

Selection of Phages Active against Multiple Antibiotic-Resistant *P. stutzeri*

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ABSTRACT. Total of 26 strains of *P. stutzeri* were isolated from the upper respiratory tract and patients ears. 11 clones of phages active against them were selected in 2002-2004. After investigation of their morphology the above clone virions were ascribed to the *Myoviridae* family. According to the studies of biologic properties of 11 clones of *P. stutzeri* phages, it was found that the five phages were characterized by the highest lytic activity ($3 \cdot 10^{11}$ - $7 \cdot 10^{11}$) and wide range of action (40-80%). © 2007 Bull. Georg. Natl. Acad. Sci.

Key words: *Pseudomonas stutzeri*, bacteriophage, virion, lytic activity.

The *Pseudomonadaceae* family and representatives of the genus *Pseudomonas* are of great importance in the etiology of purulent-inflammatory infections [1, 2]. In the above-mentioned complications *P. aeruginosa* was considered to be the major infectious agent. However, today the contribution of other representatives of the *Pseudomonas* family has increased [3]. *P. stutzeri* was known as a non pathogenic microbe for humans, but recently it has proved feasible to isolate it from the ears, upper respiratory tract and urine of patients. Very often they were isolated from water and disinfection fluids. *P. stutzeri* infections were associated with pneumonia, otitis, osteomyelitis, keratitis, wound infections, etc. The increased role of *P. stutzeri* microbes in purulent-inflammatory complications is determined by their well-expressed resistance to many antibiotics and antiseptics. Also they can develop resistance under the influence of new antimicrobial agents, survive in the environment for a long time, and produce different types of non-cellular toxins that cause reduction of natural resistance of macromolecules [3, 4].

The goal of our work was the isolation of antibiotic-resistant strains of *P. stutzeri*, selection of active phages and investigation of their biological features.

Materials and Methods. Bacteriophages - collection of active phages against *P. aeruginosa*, preserved

in the laboratory, and the medicinal-prophylactic polyvalent preparation Pyobacteriophage have been used. An antiphage serum of *P. stutzeri* - PST7 phage is obtained.

Bacterial strains - 327 strains of freshly isolated *P. aeruginosa*; 27 strains of *P. fluorescens*, 26 strains of *P. putida*, 27 - of *P. stutzeri*, 47 - of *P. cepacia*; standard strains obtained from the Moscow Tarasevich Control Institute - *P. aeruginosa* - 45, *P. putida* - 9, *P. stutzeri* - 8, *P. cepacia* - 6, *P. fluorescens* - 10; thirty strains of *P. aeruginosa* from the laboratory collection have been used.

Nutrient media - Hottinger's 0.7%, 1.5% agar; Hottinger's broth was used. Selection and investigation of biological properties of the phages were made according to the well-known methods [5, 6]. The morphology of the phage virion was determined by electron microscope JEM-EX - 1200.

Results and Discussion. A total of 26 *P. stutzeri* strains were isolated from the clinical material gathered in 1999-2004 and provided by Tbilisi, Kutaisi, and Rustavi hospitals (upper respiratory tract, ear). Morpho-cultural and biochemical examination of the strains was carried out. Antibiotic- and phage-resistant *P. stutzeri* 26 strains were chosen for further phage selection. Within the spring-summer-autumn period 10 phages, active against

P. stutzeri, were selected from 29 samples of the sewage water.

The range of action of the newly-isolated phages has been examined against homological strains. It was found that *P. stutzeri* phages produced lysis from 12% to 50% of strains. The content of the phages in the *P. stutzeri* strains was determined by the Fisque method. It was found that microorganisms did not contain the phage. Five phages were chosen for further work. After cloning we obtained 11 clones of *P. stutzeri* phages.

According to the morphology of the negative colonies, the phages were divided into four groups. Group I consisted of the phages *PST1*, *PST2*, *PST8II*, which were characterized by large (3.5-4mm) size colonies of poorly visible center and vague border. The phages of Group II - *PST14*, *PST9* - were characterized by 3.5-4 mm negative colonies of equal light center and vague border. The phages of Group III - *PST3*, *PST4* and *PST6* - had vague colonies of moderate size (2.5-3mm). The phages *PST8*, *PST7* and *PST5* of Group IV were represented by polymorphic negative colonies of moderate size (2-2.5mm).

The range of action of the obtained phages was examined against the freshly-isolated strains of the genus *Pseudomonas*. According to the action against homo-

logical strains, the clones were divided into three groups. Group I covered the phages *PST1*, *PST2*, *PST8* which were specific, they only caused lysis of the host; Group II phages *PST5*, *PST9*, *PST4*, *PST6*, *PST7* produced lysis of 35-50% of the strains. Phages of Group III - *PST8II*, *PST3* and *PST14I* induced lysis of 80% of the *P. stutzeri* strains. It was found that the *P. stutzeri* phages did not induce lysis of *P. putida*, *P. cepacia*, *P. aeruginosa* and *P. fluorescens* strains at all. The lytic activity of *P. stutzeri* phages was within $3 \cdot 10^{11}$ - $7 \cdot 10^{11}$ range.

With the aid of electron microscope, the virion morphology of *P. stutzeri* phages was investigated and it was found that they belonged to the Myoviridae family. According to their sizes, the phages were divided into two groups. In the first group the phages - *PST8II*, *PST14I*, *PST6*, *PST1*, *PST4* - $830 \times 830 \text{A}^0$; $1050 \times 220 \text{A}^0$ were incorporated and the phages - *PST5*, *PST7*, *PST8*, *PST2*, *PST3*, *PST9* - $830 \times 830 \text{A}^0$; $940 \times 6 \text{A}^0$ were included in the second group.

After investigation of the serological properties of the freshly isolated *P. stutzeri* phages, it was found that the *PST5*, *PST8*, *PST9* phages were related to the *PST7* phage.

მიკრობიოლოგია და ვირუსოლოგია

ანტიბიოტიკებისადმი რეზისტენტული *P. stutzeri*-ს მიმართ აქტიური ფაგების სელექცია

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2002-2004 წლებში პაციენტთა ზედა სასუნთქი გზებისა და თვალის ჩირქოვან-ანთებითი გართულებებიდან იდენტიფიცირებულ იქნა ანტიბიოტიკებისა და ფაგებისადმი რეზისტენტული *P. stutzeri*-ს 26 შტამი. მიღებულ იქნა აღნიშნული მიკროორგანიზმების მიმართ აქტიური ფაგების 11 კლონი. აღნიშნული კლონები ვირიონის მორფოლოგიის მიხედვით მიეკუთვნება *Myoviridae*-ს ოჯახს. *P. stutzeri*-ს ფაგის 11 კლონის ბიოლოგიური თვისებების შესწავლის საფუძველზე დადგინდა, რომ მაღალი ლიზისური აქტივობით ($3 \cdot 10^{11}$ - $7 \cdot 10^{11}$) და ფართო დიაპაზონით (40-80%) ხასიათდებოდა 5 ფაგი.

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