

## What can bacteriophages do for us?

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The phenomenon of antibiotic-resistance in pathogenic microorganisms represents a worldwide hot-spot in the healthcare since new antibiotic-resistant strains are emerging at a constant rate and traditional treatments, based on antibiotic therapy, are now failing. The search of new alternatives is thus necessary and mandatory but the answer for the problem of antibiotic resistance may not be as far way in the future as might first be thought. Bacteriophages are viruses that attack and lyse bacteria. They produce two type of enzymes that can be used in therapy: holins and lysins. These enzymes are able to degrade the bacterial cell wall, thus causing their lysis and death. The search of formulations based in phage products offers a new hope for medicine in the struggle against illness.

**Keywords** phage; antibiotic-resistance; phage; holin; lysozyme.

### 1. Antibiotic resistance.

Since discovery of the first antibiotic by Fleming, the pharmaceutical industry began to produce penicillin for clinical use [1], and for 50 years the production of natural or synthetic antibiotics has been continuous. However, the number of approved antibiotics by the Federal Drug Administration (FDA) has been very reduced (Table 1), and hence we are faced with the fact that the number of new antibiotics is decreasing while the number of resistant microorganisms is increasing, this being particularly so in the case of ear infections due to pneumonia or meningitis. Natural antibiotics are produced by microbiota as defence mechanism against other bacteria or fungi. In contrast, chemical synthesis has developed important antibiotics such as fluoroquinolones, which that include Cipro or linezolid, which are effective against some resistant strains of *Staphylococcus*, *Streptococcus* and *Enterococcus* [2].

**Table 1** Approved antibiotics by FDA in last years [3].

Period	Approved antibiotics	Agents
1991-1995	26	multiple agents as temafloxacin, lomefloxacin, dirithromycin
1996-2000	11	meropenem, levofloxacin, gatifloxacin ..
2000-2003	3	linezolid, cefditoren pivoxil, gemifloxacin

There are many reasons for the antibiotic resistance phenomenon, the main one being abusive use over the past twenty years. The resistance phenomenon represents not only a important healthcare issue but also an economic problem, with an estimated cost of about 4000 million dollars per year [4]. The importance of the problem is so great that in 1999 the European Union established a directive aimed at coordinating the fight against antibiotic resistance within the different EU and the US government did the same in 2000 [5, 6]. Today, penicillin fails to completely eradicate streptococci in up to 35 % of patients [7]; the infections caused by *Streptomyces agalactiae* in pregnant women cannot be treated with antibiotics because they increase the risk of abortion [8]. Methicillin-resistant *S. aureus* accounted for

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nearly 60 % of nosocomial *S. aureus* infections in 2001 and vancomycin-resistant *Enterococcus faecium* have been reported [9].

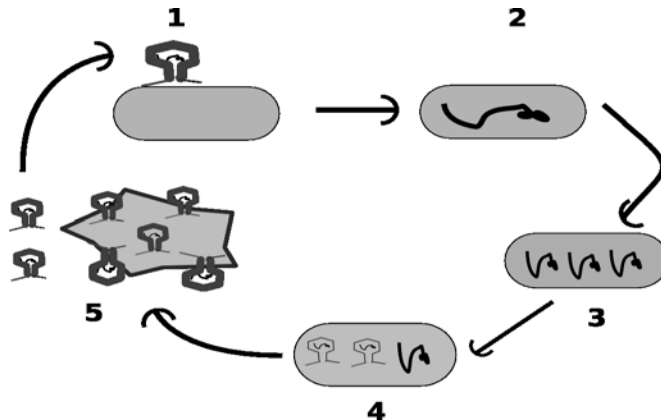
Morover, the resistance phenomenon has gained importance not only in humans but also in cattle. Thus, mastitis, caused mainly by *S. agalactiae*, *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae*, generate losses estimated at between 1.7 and 2 billion dollars [10].

## 2. Phages.

### 2.1 Life cycle of the bacteriophages

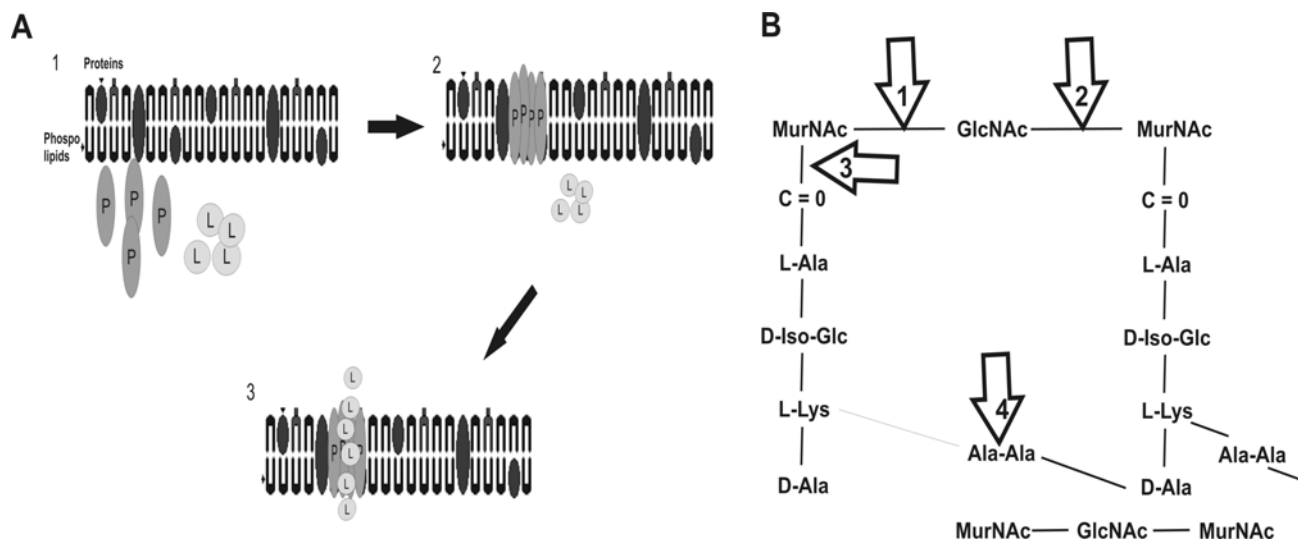
A bacteriophage is a virus able to infect and kill bacteria that, in the case of lytic phages, interferes unidirectionally with the normal bacterial metabolism, meaning that the bacteria enter a lytic cycle.

They are ubiquitous, obligate parasites and highly specific for their bacterial host. But what is an enzymatic? The most exact definition refers to a group of bacteriophage-associated enzymes that are produced actively during the lytic cycle. These enzymes are able to degrade the peptidoglycan layer of the bacterial cell wall. When this degradation has been carried out, new mature particles of bacteriophages can be released from bacterial cell (Fig. 1).



**Fig. 1** Bacteriophage life cycle. 1: bacteriophage attaches to a specific host bacterium. 2: it injects its DNA. 3: bacteriophage uses bacterial DNA and protein synthesis machinery to make the different bacteriophage parts. 4: Assembly of new bacteriophage. 5: The new bacteriophages are released after cell lysis so that new cycles can begin again.

For their release from the producing cell, most bacteriophages synthesize two type of enzymes: holins and lysins. Holins are small membrane proteins which are believed to accumulate in the cytoplasmic membrane as oligomers. They are responsible for the collapse of the membrane potential and the forming of non-specific membrane lesions, allowing lysins to access the peptidoglycan layer. They are the “clocks” of the lytic cycle (Fig. 2A).



**Fig. 2.** A: Action of holins: after their accumulation in the cytoplasm (1), formation of pores across the membrane (2), access of lysins to the peptidoglycan layer (3). B: Points of hydrolysis of the phage-encoded murein-degrading enzymes. 1: lysozyme; 2: glucosaminidase; 3: amidase and 4: endopeptidase.

Lysins are the responsible for the direct degradation of peptidoglycan layer; they are synthesized in the cytoplasm and reach the peptidoglycan layer thanks to the lesions made by the holins in the cytoplasmic membrane. The lysins are classified according to the point of hydrolysis within the peptidoglycan layer (Fig. 2B). N-acetylmuramidases (or lysozymes) hydrolyze the  $\beta$ -1,4-O-glycosidic bond between C1 of N-acetylmuramic acid (MurNAc) and C4 of N-acetylglucosamine (GlcNAc). Glucosaminidases cleave the  $\beta$ -1,4-O-glycosidic bond C1 of GlcNAc and C4 of MurNAc. Amidases (or N-acetyl-L-alanine amidases) cleave the amine bond between the L-alanine of the peptide chain to the lactyl group of the muramic acid. Endopeptidases cleave the peptide bonds that connect the peptide side-chains of adjacent glycan strands [11]. Lysozymes have found a variety of applications: as a food additive, in cheese and wine production, in household chemicals, ... [12]. Some bacteriophage-lysozymes can mediate a holin-independent host lysis, such as the lysozymes from oenococcal bacteriophage fOG44 and bacteriophage P1 [13, 14].

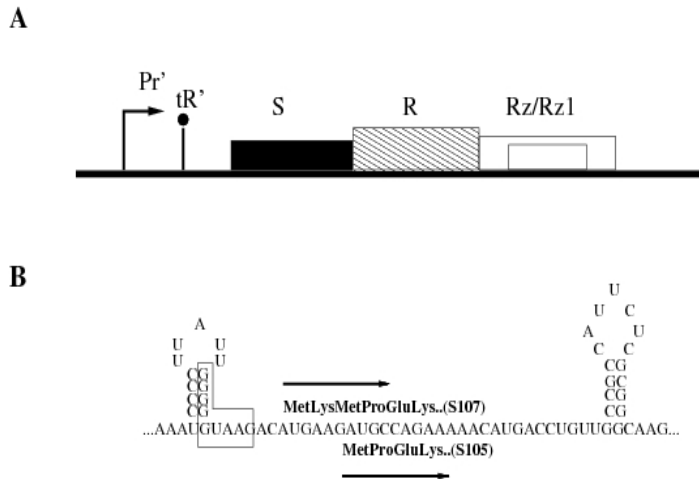
Holins are extremely diverse but can be grouped in three classes according to their known or predicted membrane topology. Class I holins have three transmembrane domains (TMD) while class II holins have two TMD [15].

## 2.2 Genetic organisation.

Usually, the genes that encode lysins and holin show a typical organisation in cluster, such that the genes encoding holins are located immediately upstream from lysins genes [16]. For example, the lysis cassette of bacteriophage  $\lambda$  has four genes, namely S, R, Rz and Rz1, but only S and R are absolutely required for lysing the host cell [17] (Fig. 3A). Many of the holin genes show the dual-start motif and thus encode two proteins (Fig. 3B). Both Met condons are used for translational initiation, giving rise to two proteins with two aminoacids difference at the N-terminal end. This difference marks the function of the protein: S105 is the holin whereas S107 is the "anti-holin" and its function concerns the inhibition of S105 [18]. Genetic evidences have revealed an mRNA secondary structure, termed sdi (site-direct initiation) that controls the translational initiations of S107 and S105 [17].

There are some cases such as bacteriophage P1 in which the holin gene is not clustered with the lysozyme gene [12]. If the holin gene of P1 is deleted, a delayed lysis on the host takes places [19]. In

this case, the lysin carries a N-terminal TMD, which acts as a normal signal-arrest-release (SAR) sequence. The lysin is transported by the host secretory system and accumulates in the periplasm in an inactive form [14], and its activation requires the release from the bilayer.



**Fig. 3.** A: Lysis cassette of bacteriophage  $\lambda$ . B: Dual-start motif of holin. The box indicates the Shine-Delgano sequence for the dual translational starts of S.

ssRNA bacteriophages code for neither a lysin nor a holin, but for proteins that interfere with bacterial peptidoglycansynthases [20].

Lysins are often chimaeric proteins, with a well-conserved catalytic domain fused to a divergent binding domain [21]. Although a rare occurrence, lysins of *Streptococcus* bacteriophages may contain introns that split the gene [22]. The binding between lysin and cell wall is site-specific event. When lysin from different bacteriophages of *S. pneumoniae* were analysed, it was also found that binding domain established bonds with choline residues present in the teichoic acids of the cell wall [23]. It was found that the cell-wall anchoring domain was formed by six repeats that were responsible for the recognition and union [24].

### 3. Bacteriophages and medicine

#### 3.1 A short walk across history.

Bacteriophages were discovered independently by Twort and D'Herelle in 1915 and 1917 respectively [25,26], and in fact D'Herelle successfully applied bacteriophage therapy to treat dysentery in Paris as early as 1918 [27]. Early on, the pharmaceutical industry began to sell bacteriophage products for human use [28]. However, following the discovery of antibiotics and their general application, the uses of bacteriophages in medicine waned, leaving some countries as the former Soviet Union to continue actively isolating bacteriophages and using them to treat serious infections, specially in the Eliava Institute, established by Giorgi Eliava, a student of D'Herelle [1, 29]. The Eastern Block situation has meant that these approaches have largely remained unknown in the West.

However, changes in political circumstances together with the antibiotic-resistance phenomenon have reversed this lack of communion between the East and the West, to such an extent that Western countries have turned their eyes to the bacteriophages-based therapies that were carried out for decades in the Eastern countries. Most recent articles appearing in the Western journals reflect little knowledge of the extensive Eastern European research and clinical utilization of the phage therapy.

### 3.2 What was happening in Western?

While in Eastern Europe, investigators applied bacteriophages for therapy, in the Western Block studies on bacteriophages focused on the new field of molecular genetics: the identification of DNA, the genetic code, mRNA and so on, mainly with the phages  $\lambda$  or T. The bacteriophages were proposed as vehicles for displaying proteins. In this case, the phage would display a protein on the surface through transcriptional fusion of the target gene with a coat-protein gene [30]. Bacteriophages-display libraries can be used to isolate proteins or peptides, with practical applications [31]. Bacteriophages have also been proposed as potential gene delivery vectors in genetic therapy or as vaccine delivery vehicles. Because of the inherent immunogenicity of phages, the virions are quickly engulfed by antigen-presenting cells and broken down, allowing release of the DNA vaccine [32].

The uses of bacteriophages were considered, first, in fields such as aquaculture, plague control in agriculture or veterinary medicine [10, 33, 34, 35]. Thus, bacteriophages therapy was successfully applied to the treatment of septicaemia and meningitis in chickens and calves [36]. Bacteriophages have been proposed as biocontrol agents to reduce *Salmonella* in poultry products [37], or to prevent food-borne pathogens.

The political changes in the Eastern Block plus the antibiotic-resistance phenomenon in bacteria spurred the Western Block turned its eyes back bacteriophages as possible therapeutic agents. The development of molecular techniques enabled the possibility to be considered of therapies based on intact bacteriophages or phage components. The main bacteriophage components for therapy are the cell wall hydrolases or lysins. The killing effect of a lysin can be obtained when purified recombinant forms are applied directly to sensitive bacteria. Also, lysin engineering can be carried out in order to increase their effectiveness. For example, it has been possible to swap different lysin domains with different bacterial and catalytic domains, resulting in a new enzyme that cleaves different bonds in the peptidoglycan but with the same specificity [22]. Recently, successful bacteriophage therapy in the treatment of lethal and vancomycin-resistant *Enterococcus faecium* infections in mice has been described [38]. Bacteriophage M13R merits special mention in that it presents the holin gene altered [39]. This bacteriophage is thus able to kill *E. coli* without actually lysing the cells. This engineered form prevents the release of endotoxin from lysed bacteria and, hence undesirable side effects in patients. A development of this model was introduced by Westwater et al., who developed non-lytic bacteriophages with a system of programmed death controlled by two modules: a stable toxin and an unstable antidote that neutralised the toxin effect [40].

Bacteriophages show normal specificity towards the bacterial host. However, bacteriophages such as P1 are able to inject their DNA into a broad range of Gram -negative bacteria [41]. The lysin plyGBS isolated from the GBS bacteriophage NCTC 11261 shows different lytic activity within the different groups of streptococci and exhibits enzymatic activity against other species such as *S. salivarius*, *S. gordonii* and *S. mutans* [42]. This enzyme can be used in treatment against infections caused by *Streptococcus agalactiae* located in the vagina or oropharynx. The  $\gamma$  bacteriophage produces a lysin effective against *Bacillus anthracis* [43]. The virion p68 produces the p17 lysin, effective against *Staphylococcus aureus* [44]. The lysins  $\phi$ 13 and  $\phi$ 6 from bacteriophages  $\phi$ 13 and  $\phi$ 6 have been shown to be efficient against *Pseudomonas syringae* [45].

The growth of *Helicobacter pylori*, a pathogen associated with gastritis, peptic ulcers and gastric cancer, is inhibited by a recombinant bacteriophage based on the filamentous bacteriophage M13 [46].

An interesting perspective concerns the possibility of combining the bacteriophage lytic enzymes and antibiotics. Thus it has been possible to successfully combine the amidase Pal and the lysozyme Cpl-1 against *S. pneumoniae* [23, 47] and lysin Cpl-1 with the antibiotics gentamicin and penicillin [22].

## 4. Antibiotics, enzybiotics or the other way around?

It is not possible to state that either (antibiotics, enzybiotics) is better than the other. Both have advantages and disadvantages. The main advantage of antibiotics over enzybiotics is their broad spectrum of action, and it is not necessary to identify the bacterium causing the infection before treating

the patient; however, in bacteriophage therapy, an *a priori* identification of the causal agent is required. Currently, it is possible to identify a given strain in a matter of minutes but the cost of this type of diagnosis means that molecular biology techniques cannot be used on a routine basis [48]. Accordingly, the specificity of bacteriophages can be a problem, as stated above, but it can also be an advantage because it minimizes the undesirable side-effects of antibiotics: treatments with phages do not affect the normal microbiota of the organism. As also indicated above, other undesirable side-effect of phage therapy, and also of the antibiotic-based therapies, such as the release of endotoxin from killed bacteria, can be avoided when bacteriophages without a lysis system are employed.

When therapy with intact bacteriophage particles is considered, a clear advantage must be taken into account: bacteriophages replicate inside the target bacteria and hence a single-injection of bacteriophages elicits multiple progeny inside the organism and a single injection may be enough for the treatment. Although there are data concerning the persistence of administered bacteriophages for several days in the human body, no evidence at all of their replication has been reported [28].

Since phages are the most abundant and ubiquitous “creatures” on the earth, humans are exposed to bacteriophages as from the very moment of birth, and this explains the good tolerance for bacteriophage-based treatments.

Bacteriophage therapies are not effective against dormant spores, because these latter have barriers that protect the peptidoglycan layer. However, such barriers disappear after 10 minutes after germination. At this moment, phages are able to attack the peptidoglycan and to destroy the bacteria [43].

Moreover, the treatment with purified lysins shows absence of immunotoxicity in rabbits or toxic side effects [49, 50]

The production cost of bacteriophages is very low as compared to antibiotic production. If the costs of development of new antibiotics are taken into account, then the advantage of phages over antibiotics is clear.

Another interesting point related to phage therapy is that all the effects will be located at the infection site, while antibiotics do not necessarily concentrate at the site of infection. A bacteriophage cannot replicate without the presence of the target bacteria, and they therefore only act when and where the bacteria in question become available. Today, however, this advantage can also be considered a disadvantage as well. The size of bacteriophages suggests poor tissue distribution [51]. Nevertheless, this can be overcome by using different methods of bacteriophage administration to allow them to reach the affected tissues. In this sense, some studies have shown that after intravenous injections bacteriophages can be detected in nearly all organs [52].

Bacteria also develop resistance to bacteriophages, but as yet it is easier to develop or find new bacteriophages able to attack bacteria than new antibiotics. Bacteriophage-resistant bacteria remain susceptible to other bacteriophages having a similar target range.

## 5. And now, what?

It is mandatory that further investigation be carried out. The efficacy of phage-based products has been demonstrated both *in vitro* and *in vivo*, but it is now necessary to open the door and allow their use in a much more open sense. Recently (2006), the FDA has approved a cocktail of bacteriophages for use against *Listeria monocytogenes* contamination in ready-to-eat meat and poultry products [53], and indeed this is the first time that a phage preparation has been approved as a food additive. The Phage Therapy Center of Tbilisi (Georgia) is a clinic specializing in bacteriophage therapy in two situations: i) infections in tissues where circulation is poor, thereby hindering the delivery of antibiotics to the infected area, and ii) infections with bacteria resistant to antibiotics [54].

The Southwest Regional Wound Care Centre (Texas, USA) has been using phages in therapy to treat antibiotic-resistant infections [31]; biodegradable patches impregnated with bacteriophages have been used in Georgia to treat patients with chronic infections [53]. The “Phage International” company has developed a product called “PhagoBioDerm”, a biodegradable polymer impregnated with

bacteriophages, antibiotics and proteolytic enzymes that can be used for both prophylaxis and therapy [55]. This product showed very promising results when it was assessed in the treatment of infected venous stasis ulcers and other poorly healing wounds, where antibiotics are unable to penetrate because of poor wound vascularization; it also demonstrated a fair degree of efficacy in the eradication of multidrug-resistant *S. aureus* in patients with skin damage [56, 57]. Additionally, there is a version called “PhageDent”, which was formulated for periodontal applications [58].

One of main problems countered by the FDA in this type of study is the difficulty involved in obtaining a lot-to-lot stable composition of all components [53]. It should also be taken into account that clinical trials are costly.

Bacteriophage components have been explored for developing tools to control processes such as milk fermentation, cheese ripening; indeed, even transgenic cattle able to secrete a recombinant lysin-like hydrolase into their milk to protect against *S. aureus* mastitis have been investigated [53].

As mentioned above, bacteriophage-based therapies have been focused on whole particles or bacteriophage components. When bacteriophage components were considered, the works based on lysin outnumbered the rest. However, recently, the importance of holins, as well as their application, has been brought to the attention of researchers. Thus, recent studies have addressed the cytotoxic activity of the holin from  $\lambda$  bacteriophage against mammary cancer cells *in vivo*, suggesting its potential therapeutic use in cancer therapy [59].

One relevant factor in the development of all bacteriophage-based therapies is their intellectual property status, because the idea of using bacteriophages as therapeutic tools is almost a hundred years old, and as such, unpatentable. The question is then obvious: are companies willing to risk the investment of huge amounts of money if they are not going to be able to progress through a single international patent? Despite all this, the foundation of companies for development of bacteriophage-based products has already begun [9], thus already offering different applications for different fields, ranging from agriculture to medicine (Table 2).

**Table 2** Examples of some bacteriophage-based companies

Company	Main Focus
Exponential Biotherapies (USA)	Phase 1 clinical trial completed of vancomycin-resistant <i>Enterobacterium</i>
Phage Therapeutics International (Canada)	Phage preparations for antibiotic-resistant <i>Staphylococcus</i>
Biophage Pharma (Canada)	Cancer, infection/inflammation and immune modulation
Intralytix (USA)	Environmental, food processing and medical applications
PhaGen AB (Sweden)	Phage therapy technology
Hexal Gentech (Germany)	Phages deliver vehicles
Phage Biotech (Israel)	Clinical, veterinary, agricultural, industrial and ecological applications
Enzobiotics/New Horizons Diagnostics (USA)	Diagnostic agents and topical therapeutics
ImBio (Rusia)	liquid, tablet and formulations for treating bacterial infections
BioPhag (Rusia)	two complex phage preparations
Biomed (Rusia)	First bacteriophage manufactured against dysentery (1940). First commercial batches of staphylococcal phages (1994)
CMBP (Georgia)	PhagoBioDerm
Eliava Institute of Bacteriophage (Georgia)	therapeutic phage preparations

## 6. Conclusions

The efficacy of bacteriophages in therapy has been widely tested, although more studies are necessary, and advances in pharmacokinetics, studies on (+bio?)compatibility and those addressing synergistic effects with other substances such as antibiotics, probiotics, and so on, are required.

Additionally, the study of bacteriophage genomes and the role of the bacteriophage genes in the lytic cycle, as well as the mechanisms of bacteriophage-bacteria interactions, may identify novel therapeutic targets to improve current bacteriophage-based therapies.

**Acknowledgements** The authors wish to express their deepest gratitude to the Xunta de Galicia, the Spanish Ministry of Education and the Ramon Areces Foundation of Madrid.

## References

- [1] A. Sulakvelidze, *Drugs Discovery Today*, **12**,807–809 (2005)
- [2] T. M. Powledge, *Public Library of Science Biology*, **2**,151-154 (2004)
- [3] [www.fda.org](http://www.fda.org)
- [4] Workshop summary. Institute of Medicine. Antimicrobial resistance: issues and options, National Academy Press, Washington D. C. (1998)
- [5] European Union Council. Resolution 8.7.1999. C195 17.7.1999
- [6] US Centers for Disease Control, the Food and Drug Administration and the US National Institutes of Health. ([www.cdc.gov/drugresistance/actionplan/aractionplan.pdf](http://www.cdc.gov/drugresistance/actionplan/aractionplan.pdf)) (2000)
- [7] D. Nelson, L. Loomis and V.A. Fischetti, *Proceedings of the National Academy of Sciences of the United States of America*, **98**,4107-4112 (2001)
- [8] Q. Cheng, D. Nelson, S. Zhu and V.A. Fischetti. *Antimicrobial Agents Chemotherapy*, **49**,111-117 (2005)
- [9] K. Thiel, *Nature Biotechnology*, **22**,31-36 (2004)
- [10] D.M. Donovan, S. Dong, W. Garrett, G.M. Rousseau, S. Moineau and D.G. Pritchard, *Applied and Environmental Microbiology*, **72**,2988-2996 (2006)
- [11] E.A. Stojkovic, L.B and Rothman-Denes, *Journal of Molecular Biology*, **366**,406-419 (2006)
- [12] C. Lee, J. Lin, T. Chow, Y. Tseng and S. Weng, *Protein Expression and Purification*, **50**,229-237 (2006)
- [13] C. Sao-Jose, R. Parreira, G. Vieira and M.A. Santos, *Journal of Bacteriology*, **182**,5823-5831 (2000)
- [14] M. Xu, A. Arulandu, D. K. Struck, S. Swanson, J.C. Sacchettini and R. Young, *Science*, **307**,113-117 (2005)
- [15] R. Young, *Journal of Molecular Microbiology and Biotechnology*, **4**,21-36 (2002)
- [16] J. Farkasovska, A. Godany and C. Vlcek, *Folia Microbiology*, **49**,679-684 (2004)
- [17] A. Grundling, D. L. Smith, U. Blasi and R. Young, *Journal of Bacteriology*, **182**,6075-6081 (2000)
- [18] T. Park, D. K. Struck, J. F. Deaton and R. Young, *Proceedings of the National Academy of Sciences of the United States of America*, **103**,19713-19718 (2006)
- [19] C. Schmidt, M. Velleman and W. Arber, *Journal of Bacteriology*, **178**,1099-1104 (1996)
- [20] I. Young, I. Wang and W. D. Roof, *Trends Microbiology*, **8**,120-128 (2000)
- [21] I. N. Wang, D. L. Smith and R. Young, *Annual Review Microbiology*, **54**,799-825 (2000)
- [22] V. A. Fischetti, *Trends Microbiology*, **13**,491-496 (2005)
- [23] I. Jado, R. Lopez, E. García, A. Fenoll, J. Casal and P. García, *The Journal of Antimicrobial Chemotherapy*, **52**,957-973 (2003)
- [24] J. A. Hermoso, B. Monterroso, A. Albert, B. Galan, O. Ahrazen, P. Garcia, M. Martinez-Ripoll, J. L. Garcia and M. Menendez, *Structure*, **11**,1239-1249 (2003)
- [25] F. W. Twort, *Lancet*, **ii**,1241 (1915)
- [26] F. D'Herelle, *Les Comptes rendus de l'Académie des Sciences*, **165**,373–375 (1917)
- [27] W. C. Summers, Felix D'Herelle and the origins of molecular biology, 1 st. Ed. New Haven, Yale University Press (1999)
- [28] A. Sulakvelidze, Z. Alavidze Jr. and J. G. Morris, *Antimicrobial Agents Chemotherapy*, **45**,649–659 (2001)
- [29] W. E. Huff, G. R. Huff, N. C. Rath, J. M. Balog and A. M. Donoghue, *Poultry Science*, **84**,655-659 (2005)
- [30] G. P. Smtih, *Science*, **228**,1315-1317 (1985)
- [31] J. R. Clark and J. B. March, *Trends in Biotechnology*, **24**,212-218 (2006)
- [32] J. R. Clark and J. B. March, *FEMS Immulogy and Medical Microbiology*, **40**,21-26 (2004)
- [33] H. Heuer, R. M. Kroppenstedt, J. Lottman, G. Berg and K. Smalla, *Applied and Environmental Microbiology*, **68**,1325-1335 (2002)
- [34] L. D. Goodridge, *Trends in Biotechnology*, **22**,384-385 (2004)



- [35] J.P. Higgins, S. E. Higgins, K. L. Guenther, W. Huff, A. M. Donoghue, D. J. Donoghue and B. M. Hargis, *Poultry Science*, **84**,1141-1145 (2005)
- [36] P. Barrow, M. Lowell and A. Berchieri, *Clinical and Diagnostic Laboratory Immunology*, **5**,294-298 (1998)
- [37] W. E. Huff, G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue, *Poultry Science*, **84**,655-659 (2005)
- [38] B. Biswas, S. Adhya, P. Washart, B. Paul, A. N. Trostel, B. Powell, R. Carlton and C. R. Merril, *Infection and Immunity*, **70**,204-210 (2002)
- [39] S. Hagens and U. Blasi, *Letters in Applied Microbiology*, **37**,318-323 (2003)
- [40] C. Westwater, L. M. Kasman, D. A. Schofield, P. A. Werner, J. W. Dolan, M. G. Schmidt, and J. S. Morris, *Antimicrobial Agents Chemotherapy*, **47**,1301-1307 (2003)
- [41] G. K. Schoolnik, W. C. Summers and J. D. Watson, *Nature Biotechnology*, **22**,505 (2004)
- [42] Q. Cheng, D. Nelson, S. Zhu and V. A. Fischetti, *Antimicrobial Agents Chemotherapy*, **49**,111-117 (2005)
- [43] R. Schuch, D. Nelson and V. A. Fischetti, *Nature*, **418**,884-889 (2002)
- [44] M. Takac and U. Blasi, U., *Antimicrobial Agents Chemotherapy*, **49**,2934-2940 (2005)
- [45] R. Dangelavicius, V. Cvirkaite, A. Gaidelyte, E. Bakiene, R. Gabrenaite-Verkhovskaya and D. H. Bamford, *Journal of Virology*, **79**,5017-5026 (2005)
- [46] J. Cao, Y. Sun, T. Berhlindh, B. Mellgard, Z. Li, B. Mardh and S. Mardh, *Biochimica et Biophysica Acta*, **1474**,107-113 (2000)
- [47] J. M. Loeffler and V. A. Fischetti, *Antimicrobial Agents Chemotherapy*, **47**,375-377 (2003)
- [48] S. Projan, *Nature Biotechnology*, **22**,506 (2004)
- [49] J. M. Loeffler, D. Nelson and V. A. Fischetti, *Science*, **294**,2170-2172 (2001)
- [50] R. Schuch, D. Nelson and V. A. Fischetti, *Nature*, **418**,884-889 (2002)
- [51] S. Projan, *Nature Biotechnology*, **22**,167 (2004)
- [52] K. Dabrowska, K. Switala-Jelen, A. Opolski, B. Weber-Dabrowska and A. Gorki, *Journal of Applied Microbiology*, **98**,7-13 (2005)
- [53] V. A. Fischetti, D. Nelson and R. Schuch, *Nature Biotechnology*, **24**,1508-1511 (2006)
- [54] [www.phagetherapycenter.com](http://www.phagetherapycenter.com)
- [55] [www.phageinternational.com](http://www.phageinternational.com)
- [56] K. Markoishvili, G. Tsitlanadze, R. Katsavara, J. G. Morris and A. Sulakvelidze, *International Journal of Dermatology*, **41**,453-458 (2002)
- [57] D. Jikia, N. Chkhaidze, E. Imedashvili, I. Mgaloblishvili, F. Tsitlanadze, R. Katsarava, J. G. Morris and A. Sulakvelidze, *Clinical and Experimental Dermatology*, **30**,23-26 (2005)
- [58] T. E. Shishniashvili, *Medical Journal of Australia*, **2**,71-78 (1999)
- [59] C. A. Agu, R. Klein, S. Schwab, M. Konig-Schuster, P. Kodajova, M. Ausserlechner, B. Binishofer, U. Blasi, B. Salmons, W. H. Gunzburg and C. Hohenadl, *The Journal of Gene Medicine*, **8**,229-241 (2006)