The history of bacteriophages discovery starts in 1896, when British bacteriologist, Ernst Hankin observed antibacterial properties of Ganges water. After the studying the impact of the water on the presence of bacteria sp. Vibrio cholerae, he has found that substances contained in the water prevent spread of cholera epidemic [1]. Two years later Russian bacteriologist, Gamaleya and several other researchers have observed similar phenomenon for Gram-positive Bacillus subtilis [2]. However, only 20 years later British bacteriologist, Frederic Twort, has postulated that the antibacterial agent may be a virus [3]. Twort did not continue his research and two years later microbiologist Felix D’Herelle started to study bacteriophages. D’Herelle for the first time has isolated phages, and used them subsequently for treating bacillary dysentery in children [4].

Bacteriophages are viruses that infect bacterial cells. Phage particle is named virion and is made of protein or lipido-protein capsid, which coats its genome. The important property of phages is their high specificity towards the host trough complementary receptor related to the presence of suitable receptor on bacterial surface [5]. Bacteriophage, after infecting bacterium may, destroy host cell by lytic cycle or may incorporate nucleic acid into bacterial chromosome thus initiating lysogenic cycle. During lytic cycle, phage activates processes leading to the multiplication of virus particles, causing formation of descendant virus particles, causing bacterial cell lysis and release of descendant phages. In the lysogenic cycle of bacteriophage, phage DNA is integrated with bacteria chromosome. Under stress conditions, the lytic cycle is induced [6, 7].

Bacteriophages, which are in equilibrium with bacterial world, are an important part of the ecosystem. It is estimated that on Earth there are approx. 10^{31} of such particles of a high diversity of genotypes [8]. In such a large virus pool, one may expect that there are nanoparticles of broad application spectrum. Therefore, intensive research in the field of applying these viruses focuses on looking for particles of potential applications in the treatment of bacterial infections.

Bacteriophages have been used in therapy as early as 1930s and 1940s, but they have lost competition with antibiotics. For many years, there was a growing interest in phages as particles of potential therapeutic application, among others, due to the fact that unjustified use of antibiotics for decades has led to the formation of new, antibiotic-resistant bacterial strains [9, 10]. The first centre for bacteriophage therapy within European Union was established in 2005 at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wroclaw. The Institute of Immunology possesses the collection, started originally by Ludwik Hirszfeld himself in 1948, consisting of 500 virulent phages of various origin [9]. Safety of phage therapy requires comprehensive understanding of interactions of bacteriophage particles in vivo systems. Thus it is important end, it is important to understand specificity of phage-bacteria interactions, pharmacokinetics of bacteriophage preparations, bacteriophage physicochemical properties, as well as an impact of stabilizer and impurities on biological properties of the preparation. The fact that bacteriophage preparations are self-replicating systems and thus in vitro studies do not translate directly to in vivo conditions is of special importance [11].

New aspects of bacteriophage application in treatment of bacterial infections are under development, e.g. recombinant phages, causing bacterial cell death, but causing bacteriolyis [12]. There is also a new approach involving use of bacteriophages as adjuvants in antibiotic therapy. The advantage of such combined therapy is a less likely development of probability of occurrence of antibiotic-resistant bacterial strains [13, 14].

Many biotechnology companies interested in application of bacterial viruses were established. An example may be Omnalytics, which has obtained approval of US Environmental Protection Agency for use of AgriPhage against plant pathogenic bacteria. EBI Ford Safety has put on the market product under name Listex™P100 for control of Listeria in meat and cheese [15]. In 2006, US Food and Drug Administration (FDA) have approved adding phage preparation LMP 102 (Intralytix, Inc.) to the food for control of dangerous bacteria sp. Listeria. In Poland, there is Proteon Pharmaceuticals working on designing products based on bacteriophages.

Bacteriophages have found broad application scope in genetic engineering, e.g. as vectors. Phages are used also for “display” of modified proteins on capsid surface. The technique called phage-display allows creating peptide libraries, which can be tested [16, 17].

Moreover, bacteria identification methods are developed. They use high specificity of phages in binding and infecting certain species of microorganisms, e.g. test involving phage infection for detection of MRSA (methicillin-resistant Staphylococcus Aureus). In the future, such methods in combination with phage therapy may become an effective, fast and relatively cheap way of combating bacteria [18, 19].

The important aspect of bacteriophage research is defining and understanding the properties of phage preparation impurities, whose source might be ingredients of medium or bacteriolysis products. The most important impurity for preparations obtained using Gram-negative bacteria is lipopolysaccharide (endotoxin, pyrogen), which is a strong immunostimulant. Its elevated concentration in blood causes many pathophysiological responses [20]. Under physiological conditions, human organism is constantly exposed to contact with endotoxins, as they are ubiquitous part of our environment. In case of injectable preparations endotoxin concentration standards depend on the type of products and thus, for inhaled air it is 20 ng m^{-3} [21]. EU (Endotoxin Unit) specifies pyrogenic properties of preparation. 1 EU is equivalent to 120 pg/ml of Escherichia coli endotoxin O111:B4 [22]. For preparations administered intravenously, the limit is specified by Pharmacopoeia and it is 5 EU kg^{-1} body weight per hour (European Pharmacopoeia, 1997) [23]. For preparations for administration to the central nervous system, endotoxin concentration in the preparation cannot exceed 0.2 EU kg^{-1} body weight per hour.

There are many methods for removal of endotoxins from preparations. Preparation of pyrogenic aqueous salt solutions poses no problems, while removal of impurities of biological preparations,
e.g. protein-based, each time requires specific solutions. While selecting purification method, number of factors must be taken into account: source of impurity, structure and molecular weight of endotoxin, character and molecular weight of purified biomolecule. Thus end such procedures as: ultrafiltration, gel filtration on polysaccharide carriers or phase separation are used. These methods use differences of molecular weight, ligand interactions and differences in hydrophilic and hydrophobic properties [24–29].

Bacteriophages are nanoparticles of diverse size, made of macromolecules of different charges and hydrophobicity. Biochemical complexity of phage virion and its size (as high as hundreds of nanometres) cause that not all purification methods used in biotechnology and medicine might be used. Diversity of particles forming bacteriophage capsid might result in that strong interaction with chromatography matrix panel will hinder or block elution of pure preparations. The exception is cellulose bed having low presentation due to the limited number of ionic bonds with bacteriophages [30]. Membrane filtration used in subsequent stages allows obtaining chromatography [30], uses heterophase extraction, which allows approach, is EndoT rap® – a commercial preparation [31].

Uses affinity of endotoxins to phage protein. An example of such an approach, is EndoTrap® – a commercial preparation [31].

The Biomedical Chemistry Lab “Neolek” of the Institute of Immunology and Experimental Therapy PAS, apart from ion-exchange chromatography [30], uses heterophase extraction, which allows effective removal of pyrogens using solvents hardly miscible with water [32]. Membrane filtration used in subsequent stages allows obtaining bacteriophage preparations, which are practically pyrogen-free. The method allows removal of pyrogens from bacteriophage preparations grown in Gram-negative bacteria with 99% efficiency.

Satisfying the requirements of pharmaceutical preparations is a major challenge for bacteriophages. It is not just about product purity, but also its stability. It was shown that highly purified preparations in poor culture media quickly lose their antibacterial activity and the addition of triblock copolymer (pluronic) greatly decreases loss of activity [33].

The used technologies guarantee repeatability of batches. Bacteriophages can be treated as nanoparticles constructed from many proteins of various features, which determine their physicochemical properties. Studying these properties may prove to be crucial for the development of purification methods, as well as conditions for storage, sterilization and stabilization of phase preparations.

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Bozena SZERMER-OLEARNIK – Ph.D., Eng., graduated from the Faculty of Chemistry at Wrocław University of Technology (2005). She got her Ph.D. in 2013. She is an assistant in the Laboratory of Biomedical Chemistry “NEOLEK” at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław. Scientific interests – physical chemistry of bacteriophages.

Literature


*e-mail: borat@istd.pan.wroc.pl

JANUSZ BORATYNYSKI – Ph.D., D.Sc., Eng., graduated from the Faculty of Chemistry of Wrocław University of Technology (1972). He got her Ph.D. in 1980 and his D.Sc. in 2000. He became a full professor in 2009. He is the Head of the Laboratory of Biomedical Chemistry “NEOLEK” at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław. Scientific interests – chemical modification of biological macromolecules.