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Microbiology

Characterization of New Bacteriophages of *Salmonella* and *Shigella*

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New, highly active clones of bacteriophages against *S. typhimurium, S. sonnei* and *S. flexneri* strains have been isolated. Susceptibility of *S. typhimurium* strains to the phages made up 96.3%. The phage adsorption period on the host cell varies within 10-15 min, the latent period is 22-28 min, and the yield of the phage makes up 110-180 phage particles per virion. It has been established that *Salmonella* and *Shigella* phages have a binary type of symmetry, a head and a tail. The head of phages is characterized by hexagonal symmetry, the dimensions of the phage head are different. The main differences concern the structure of the tail. Based on the morphology, *Salmonella* phages were classified into *Myoviridae* or *Siphoviridae* family, while *Shigella* phages belong to the morphological types of *Siphoviridae*, *Podoviridae* and *Myoviridae*. Considering high lytic activities and broad spectrum of these phages, they can be used for the creation of the polyvalent phage preparation for prevention and treatment of food-borne pathogens. Control of these pathogens will reduce their occurrence in the environment and food line. *© 2020 Bull. Georg. Natl. Acad. Sci.*

Salmonella, shigella, bacteriophage, morphology, biological properties

Salmonella is important food-borne pathogen that causes food poisoning. Salmonellosis is a major illness accompanied by headache, diarrhea, vomiting, and high fever due to *Salmonella* infection in the epithelial tissue of animals and humans via contaminated food [1]. *Shigella* is another wide-spread food-borne pathogen causing human diarrhea termed "shigellosis" [2]. These bacteria are responsible for food poisoning outbreaks worldwide through various foods, such as ground meat, raw milk, apple cider, and fresh vegetables. In the United States, approximately 500,000 cases of shigellosis and more than 1.4 million cases of food-borne salmonellosis have been reported per year, with 17,000 hospitalizations and 600 deaths [3, 4]. Wide and not always justified use of antibiotics and chemopreparations in medicine, especially of a broad spectrum, promoted the ubiquitous spread of bacteria with natural and acquired resistance. At the same time, intensive antibiotic therapy is often accompanied by a number of complications – changes in the immune reactivity, development of infections caused by opportunistic microorganisms.

Even though various antibacterial preparations have been developed to control these pathogens, development of safe and effective new agents to control food-borne pathogens is urgently needed [5].

Existing in nature abundance of bacteriophage clones with different morpho-biological properties and molecular organization, differing from one another both in mechanism of interaction with host cells and reproductive capacity, requires a clear differentiation and in-depth study of principal and some subsidiary taxonomic characters for their purposeful use in the treatment and prevention of bacterial infection.

The aim of this work is to study the morphological and some biological properties of new phage clones (pure lines) of *salmonella* and *shigella* phages.

thermostat at 37°C for 24h. After 18-24h of incubation, material is filtered through Millipore filters and checked for the presence of phage.

The study of a number of theoretical and practical problems requires availability of pure phage lines. For this purpose, the cloning method is used. Cloning of bacteriophages is carried out by passage of morphologically homogeneous negative colonies on homologous bacterial cultures. To study the interaction of the phage with the host cell, adsorption and phage yield are examined. To study the morphology of the phage itself, an electron microscope is used.

Results and Discussion

Isolation of new phages of salmonella was conducted from wastewater, the Mtkvari River and the stool of patients with salmonellosis. Five

Table 1. Electron microscopic parameters for 5 phages of S. *typhimurium*

Name of the phage	Host strain	Morphological group	Dimensions of the phage head	Size of the phage tail
			Length × width	Length × width
S.typhimurium S-25	*S.typhimurium* Q	*Myoviridae*	60 nm × 50 nm	95 nm × 20 nm
S.typhimurium S-146	*S.typhimurium* Q	*Myoviridae*	60 nm × 50 nm	95 nm × 15 nm
S.typhimurium SK-18	*S.typhimurium* Q	*Myoviridae*	75 nm × 70 nm	10 nm × 15 nm
S.typhimurium SS-19	*S.typhimurium* Q	*Siphoviridae*	45 nm × 45 nm	125 nm × 15 nm
S.typhimurium S-171	*S.typhimurium* Q	*Siphoviridae*	45 nm × 45 nm	100 nm × 15 nm

Table 2. Electron microscopic parameters for the 6 phages of *S. sonnei* and *S. flexneri*

Phage name	Host strain	Morphological group	Dimensions of the phage head	Size of the phage tail
			Length × width	Length × width
S. flexneri 8	*S. flexneri* 2a 25	*Siphoviridae*	50 nm × 50 nm	140 nm × 15 nm
S.flexneri 3a 25	*S. flexneri* 2a 25	*Siphoviridae*	45 nm × 45 nm	125 nm × 15 nm
S. flexneri 7	*S. flexneri* 2a 25	*Siphoviridae*	50 nm × 50 nm	140 nm × 15 nm
S. flexneri 3	*S. flexneri* 1в	*Podoviridae*	50 nm × 50 nm	15 nm × 10 nm
S. sonnei 442	*S. sonnei* 1148	*Myoviridae*	60 nm × 60 nm	95 nm × 15 nm
S. sonnei 3	*S. sonnei* 32	*Podoviridae*	45 nm × 45 nm	20 nm × 10 nm

Materials and Methods

Isolation of bacteriophages from wastewater: 10ml of concentrated broth is added to 90ml of wastewater, and to isolate a particular phage, 1ml of the corresponding 18-hour culture is added to this mixture [6]. The mixture is placed in a

phages, conditionally named as S-25, S-146, SK-18, SS-19, S-171, were isolated. 110 strains of *S. typhimurium* were isolated from different clinics in Tbilisi (Republican Children's Hospital, Republican Infectious Diseases Hospital, Republican Hospital).

The susceptibility of freshly isolated strains to new bacteriophages was determined. As the results showed, 96.3% of *S. typhimurium* strains were sensitive to 5 new phages.

To characterize the biological properties of *S. typhimurium* bacteriophages, the following bacterial features were used in the study: the morphology of negative colonies, the spectrum of the lytic action, the rate of adsorption and the average yield per one cell.

On the lawn, *S. typhimurium* phage culture gave two types of negative colonies: medium – from 1.5 to 2.0mm in diameter with smooth edges and a transparent center and large – from 1.5 to 4.0mm in diameter with a transparent center and even edges.

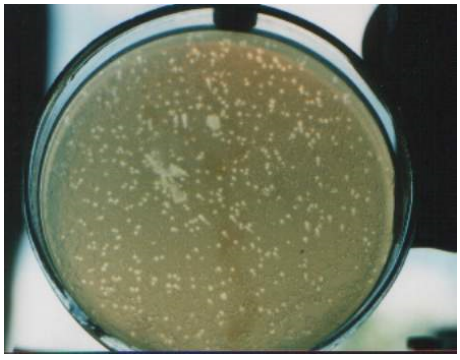

Fig. 1. Plaques of phage *S. typhimurium* S-25 on the strain *S. typhimurium*. Q plaque's diameter is 1-**2** mm.

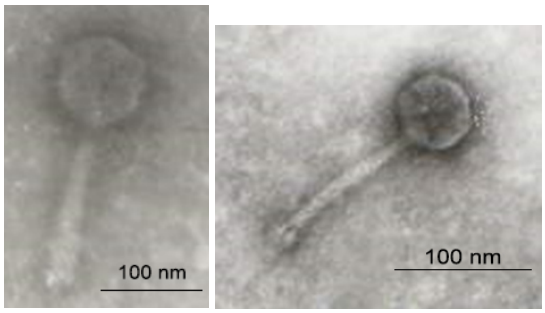

Fig. 2. *S. typhimurium* SK-18 and *S. typhimurium* SS-19 phages electron micrograph.

The adsorption time of the studied phages varied within 11-14 min, the latent period was 22-28 min, and the yield was 120-130 phage particles per virion.

It has been established that *S. typhimurium* phages have a binary type of symmetry, a head and a tail. They belong to the morphological types of *Myoviridae* and *Siphoviridae*. The head of phages is characterized by hexagonal symmetry, the dimensions of the phage head are different (Table 1). The main differences concern the structure of the tail.

The isolation of new phages against *S. sonnei* and *S. flexneri* strains was carried out from wastewater as well as from the Mtkvari River. Six phages in all were isolated, 4 phage clones against *S. flexneri* and 2 clones against – *S. sonnei*.

Phage clones were characterized by the following parameters: morphology of negative colonies, adsorption rate, and average yield per one cell. On the host strain, *S. flexneri* phage clones gave negative colonies from 4 to 5mm in size, and *S. sonnei* phage clones – from 4 to 6mm, with a transparent center and even edges.

The adsorption time for the studied clones of *S. flexneri* phages varied from 10 to 15 min, the latent period was 22-26 min, and the yield was 110-180 phage particles per virion.

For *S. sonnei* phage clones, the adsorption time varied from 10 to 15 min, the latent period was 22-26 min, and the yield was 110-180 phage particles per virion.

It is established that the *Shigella* phages have a binary type of symmetry, a head and a tail. They belong to the morphological types of *Siphoviridae*, *Podoviridae* and *Myoviridae*.

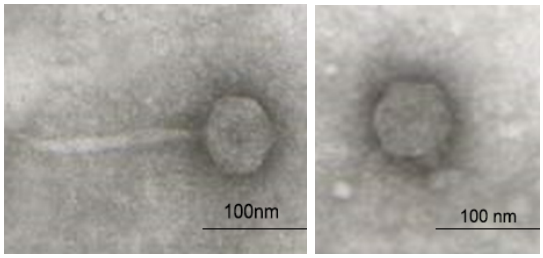

Fig. 3. *S. flexneri* phage 7 and *S. flexneri* phage 3 electron micrograph.

The head of phages is characterized by hexagonal symmetry, the dimensions of the phage head are different (Table 2). The main differences concern the structure of the tail.

Based on the data obtained, it can be concluded that the isolated new *S. typhimurium* phages are highly active against the strains of *S. typhimurium* – 96.3%. The isolated *S. typhimurium* phages belong to the morphological groups of *Myoviridae* and *Siphoviridae*. The isolated *S. sonnei* and *S. flexneri* phages belong to the morphological groups of *Siphoviridae*, *Podoviridae* and *Myoviridae*. They have the same adsorption time of 10-15 min, the latent period of 22-26 minutes and the yield of 110-180 phage particles per virion. Considering high lytic activity and broad spectrum of these phages, they can be used for the creation of the polyvalent phage preparation for prevention and treatment of food-borne pathogens. Control of these pathogens will reduce their occurrence in the environment and food line.

მიკრობიოლოგია

Salmonella-ს და *Shigella*-ს ახალი ბაქტერიოფაგების დახასიათება

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გამოყოფილ იქნა *S. typhimurium, S. sonnei* და *S. flexneri*-ს საწინააღმდეგო ახალი, ეფექტური ბაქტერიოფაგების კლონები. *S. typhimurium*-ის შტამების მგრძნობელობა ფაგების მიმართ შეადგენდა 96.3%-ს. შესწავლილ იქნა გამოყოფილი ბაქტერიოფაგების მორფოლოგია და ბიოლოგიური თვისებები, როგორიც არის ადსორბციის დრო, ლატენტური პერიოდი და ფაგების გამოსავლიანობა. მასპინძელ უჯრედებზე ფაგების ადსორბციის დრო შეადგენდა 10-15 წთ, ლატენტური პერიოდი მერყეობდა 22-28 წთ-ის ფარგლებში, ხოლო გამოსავლიანობა შეადგენდა 110-180 ფაგურ ნაწილაკს. ფაგების მორფოლოგიური შესწავლის შედეგად დადგინდა, რომ *Salmonella*-ს ფაგები მიეკუთვნებიან *Myoviridae* და *Siphoviridae* ოჯახს, ხოლო *Shigella*-ს საწინააღმდეგო ბაქტერიოფაგები – *Siphoviridae, Podoviridae* და *Myoviridae* ოჯახს. აღნიშნული ბაქტერიოფაგები შესაძლებელია გამოყენებულ იქნას ფაგური პრეპარატების შესაქმნელად.

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