incubation of a culture lysed by phage, resistant bacteria can appear

3. Potential solutions:

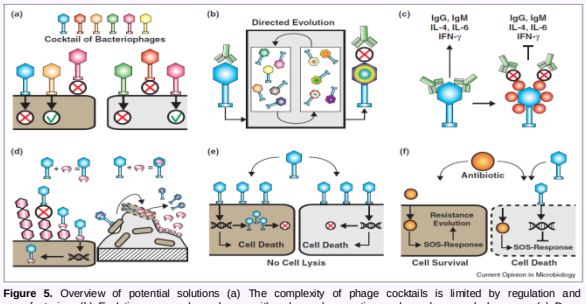

Figure 5. Overview of potential solutions (a) The complexity of phage cocktails is limited by regulation and manufacturing. (b) Evolution can produce phages with enhanced properties such as decreased clearance. (c) Drug-delivery technologies can enhance systemic phage delivery and reduce phage inactivation and clearance. (d) Enzymatic bacteriophages can be engineered to degrade barriers to phage adsorption (left) and disrupt the structure of bacterial biofilms (right). (e) Non-lytic and/or non-replicative phages can reduce the release of bacterial endotoxins. (f) Bacteriophages can be engineered to target intracellular defense pathways, which protect bacteria against antibiotics. *From* [4]

4. Comparing phage and antibiotics

	Bacteriophages	Antibiotics
Environment	Are the most abundant living entities on the planet and natural enemies of bacteria	Normally synthetics or semisynthetic
Host range	Narrower	Destroy all bacterial cells disturbing the normal microflora
Side effects	Have not been described	Have severe side effects
Concentration	Decrease as soon as bacterial cells are eliminated	Decreases with time

Figure 6. Color enhanced Transmission Electron Micrograph (TEM) of T4 enterobacteria phages attacking an *E. coli* cell.
From © Eye of Science/Science Source

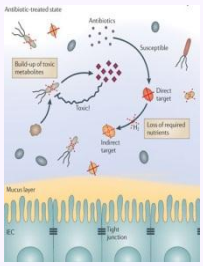

Figure 7. Antibiotics have direct and indirect effects on the microbiota. *From* [6]

Conclusions

- ✓ Currently, many pathogenic bacteria have acquired multiple drug resistance. In this context, one intriguing approach is to use bacteriophages therapy.
- ✓ Phages could play an important role in treating bacterial infections in humans, animals and crops, as well as in decontaminating food supplies and communal environments.
- ✓ However, it is also quite clear that the safe and controlled use of phage therapy will required detailed information on the properties and behavior of the specific phage-bacterium system.

References

1. Matsuzaki, S. *et al.* (2005) J infect Chemother 11:211-219.
2. Hagens, S. *et al.* (2004) Antimicrob Agents Chemother 48:3817-3822.
3. Fenton, K. *et al.* (2010) Bioengineered Bugs 1:1,9-16.
4. Lu, KT. *et al.* (2011) Current Opinion in Microbiology 14:524-531.
5. Kutateladze, M. and Adamia, R. (2012) Trends in Biotechnology 28:12 591-595.
6. P. Willing B. *et al.* (2011) Nature Reviev in Microbiology 9: 233-243.

Universitat Autònoma de Barcelona