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EXPERIMENTAL WORKS

Bacteriophages of Soil Bacilli: A New Multivalent Phage of *Bacillus altitudinis*

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Abstract—*Bacillus* are soil saprophytes, facultative anaerobes developing in the temperature range of 28–37°C. 16S rRNA cataloging shows that these bacteria form a coherent class with broad variability of virulence. *Bacillus* phages can be extensively used for phagotyping bacteria in the process of soil, water, and food monitoring. *Bacillus* phages can also be used as vectors in horizontal gene transfer and potential therapeutic agents. Thus, description of the biological diversity of the *Bacillus* phages is useful for further development of tools used in molecular biology and biomedicine. In this work, the scheme for isolation of soil bacteriophages was unified, which allowed ten bacillus phages to be isolated from different types of soil. It was shown that the number of phages depended on the soil fertility, decreasing as the soil changed from black soil to chestnut soil to gray forest soil to uncontaminated urban soil to oil-contaminated urban soil. A new polyvalent DNA-containing bacteriophage SRT01hs of *B. altitudinis* (it is also able to infect *B. subtilis*, *B. cereus*, and *B. pumilus*, but not *B. licheniformis* and *B. atrophaeus*) was described in detail. It has a typical structure: a total length of 360 nm and an icosahedron-shaped head 100 nm in diameter. Several phages simultaneously attack a *B. altitudinis* cell by increasing the level of intracellular low-molecular RNA. Infection with the phage virtually eliminates the stationary growth phase of infected bacilli and leads to a permanent increase in the number of phages in cultural liquor, with the exception of the time period of high activity of the secreted ribonuclease.

Keywords: soil phages, *Bacillus*, *B. altitudinis*, bacteriophage of *B. altitudinis*, intracellular RNA, secreted ribonuclease

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INTRODUCTION

Bacteriophages are the most numerous and widespread group of viruses, typically living in fertile soil. Lysing cells of bacteria and archaea phages control the size of microbial populations. The number of bacteriophages (10^{32}]) [1] on the Earth is many times greater than the total number of bacteria ($(4.1–6.4) \times 10^{30}$) [2]. In 1 s on our planet, 10^{23} infections [3] take place, and, during each reproduction cycle of phage, the explosion of a single cell results in the release of several hundreds of new virus particles. In the process of transduction, phages can transfer their own and trapped genes from one host to another. Bacteriophages are used as vectors in genetic engineering, on their basis a method of phage display was developed to study protein–protein and DNA–protein interactions [4–6]. Phagotherapy of bacterial infections, especially caused by antibiotic-resistant microorganisms, has not still fully realized its potential. In this regard, a significant number of studies dedicated to the ability of phages to affect pathogens [7] and to transfer genes of antibiotics resistance [8]. The number of publications devoted to phage integrons and gene cassettes and their functioning and means of dissemination among clinical strains of bacteria is growing [9, 10].

The biodiversity of soil phages is poorly described. In this regard, the goal of the present study was to isolate and characterize new soil bacteriophages affecting the most common types of bacilli. *Bacillus*' activity of secreting a variety of hydrolytic enzymes, ribonuclease, protease, chitinase, lipase [11–14], etc., contributes to soil fertility, leading to the formation of a pool of readily available substances that stimulate plant growth [15]. Bacilli vary widely in level of pathogenicity. As a result of horizontal transfer of genes largely associated with integerons in phages, the functional properties of soil bacilli are different. Cases of sepsis caused by transit *B. pumilus* are documented, although usually it does not have any clinical manifestations [16]. *B. subtilis* possesses genes encoding the polyglutamate hydrolase, which is used for the destruction of the cell capsule; owing to horizontal transfer, these genes have been found in other soil bacteria [17]. Phage enzymes, endolysins destroying bacterial cell