Review paper

Application of bacteriophages – selected data

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Abstract

Specific properties and life cycle of bacteriophages determine their influence on evolution bacteria genomes and make them as potential tool in the face of crisis evoked by bacteria resistance to antibiotics. Those positive results obtained while using them in pharmacy, food industry, environmental protection and tumor therapy are a proof of their vast exploration.

Key words: *bacteriophages, characteristics of bacteriophages, application of bacteriophages.*

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Almost 100 years ago, British bacteriologist, Frederick Twort, employee at the Brown College in London, discovered a factor which is presently described as presented in Figure 1, and which has a damaging effect on bacteria [1]. Another independent co-discoverer of such bacteria [1], was Felix d'Herelle, who almost simultaneously shared his discovery with the world, for the first time using the term bacteriophages (from Greek: *phagein* – to eat), and later used them in pioneering experiments on fighting against pathogenic bacteria. Notwithstanding the facts described by those two researchers and other data on bacteriophages [3, 4], it was not envisaged that they would become very important for many areas of natural science, including biology and medicine. This was partly predicted by Felix d'Herelle already in 1930, who was "ahead of his time", as the bacteriophage described by him, as the simplest biological system, is presently a foundation for many studies i.a. in the present molecular biology [5]. It is assumed that bacteriophages, also referred to as phages, being one of the greatest enemies to bacteria, are among the most complex viruses in the structural aspect. As all viruses, they are incapable of individual replication, but infect bacteria specifically for the species, or even strain. Genetic material of the phages is the single- or double-stranded DNA, linear or closed as a circle, but also single- or double-stranded linear RNA [6]. According to the present ordination [6], bacteriophages described so far are classified into 15 families, yet due to the infection process, the following are differentiated among them: lytical phages (malicious), which cause lysis of the cell due to production of filial viruses, as a result of full development cycle [7]; lysogenous phages (mild), whose development cycle stops in the eclipse phase, which as a consequence does not lead to lysis of the infected cell [3, 4, 6, 8]; and filamenting phages, which cause chronic infections, not leading to cell death, yet forcing them to continuous production of filial phage particles [7].

Bacteriophages "enter" bacterial cells through absorption via appropriate receptors on their cellular wall, which constitutes the first phase in their lifecycle. Using own enzymes, they cause lysis to bacterial wall, which is the second phase of the cycle, during which own genome is "injected" into the bacterial cell [3, 4, 6, 8]. The third phase in bacteriophage development is the eclipse phase, which comprises subordination of the host's metabolism to phage's genome. In the case of mild phages, this is the last phase of their development, where their replication stops [3, 4, 6, 8]. Phase four involves replication of bacteriophages' genetic material and synthesis of their kapsids, while phase five – release of bacteriophages in the form of "replicated" copies, which leads to lysis of the bacterial cell [3, 4, 6, 8]. Such a lifecycle, in theoretical sense, makes them have an impact on the evolution of bacterial genomes, while in practical sense, may serve as a potential tool e.g. for searching for alternative substances at the age of crisis caused by bacterial resistance to antibiotics. They may also

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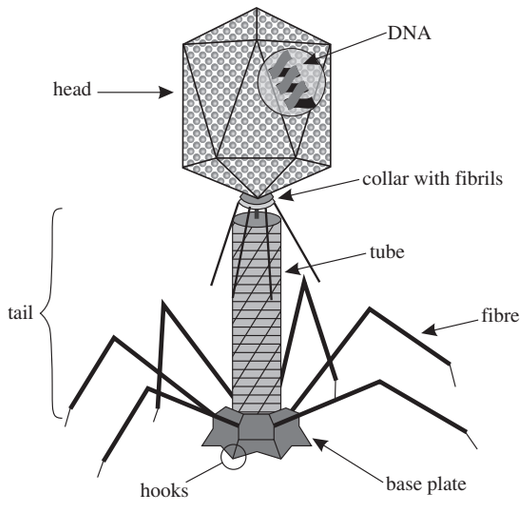

Fig. 1. T4 bacteriophage structure diagram [2]

play an important role in medicine and pharmacotherapy, as well as in molecular and food industry [3, 4, 9].

Bacteriophages and bacteria

At present, a significant problem of pharmacotherapy is the growing bacterial resistance to the operation of chemical compounds, including antibiotics, and fight against side effects resulting from their administration, which is related to drug intolerance, allergies, or reduced immunity [9-11]. Such a situation results in the increasing interest in alternative substances to chemical compounds, including antibiotics, which may result e.g. in a new treatment method for dangerous infections [10, 11]. Such facts is actually a return to methods from before the antibiotic era, namely the time of experiments with fighting against bacterial infections based on phages and products synthesised by bacteria infected with phages [12]. It must be stated that despite the passage of time, there are still rather few studies on phage treatment in humans, but also in animals. Such studies certainly have both advantages and disadvantages. Among the advantages of phage treatment, the following must be listed [12, 13]:

- host specificity – namely narrow spectre of bacteriophage action, which causes a situation where a phage used for treatment destroys specific – selected, yet harmful to the body bacterial species, while the commensal bacterial flora remains intact;
- genetic engineering of bacteriophages – namely possibility of genetic modification of bacteriophages leading to expression of new, very desired properties, e.g. regarding their maliciousness;
- cooperation of phages and antibiotics – which provides and ensures that the bacteria will not be capable of acquiring resistance to bacteriophages not to antibiotics;
- dynamic and quick replication of phages as compared to pathogenic bacteria, which gives them advantage over such pathogens.

Such advantages are, therefore, a sufficient pretext to continue studies on such bacterial viruses and to introduce measures aimed at elimination of possible drawbacks of bacteriophage treatment, including on the basis of nano-particles. As evidence for such an effect, one may use an experiment where bacteriophages infecting *Staphylococcus aureus* were used in treatment of patients with pyopneumonia and pyopleuritis [14]. Patients were divided into two groups, namely Group A – 223 patients receiving phages, and Group B – 117 patients receiving antibiotics. Complete healing was observed in 82% patients in the group treated with phages, in contrast to 64% patients in the group treated with antibiotics, and also the percentage of healing cases in the group administered phages intravenously was even higher, amounting to 95%. Other observations showed [15] that, in some cases, just one dose of phages is more "efficient" in treatment of infections than multiple administration of antibiotics. A further positive elements of bacteriophage treatment is the fact that their oral administration does not cause distortions to the natural bacterial flora of the digestive system, and does not cause changes to intestinal mucosa, which is very frequently observed in the case of antibiotics [16]. These results are of important practical significance, as they suggest that administration of phage preparations to patients should not weaken activity of cells conditioning natural immunity in humans [9]. The study [12, 15] also indicates that presently bacteriophage are not only used for treatment and prevention of contagious diseases caused by Gram-negative and Gram-positive bacteria, but there are also known attempts to apply phages in treatment of infections caused by antibiotic-resistant strains of *Mycobacterium* sp. It must be, however, added that such treatment, despite many aforementioned advantages, is still not deprived of negative effects. One of them, is the complex and complicated process of bacteriophage replication, which causes that the results of *in vitro* results do not always have to translate to *in vivo* studies. A further issue rendering this treatment difficult is the fact that the results of studies of just one bacteriophage species do not correlate with results for another species [17]. Another obstacle is formed by bacterial resistance caused e.g. by the loss of surface receptors of a bacterial cell through which phages get inside the cell [18]. Such a negative side of phage application is the resistance of the bacteria formed after bacterial cell infection with lysogenic bacteriophages [4] and their rather quick degradation from the organism, which may hinder the activities in their fighting bacterial infections, particularly in the case of chronic infections [16].

Bacteriophages and tumours

The role of bacteriophages in cancer treatment is related to the use of genetically modified filamentous bacterio-

phages, manipupated i.a. by their accompanying with a large volume of cytotoxic anti-cancer drug. It must be added that classic chemotherapy drugs used in anti-cancer treatment may cause non-targeted transfer of drugs in the organism, which may lead to their non-specific toxic effect onto other tissues of the body. This imperfection, or drawback, may be overcome by the use of platforms – transporters formed by bacteriophages, which transfer drugs to the tumour in a targeted manner and limit the exposure of non-targeted tissues or organs to the possible harmful effect of the drugs. An example of this is formed by the use of antibodies anti-ErbB2 and anti-ERGR, as "target substances" [19]. This was done using hygromycin, which was genetically conjugated with a phage by specifically modified place of katepsin-B in the phage's envelope. Such directing of phage nano-drugs via specific antibodies to receptors on tumour cell membranes will end with endocytosis, intracellular degradation, and release of medicinal substance, which will lead to inhibition of tumour cells [19].

Bacteriophages in environment protection

In the recent years, the increased share of bacteriophages is recorded in environment protection, including protection of water environment, the pollution of which is caused by many severe consequences of ecological and epidemiological nature. Therefore, appropriate and possibly quick identification of the source of pollution is important, and this forms the first phase of fighting for cleanliness of water environment. It was evidenced [20-23] that increasingly more often, among the methods applied to detect the source of contamination in the environment, bacteriophages are used as specific bio-indicators. It is assumed that specific phages introduced to infected water replicate, which proves the presence of a particular contagious factor, and in the context of their high specificity as to the host, one may use it as indicator to define microorganisms contaminating a particular water environment [24]. The most frequently analysed, among alternative bio-indicators assessing the pollution of water environments (underground waters, sewerage, lakes, rivers, estuaries, bays and marine waters), are somatic bacteriophages [20, 21], F-specific RNA bacteriophages (FRNA) [22] and F-specific DNA bacteriophages [23]. Furthermore, in the case of F-specific RNA bacteriophages showing similarity to pathogenic viruses to mammals, their applications creates an opportunity for their use as markers for water pollution with such viruses [25]. Moreover, the four genogroups described among FRNA bacteriophages detected in the analysed environment mark the level of resistance of the water environment to cleaning processes and the capacity of bacterial survival in the environment [26]. Also, the application of F-specicif DNA bacteriophages may supplement such studies exactly in the area of pollution level of the water environment [23]. Further advantage of bacteriophage application in water environment is the fact that they ensure rather quick results, and allow for simple detection. It must also be added that bacteriophages as biomarkers and parameters used in environment protection are biologically natural elements, therefore safe, as they are also easily degraded [24]. Also, in the case of using FRNA bacteriophages for bio-indication, one may determine not only the level of pollution of water reservoirs, but also the source of such pollution – animal or human, which is important from the epidemiological point of view [21, 23, 25, 26]. However, a certain disadvantage of phage use in the technologies described is the fact that their frequent presence in such an environment as natural elements, they may lead to an error in the number of phages detected in water reservoirs. Also, a certain limitation to their use is the small number of phage bio-indicators designed for practical analyses [24]. In order to eliminate the aforementioned limitations, genetic manipulations to bacteriophages were performed, as using the phage genome M13mp18, marked by adding a short DNA sequence, the identification of this biomarker is facilitated, by its differentiation from the remaining biotic elements of the water environment [24]. It must be added that studies of water environments with the use of bacteriophages are not only limited to diagnostics, but also expand the knowledge on microbiology of waters. It must be remembered that the poor pool of data regarding the presence of bacteriophage and pathogenic viruses in water reservoirs may be a cause for improper determination of water quality, and may limit the methods for development of methodologies aimed at reduction of bacterial and viral infections in the water environment [3, 4, 21, 22].

Bacteriophages in food industry

The increase in incidence of poisonings and infectious diseases in humans and animals causes our need for hygienic agents in food production to grow significantly. This results from the fact that even state-of-the-art production techniques with intensive food monitoring programme are incapable of successfully controlling such processes, as the need for food distribution to an increasingly greater number of consumers enforces the production volume which, in the event of contamination, may lead to mass diseases, even epidemics. Therefore, the studies oriented at the improvement of food safety and hygiene also progress owing to discoveries of new methods and technologies, one of which involves the use of bacteriophages. This is because they selectively kill dangerous and specific bacteria [27-29]. The properties of bacteriophages or phage-like proteins allow for quick and accurate identification of undesired pathogenic factors in food, as well as in the food production environment. It must be added that specificity of phages as regards bacteria offers the opportunity of using them for bio-control of bacteria without distortion of the natural microflora or cultures, e.g. in fermented products [28]. Also,

phage selection is a good tool for differentiation of bacteria isolated from foods, as they are used for identification and characteristics of many bacteria causing diseases and possible epidemics [30-35]. The latter method has more advantages as compared to other methods, as it is more specific, as well as quick, while also being less costly [27, 29]. It is worth mentioning that an important issue while using phages for antibacterial protection of foods is the question still asked by researchers whether bacteriophage alone are not harmful, and whether, as very specific elements in bacterial recognition, have no harmful effect on commensal bacteria, particularly when administered orally [28, 36, 37]. The answer to such questions was given by toxicity studies in rats receiving e.g. high doses of Listeria P100 phage, which did not reveal any side effects [37]. Also, studies in humans using *E. coli*-specific phages revealed that they are safe for oral administration [36]. It must be added that while bacteria developed their specialist defence mechanisms against phages, phages also catch up and continuously adjust to such changes. The problem may be partly avoided by alternate application of various phages, e.g. in the "cocktail", or by using consecutive treatments, which may reduce the incidence of bacterial resistance to bacteriophages. Several existing strategies of fighting against pathogens to household animals, such as toxin-producing *E. coli*, *Campylobacter* sp., *Salmonella* sp., form a direct extension to "classic" methods of phage therapies also focused on elimination of bacteria in animals before slaughter [38, 39]. It must also be added that food contamination e.g. by *Listeria monocytogenes* is more probable during food processing than in the course of animal life, which, by impacting on food production, causes phage treatment to form good bio-control of the pathogen, and reduces the risk of poisonings and infectious diseases caused by such bacteria. This is confirmed by the fact that in the USA in 2006, FDA (Food and Drug Administration) approved the application of anti-Listeria phages as additives to food [38, 39].

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