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Search and analysis of prophages related to phage AP-16-3 in genomes of *Sinorhizobium* spp.

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Motivation and Aim: Bacteriophages can cause lytic infection of bacteria-host which is accompanying by metabolism reprogramming of the host and its destruction as a result. Alternatively, bacteriophages can initiate lysogenic infection of bacteria cell and integrate into chromosome as prophages and then they are transmitted to subsequent generations. Rhizobiophage 16-3 [NCBI RefSeq NC_011103] was identified in the genome of the *Rhizobium (Sinorhizobium) meliloti* Rm41in the 1960s. This is one of the first well studied bacteriophages of nodule bacteria of *S. meliloti* species. In this paper data on the AP-16-3 phage isolated by us in mountainous region of Dagestan is presented. The genome of this phage showed high similarity to genome of the phage 16-3. So, it was a point of interest to evaluate an abundance of phage sequences similar to the AP-16-3 phage in strains from the genus *Sinorhizobium*.

Methods and Algorithms: The AP-16-3 phage genome was sequenced using MiSeq, Illumina, assembled (SPAdes, Flye, Racon and Medaka modules, Pilon), and annotated by Prokka. The BLASTn and BLASTp tools were used for genome analysis. Prophage sequences were searched in genomes of 53 strains of *S. meliloti*, of 9 strains of *S. medicae*, and of 7 strains of *S. fredii* (NCBI database) using the PHASTER web server. The phylogenetic tree was constructed using the OPTSIL algorithm of the VICTOR web tool.

Results: The object of the study was the soil bacteriophage AP-16-3 showed a high similarity with the temperate rhizophage 16-3. The phage was isolated from a soil sample collected at the northern mountainous region of Dagestan, Caucasus. The AP-16-3 phage genome was 61 kb and 94 ORFs were identified, among which were those whose predicted products are necessary for phage infection cycle. Sequences similar to Rhizobium phage AP-16-3 were detected in genomes of 12 strains of *S. meliloti* and in single strains of *S. medicae* and *S. fredii*. Nucleotide sequence analysis showed that 6 out of 8 prophages were intact, while other two were incomplete or defective prophages. The size of the intact prophage reached 63 kb, while the size of defective and incomplete prophages were 33.1 and 21.7 kb respectively. Prophage sequence identity with AP-16-3 ranged from 79.74 to 92.45 with up to 43 % coverage. The number of ORFs encoding protein sequences in intact prophages were up to 144, while in defective and incomplete prophages were 29 and 27 respectively. It was revealed that all prophages contained ORFs encoding tail proteins but prophages were distinct by ORFs determining synthesis of structural elements of virion. A phylogenetic analysis of the identified prophage sequences was carried out. The application of the OPTSIL algorithm showed that a set of identified phage sequences that were similar to the AP-16-3 phage nevertheless belonged to at least 7 genera of viruses (bootstrap 57 %), presumably of the Siphoviridae

family. Thus, at least 23 % of strains of *S. meliloti* harbored sequences homologous to Rhizobium phage AP-16-3 but these sequences were distinct and assigned to different phage genera.

Conclusion: The obtained data support the high prevalence and wide range of lytic activity of Rhizobium phage 16-3 and its related bacteriophages.

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