Genome characterization of *Pseudomonas* phages BIM BV-45 and BIM BV-46 – the components of biopesticide Multiphage to control bacterial diseases of vegetable crops

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Key message. The conducted investigation resulted in full nucleotide sequencing of *Pseudomonas* phage BIM BV-45 and *Pseudomonas* phage BIM BV-46 (registration numbers in GenBank NCBI MT094430 and MT094431) – components of biopesticide Multiphage

Keywords: genome, bacteriophages, *Pseudomonas*

Nowadays due to the growing challenge of antibiotic resistance in pathogenic bacteria application of lytic bacteriophages appears to be an alternative strategy to combat them. It seems natural therefore that search and characterization of bacteriophages will lay the basis for their target-oriented use as biological control agents. Biopesticide Multiphage developed at Institute of Microbiology, NAS of Belarus incorporates six phages protecting vegetable cultures from phytopathogenic pseudomonades.

Aim of the study was analysis of nucleotide sequences of genomes of two bacteriophages *Pseudomonas* phage BIM BV-45 and *Pseudomonas* phage BIM BV-46 from biopesticide Multiphage.

Nucleotide sequencing was carried out on MiSeq platform (Illumina). Automatic annotation of genetic fragments was performed using Prokka software complemented with databases InterProScan, CDSearch, BLASTp. Phage promoters were detected with the aid of PHIRE. Nucleotide sequences of phages BIM BV-45 and BIM BV-46 are deposited in GenBank NCBI library under registration numbers MT094430 and MT094431, respectively.

Analysis of full nucleotide sequence of phage BIM BV-45 sized 40 383 bp (58% GC content) led us to deduce that phage genome is represented by linear double-stranded DNA identical by 97% to *Pseudomonas* phage Andromeda (KX458241) belonging to genus *Befseprivirus*, family *Podoviridae*, order *Caudovirales*. 46 open reading frames probably determining stages of the lytic cycle and 4 sigma-70 promoters were revealed within phage genome sequence.

Genome of bacteriophage BIM BV-46 also comprised linear double-stranded DNA consisting of 38 860 bp (56% GC content). The closest similarity (97.8%) was shown to *Pseudomonas* phage Pf-10 (KP025626), belonging to T7 phages of genus *Autographivirinae*, family *Podoviridae*, order *Caudovirales*. 38 open reading frames, 4 sigma-70 promoters and 9 specific phage promoters were found in BIM BV-46 genome sequence.

The amino acid residue sequences of the BIM BV-45 phage were 95–99% identical to *Pseudomonas* phage Andromeda isolated against *Pseudomonas syringae* bacteria. The second similar to phage BIM BV-45 was *Pseudomonas* phage Bf7 (JN991020) inhibiting the growth of bacteria *Pseudomonas tolaasii*, the causative agent of brown spotting of champignons. The amino acid sequences of *Pseudomonas* phage Bf7 were similar to 75–96% of all investigated proteins of phage BIM BV-45 except for DNA ligase, which was 96% identical to the sequence of *Pseudomonas* phage Andromeda. The sequences of amino acid residues of DNA endonuclease from phage BIM BV-45 were different from the proteins of the above-mentioned phages but showed small similarity (63%) to *Pseudomonas* phage PollyC (MG775261), causing lysis of the sweet cherry pathogen *Pseudomonas syringae*.

Most sequences of amino acid residues of phage BIM BV-46 were similar by 97–100% to proteins of *Pseudomonas* phage Pf-10 (KP025626), which is also one of the components of biopesticide Multiphage (BIM BV-61) and is active against phytopathogenic bacteria *Pseudomonas fluorescens*, *Pseudomonas syringae* and *Pseudomonas putida*. In the bacteriophage BIM BV-46, the proteins responsible for the synthesis of endonuclease and endolysin were 99–100% identical to the *Pseudomonas* phage Phi-S1 (JX173487) proteins, while the amino acid sequences of the tail, the internal proteins of the virion and holine of BIM BV-46 showed identity 96–98%. The phage *Pseudomonas* phage Phi-S1 was isolated against the bacterium *Pseudomonas fluorescens* in a wastewater treatment plant and lysed most of the fluorescent species *Pseudomonas* and one strain of *Pseudomonas stutzeri*.

Summing up, based on genome structure, phages *Pseudomonas* phage BIM BV-45 and *Pseudomonas* phage BIM BV-46 constituting active principle of biopesticide of Multiphage are viruses referred to order *Caudovirales*, family *Podoviridae*, genera *Bifseprivirus* (BIM BV-45) and *Autographivirinae* (BIM BV-46). Genetic correlations of the studied phage genomes with nucleotide sequences of other *Pseudomonas* phages (deposited in GenBank NCBI) and small percentage of identity in the examined amino acid sequences indicate mosaic structure of the phages and mutational shifts arising in the course of phage genome evolution. The genetic diversity of bacteriophages BIM BV-45 and BIM BV-46 evidences a potentially broad spectrum of lytic activity of biopesticide of Multiphage toward phytopathogenic bacteria of genus *Pseudomonas*.

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