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Complete Genome Sequence of *Serratia* Phage 4S Isolated from Wastewater in Ukraine

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Aim. To isolate and characterize phage of *Serratia marcescens* bacteria. **Methods.** Phylogenetic analysis. **Results.** The complete genome of *Serratia* phage 4S represents a 173,061-bp double-stranded DNA (dsDNA) with a GC content of 39.9 %. The Basic Local Alignment Search Tool (BLAST) results indicated that the closest relative to *Serratia* phage 4S is *Serratia* phage CBH8 (7 % query coverage, 76 % identity). According to the electron micrograph images *Serratia* phage 4S belongs to the order *Caudovirales* and the family *Myoviridae*. Following a phylogenetic analysis of *Serratia* phage 4S MCP (Major Capsid Protein), our results showed that its MCP was highly homologous to *Acinetobacter* and *Enterobacter* phages and on the contrary distant from the MCP of *Klebsiella* phages. The phylogenetic analysis of *Serratia* phage 4S DNA helicase indicated that it was highly homologous to *Yersinia* and *Enterobacter* phages, and on the contrary distant from DNA helicase of *Klebsiella* phages. **Conclusions.** *Serratia* phage 4S has a lytic pathway, which means that it can be considered for further investigation as a control agent against bacterial infections caused by *Serratia marcescens*.

K e y w o r d s: *Serratia marcescens*, *Serratia* phage, sequencing, phylogenetic analysis

Introduction

Serratia marcescens is a Gram-negative bacterium of environmental origin like soil, water, and plant surfaces and known to be a plant as well as a human pathogen causing opportunistic infections in hospitals [1]. *Serratia marcescens* is still an underestimated bacterium that causes a range of infections in severely immunocompromised or critically ill patients with keratitis, conjunctivitis, urinary tract infections, pneumonia, surgical wound infections, sepsis, bloodstream infection, and meningitis [2]. Considering that bacterial resistance to antibiotics increases, phages are one of the most promising alternatives that have to be applied [3]. To date, the NCBI database (https://www.ncbi.nlm.nih.gov/) has at least 64 genome sequences of *Serratia* phages. Most of *Serratia* phages listed in GenBank of the

NCBI-NIH were isolated from environment like sewage, mine rock biofilms, river water, seawater, wastewater, pond water, swine farm samples, swine fecal and soil samples, river, compost, and supernatant of an overnight culture. Here, we report the complete genome of *Serratia* phage 4S isolated from wastewater in the Bortnychi aeration station (Kyiv, Ukraine).

Materials and Methods

In this study, the *Serratia* phage 4S was isolated from wastewater in the Bortnychi aeration station (Kyiv, Ukraine). *Serratia* phage 4S was detected using the bacterium *Serratia marcescens* isolate IMBG291 [4] as its host (obtained from the Institute of Molecular Biology and Genetics NAS of Ukraine, Kyiv, Ukraine). Host range was not investigated in this study.

To isolate phage a modified protocol of an enrichment procedure involving a double-layer agar method was used [5]. Briefly, a molten 1.4 % (wt/vol) meat peptone agar (MPA) was poured into Petri dishes and incubated at room temperature for 7 min. Then 500 mL of filtered water sample (i.e., filtered through 0.22-μm pores) were mixed with 100 mL of *Serratia marcescens* IMBG291 cells and added to 2 mL of molten 0.7 % (wt/vol) MPA and poured into a Petri dishes with underlay 1.4 % (wt/vol) MPA. Disposable Petri dishes were incubated overnight at 25 °C. A single plaque was picked with a pipette tip and transferred into Saline buffer (0.5 %), followed by centrifugation and vortexing to release phages from the agar plaque and stored at 5 °C. The morphology of phage 4S was determined using electron microscopy (M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, Kyiv, Ukraine). Staining was performed with 2 % uranyl acetate on freshly prepared formvar coated copper grids.

Extraction of genomic DNA from pure *Serratia* phage 4S suspension was carried out using the DNA-sorb-AM nucleic acid extraction kit (Amplisens biotechnologies, Moscow, Russia) and DNA purification was done using Zymo research DNA Clean & Concentrator (Zymo Research Corporation, Irvine, USA) according to the manufacturer's protocol. A whole-genome amplification using random priming was carried out using REPLI-g Single Cell Kit (Qiagen, Venlo, Netherlands) according to the manufacturer's protocol. DNA library preparation and phage genome sequencing were performed using Ion Torrent next-generation sequencing (Latvian Biomedical Research and Study Centre, Riga, Latvia).

The DNA library was prepared using an Ion Plus Fragment Library Kit (Thermo Fischer Scientific, USA). Trimmomatic [6] and BBNorm (https://jgi.doe.gov/data-and-tools/bbtools/bb-tools-user-guide/installation-guide/) were used for reads trimming and filtering to remove adaptor sequences as well as reads less than 30 bp. FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc) was used as the reads quality control tool, with subsequent assembly using SPAdes Genome Assembler v3.13.1[7]. *Serratia* phage 4S genome coverage was achieved 1000× (x?), which accounts for high genome coverage. Putative phage coding sequences were identified using Prodigal v1.20 [8], whereas the genome translation of a nucleotide sequence to a protein sequence was performed using DNA Master [9]. BLASTp was used to iden-

tify Query Cover (%) and Percent Identity (%) when comparing [the] isolated phages and phages from NCBI, whereas BLASTp was also used for [the] putative ORF functions prediction. The genome map was built using the online tool CGView Server (http://stothard.afns.ualberta.ca/cgview_server/). Phylogenetic trees were constructed using ClustalX alignment and the neighbor-joining method in MegaX.

Results and Discussion

Serratia phage 4S produced clear plaques (≈ 2 mm in diameter) on MPA agar inoculated with *Serratia marcescens* isolate IMBG291. Therefore, in the next step, [the] electron images of phages were obtained. *Serratia* phage 4S shows an icosahedral head (of about 68 nm in diameter) and long contractile tail (of about 107 nm in length and 18 nm in diameter). Based on this morphology it can be assigned to order *Caudovirales* and family *Myoviridae* (**Fig. 1**).

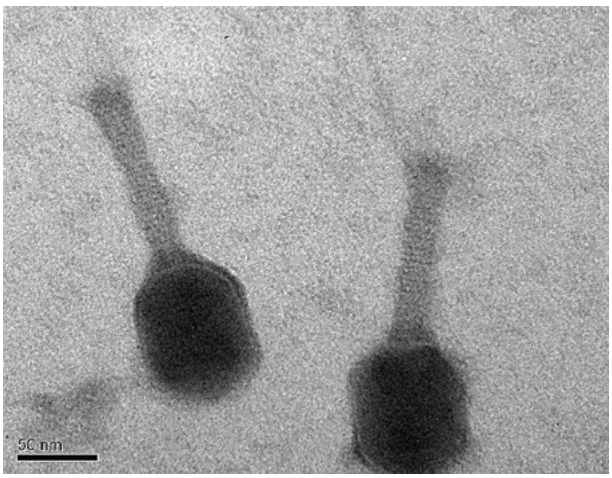

Fig. 1. Transmission electron micrograph of phage 4S negatively stained with 2 % uranyl acetate. The bar indicates 50 nm.

Subsequently, bacteriophage was sequenced by whole genome sequencing (WGS). The DNA sequence data was further subjected to genetic analysis. The genome of *Serratia* phage 4S represents a 173,061-bp double-stranded DNA (dsDNA) with the GC content of 39.9 %. Blastn results indicated that the closest relative to *Serratia* phage 4S is *Serratia* phage CBH8 (7 % query coverage, 76 % identity). The total number of coding sequences (CDS) of phage 4S was 290. *Serratia* phage 4S genome contained genes that can be grouped according to its function: structural and genes for replication/recombination/reparation, transcription, translation, nucleotide metabolism, and additional functions. The first group that includes phage structural genes encodes head structure proteins (major capsid protein, prohead protease, and core proteins, scaffold and head completion proteins), whereas the second - tail/neck structure proteins (tail tube and sheath proteins, tail sheath stabilizer, and completion proteins, tail fiber protein, neck proteins, baseplate tail tube initiator, tail tube protein, baseplate wedge, and hub subunits). Hence, the first and second phage gene groups encode proteins that allow the complete recovery of phage head and tail structures. The third group of replication/recombination/repair genes encodes replication proteins (rIIA protein, DNA topoisomerase II subunits, DNA ligase, DNA primase-helicase subunit, DNA helicases and primase, phage clamp loader subunit, sliding clamp protein, DNA polymerase and exonuclease A, DNA end protector protein, and endonucleases) and recombination/repair proteins (recombination endonucleases, repair and single-stranded DNA binding protein), which suggests that phage 4S has its

own replication/recombination/repair systems. The fourth group of nucleotide metabolism genes encodes deoxycytidylate deaminase, thymidylate synthase and kinase, and reductases. The fifth group of transcription/translation genes encodes RNA polymerase sigma factor for late transcription, late promoter transcription accessory protein, and the translational repressor protein. Additional genes encode proteins such as lysozyme, which helps phage to lyse the host cell, thus phage virions are released.

Among the all predicted CDS, on the genome of phage 4S, a Major Capsid Protein and a DNA helicase were used for phylogenetic analysis. *Serratia* phage 4S was submitted to the GenBank database under the accession number MW082584.

Phage genome map was constructed using CGView Server (**Fig. 2**). Concentric rings display gene information depending on the phage DNA sequence. A zoomed map represents a part of *Serratia* phage 4S genome, which mostly includes the genes that code for structural proteins such as tail sheath and tail tube proteins, portal vertex protein, prohead core scaffold and protease, major capsid protein, head vertex protein, inhibitor of prohead

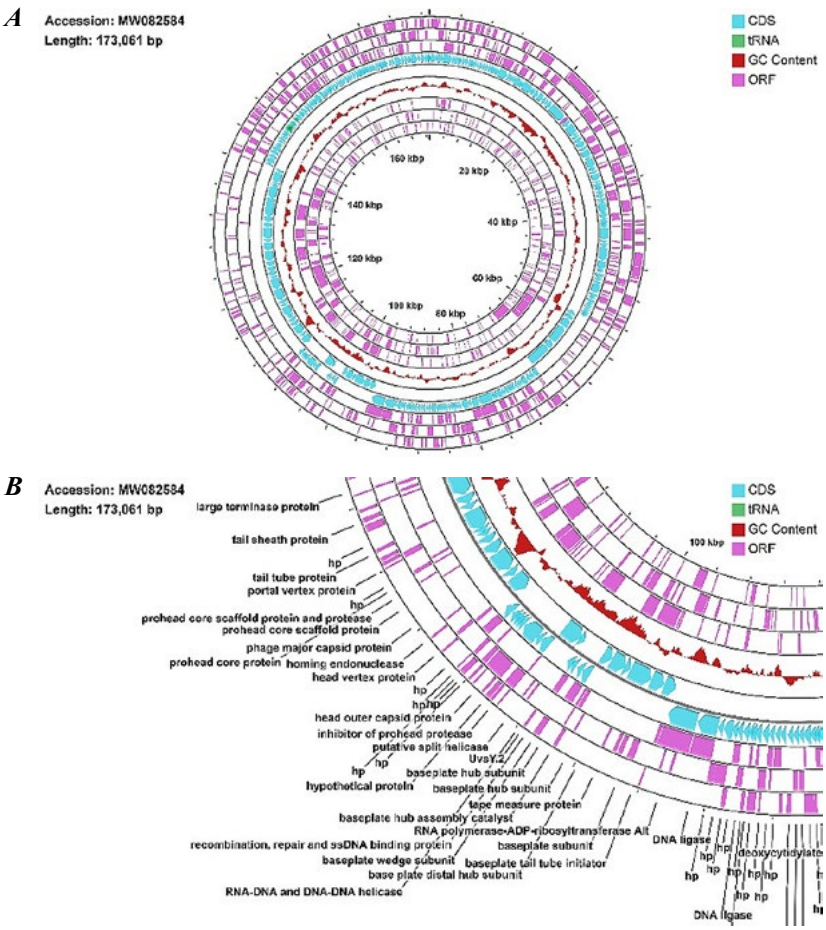

Fig. 2. *Serratia* phage 4S genome map generated using the CGView Server, whole-genome displayed (A) and a zoomed map (B). The contents of the rings (starting with the outermost ring) are as follows: Rings 1, 2, 3, 6, 7, and 8 depict features from separated open reading frames (ORFs) and strands; Ring 4 shows potential coding sequences (CDS); Ring 5 shows GC content. Labels indicate functions of proteins encoded by predicted CDS (B).

protease, head outer capsid protein, tape measure protein, baseplate hub assembly catalyst and hub subunits, baseplate wedge and distal hub subunits, baseplate tail tube initiator. A zoomed map also represents genes that code for replication/recombination/repair proteins including homing endonuclease, putative split helicase, RNA polymerase-ADP-ribosyltransferase Alt, recombination, repair and ssDNA binding protein, RNA-DNA and DNA-DNA helicase, and DNA ligase. Hypothetical proteins are indicated as hp.

Phylogenetic trees were constructed based on conserved sequences of *Serratia* phage 4S. The first phylogenetic tree was constructed using amino acid sequences of the predicted Major Capsid Protein (MCP), which is often the most conserved sequence in phage genomes. According to phylogenetic analysis of *Serratia* phage 4S MCP, the results revealed that its MCP was highly homologous to *Acinetobacter* and *Enterobacter* phages, and on the contrary distant from MCP of *Klebsiella* phages (**Fig. 3**).

The second phylogenetic tree was constructed using amino acid sequences of the predicted DNA helicase. The phylogenetic analysis of *Serratia* phage 4S DNA helicase indicated that it was highly homologous to *Yersinia* and *Enterobacter* phages and on the contrary

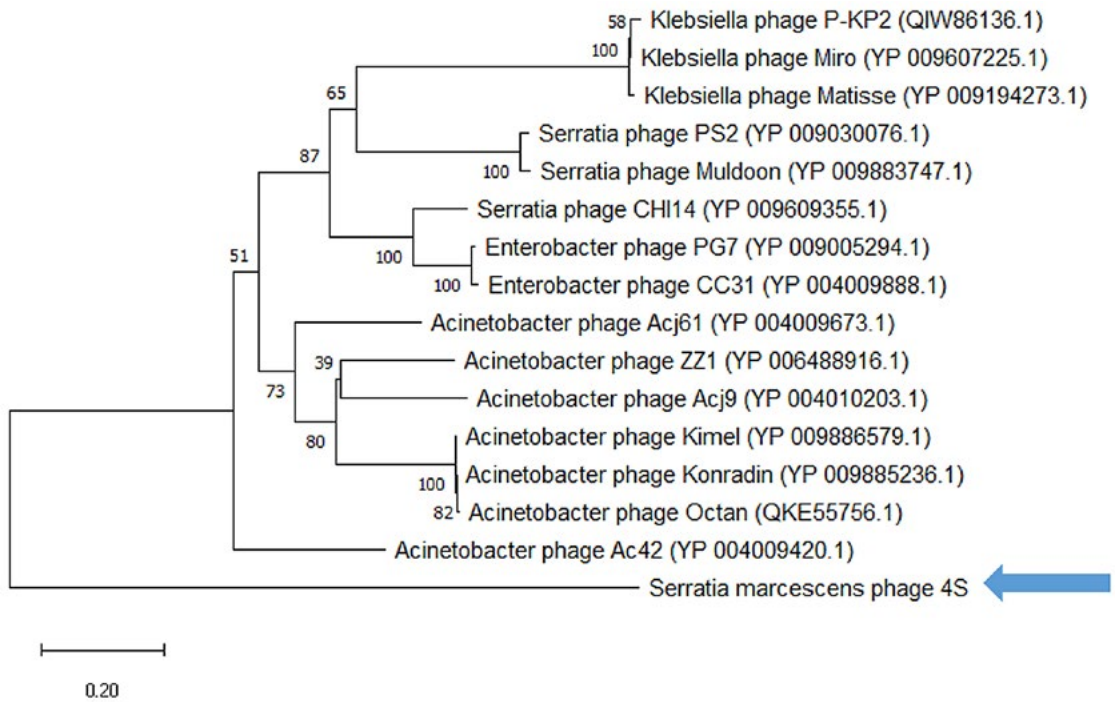

Fig. 3. Comparative phylogenetic analysis of *Serratia* phage 4S Major Capsid Protein with proteins of selected phages in NCBI-BLAST. Phylogenetic trees were constructed using ClustalX alignment and the neighbor-joining method in MegaX. The protein ID is shown after the name of each phage in parentheses. The solid arrow indicates the location of *Serratia* phage 4S. Bootstrap values are indicated at the nodes. Amino acid substitutions per site are indicated within the scale bar.

distant from the DNA helicase of *Klebsiella* phages **(Fig. 4)**.

Conclusions

In conclusion, a newly isolated *Serratia* phage 4S able to lyse *Serratia marcescens* bacterium was characterized. According to the electron micrograph results, *Serratia* phage 4S belongs to the order *Caudovirales* and the family *Myoviridae.* The complete genome of *Serratia* phage 4S represents a 173,061-bp dsDNA with a GC content of 39.9 %. The Blastn (BLASTp?) results indicated that the closest relative to *Serratia* phage 4S is *Serratia* phage CBH8 (7 % query coverage, 76 % identity), which means that *Serratia* phage 4S is new because query coverage is relatively low. According to phylogenetic analysis of *Serratia* phage 4S MCP, our results showed that its MCP was highly homologous to *Acinetobacter* and *Enterobacter* phages and on the contrary distant from MCP of *Klebsiella* phages. The phylogenetic analysis of *Serratia* phage 4S DNA helicase indicated that it was highly homologous to *Yersinia* and *Enterobacter* phages, and on the contrary distant from DNA helicase of *Klebsiella* phages. *Serratia* phage 4S has a lytic pathway, which means that it can be con-

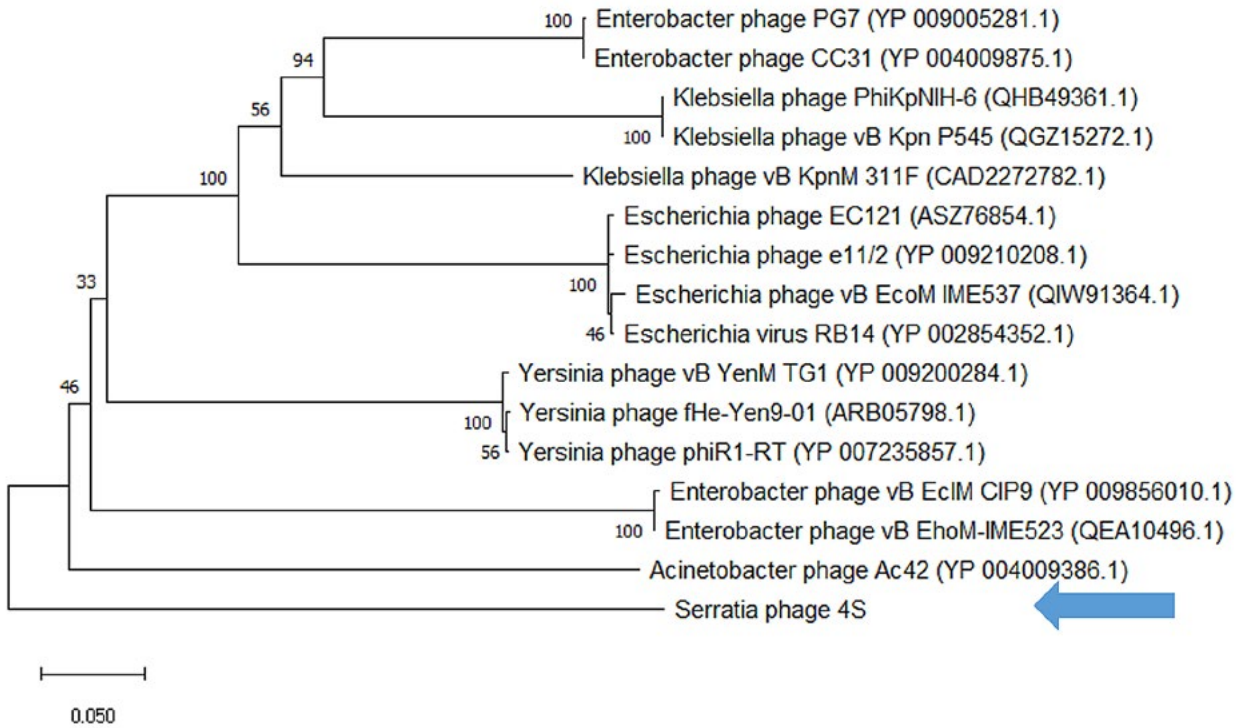

Fig. 4. Comparative phylogenetic analysis of *Serratia* phage 4S DNA helicase with proteins of selected phages in NCBI-BLAST. Phylogenetic trees were constructed using ClustalX alignment and the neighbor-joining method in MegaX. The protein ID is shown after the name of each phage in parentheses. The solid arrow indicates the location of *Serratia* phage 4S. Bootstrap values are indicated at the nodes. Amino acid substitutions per site are indicated within the scale bar.

sidered for further investigation as a control agent against bacterial infections caused by *Serratia marcescens*.

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Повна послідовність геному *Serratia* phage 4S, ізольованого зі стічних вод на території України

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Мета. Виділити і охарактеризувати фаг бактерій *Serratia marcescens*. **Методи.** У цьому дослідженні для виділення і очищення бактеріофага був використаний метод подвійних агарових шарів. Накопичення фагу проводили на твердому поживному середовищі. Морфологія виділеного фага була визначена за допомогою електронної мікроскопії, а повногеномне секвенування проведено за допомогою секвенування нового покоління Ion Torrent. **Результати.** Повний геном бактеріофага *Serratia* phage 4S містить дволанцюгову ДНК (длДНК) довжиною 173 061 п.о. з вмістом ГЦ-пар 39,9 %. Результати методу пошуку основного локального вирівнювання (BLAST) показали, що найближчим родичем бактеріофага *Serratia* phage 4S є фаг *Serratia* phage CBH8 (покриття склало 7 %, ідентичність 76 %). Згідно до електронно-мікроскопічних зображень, *Serratia* phage 4S належить до порядку *Caudovirales* та родини *Myoviridae*. Результати філогенетичного аналізу MCP (головного капсидного білка) фага *Serratia* phage 4S показали, що його послідовність MCP була найбільш подібна до послідовностей MCP фагів *Acinetobacter* і *Enterobacter* та, навпаки, найменш подібна до послідовностей MCP фагів *Klebsiella*. Філогенетичний аналіз послідовності ДНК-гелікази

Serratia phage 4S показав, що вона найбільш подібна до послідовностей ДНК-гелікази фагів *Yersinia* і *Enterobacter* та, навпаки, найменш подібна до послідовностей ДНК-гелікази фагів *Klebsiella*. **Висновки.** Бактеріофаг *Serratia* phage 4S має літичний шлях розвитку, тому може розглядатися для подальшого вивчення як засіб для боротьби з бактеріальними інфекціями, викликаними *Serratia marcescens*.

Ключові слова: *Serratia marcescens*, *Serratia* phage, секвенування, філогенетичний аналіз

Полная последовательность генома *Serratia* phage 4S, выделенного из сточных вод на территории Украины

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Цель. Выделить и охарактеризовать фаг бактерии *Serratia marcescens*. **Методы.** В этом исследовании для выделения и очистки бактериофага был использован метод агаровых слоев. Накопление фагов проводили на твердой питательной среде. Морфология выделенного фага была определена с помощью электронной микроскопии, а полногеномное секвенирование проведено с помощью секвенирования следующего поколения Ion Torrent. **Результаты.** Полный геном бактериофага *Serratia* phage 4S представляет собой двухцепочечную ДНК (дцДНК) длиной 173061 п.о. с содержанием ГЦ-пар 39,9 %. Результаты средства поиска основного локального выравнивания (BLAST) показали, что ближайшим родственником бактериофага *Serratia* phage 4S является фаг *Serratia* phage CBH8 (покрытие составило 7 %, идентичность 76 %). Согласно электронно-микроскопическим изображениям бактериофаг *Serratia* phage 4S принадлежит к порядку *Caudovirales* и семейству *Myoviridae*. Результаты филогенетического анализа MCP (главного капсидного белка) фага *Serratia* phage 4S показали, что его последовательность MCP была наиболее подобная к последовательностям MCP фагов *Acinetobacter* и *Enterobacter* и, наоборот, наименее подобная к последовательностям MCP фагов *Klebsiella*. Филогенетический анализ последовательности ДНК-геликазы *Serratia* phage 4S показал, что она наиболее подобная к последовательностям ДНК-геликазы фагов *Yersinia* и *Enterobacter,* и, напротив, наименее подобная к последовательностям ДНК-геликазы фагов *Klebsiella*. **Выводы.** Бактериофаг *Serratia* phage 4S развивается по литическому пути, соответственно он может рассматриваться для дальнейшего изучения в качестве средства борьбы с бактериальными инфекциями, вызываемыми *Serratia marcescens*.

Ключевые слова: *Serratia marcescens*, *Serratia* phage 4S, секвенирование, филогенетический анализ.

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