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ANNOTATED SEQUENCE RECORD

Complete genome sequence of Phobos: a novel bacteriophage with unusual genomic features that infects *Pseudomonas syringae*

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Abstract

The GenBank database contains over 2580 complete genome sequences from bacteriophages. However, limited reports are available concerning phages can that lyse members of *Pseudomonas syringae*, although this is a widespread bacterial species that can infect almost 200 plant species. In the present study, we isolated and characterized a new *Siphoviridae* phage, named "Pseudomonas phage vB_PsyS_Phobos" (for brevity, referred to here as Phobos). To our knowledge, this is one of the first genome sequences reported for a phage with lytic activity against *P. syringae* pv. *syringae*. The genome of Phobos is dsDNA of 56,734 bp with a GC content of 63.3%, containing 65 ORFs. Genome analysis revealed that Phobos is a novel lytic phage with unique genomic features and low similarity to other phages, suggesting that Phobos represents a new phage genus. Genome sequencing did not reveal sequences with significant similarity to known virulence factors, antibiotic resistance genes, potential immunoreactive allergens, or lysogeny-related proteins, suggesting suggests that phage Phobos is strictly lytic. Therefore, Phobos may be suitable for formulation as a biocontrol agent against *P. syringae* pv. *syringae*.

Introduction

Plant-pathogenic bacteria cause devastating damage to crops and billions of dollars in crop losses worldwide every year [1]. Members of the *Pseudomonas syringae* species complex, including *P. syringae* pv. *syringae,* are among the most important plant pathogens, causing diseases such as bacterial canker, speck, apical necrosis, spot, and blight disease on a wide range of economically and culturally useful plant species [2]. This problem is aggravated by the fact that effective management of these pathogens of plants may be difficult.

The limited availability of safe and effective alternatives for controlling plant diseases caused by bacteria has sparked renewed interest in exploring the use of bacteriophage as biocontrol agents for the control of plant diseases [3]. Bacteriophages, also known as phages, are viruses that infect bacteria, use the bacterial machinery to replicate, and lyse the host cell. These viruses can be an effective tool in integrated disease management approaches. However, a suitable phage candidate for effective biocontrol should not contain any genes that encode toxins, allergenic proteins, virulence factors, or genes responsible for the establishment and maintenance the lysogenic state [4].

Here, we report the complete genome sequence of a novel phage (named Phobos), that appears to represent a new genus and is capable of lysing several *P. syringae* strains and therefore might have biocontrol application potential.

Materials and methods

The bacteriophage was isolated from irrigation water on a farm in Mexico where tomatoes were grown (24°37′25.5″N 107°26′36.1″W), using the soft agar overlay method [5].

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Luis Amarillas and Mitzi Estrada-Acosta contributed equally to this work.

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The phage was concentrated by centrifugation, purified by filtration (0.22-µm-pore-size filter) and dialysis using 10K MWCO Slide-A-Lyzer Dialysis Cassettes. Purified virions were examined using a JEOL JEM-1011 transmission electron microscope (JEOL Ltd. Tokyo, Japan) at an acceleration voltage of 80 kV. The isolated phage was tested against a collection of 17 strains of *P. syringae* belonging to three pathovars (*syringae*, *tomato*, and *phaseolicola*) for host range determination using the soft agar plaque method. Phage genomic DNA was extracted using the SDS/proteinase K method [6]. The DNA concentration and purity were analyzed using a Qubit spectrophotometer (Invitrogen). Phage genome sequencing was performed using the Illumina NextSeq 500 platform (Illumina, USA) with an average read length of 2 × 150 bp paired-end reads. Low-quality (Q-value < 20) reads were filtered out using Trimmomatic. CLC Genomics Workbench 12.0 (QIAGEN, Toronto) was used to assemble the reads. Putative open reading frames (ORFs) were predicted using the GeneMark server with a length threshold of 90 bp [7]. Putative functions of ORFs were identified using results of searches against the non-redundant database (NCBI) by BLAST. The deduced amino acid sequences of all the ORFs were compared with the Pfam [8], TMHMM server, and NCBI Conserved Domain databases. Bioinformatic analysis was conducted on the phage genome sequence to identify putative genes encoding virulence factors, resistance factors, or genes associated with lysogenic conversion. The analysis was performed using the pipeline (specialty genes checkpoint) described by Philipson et al. [9]. Transcriptional regulatory elements (promoters, and terminators with a ΔG value of -10 kcal/mol or less) were identified using BPROM, PhagePromoter and ARNold [10]. tRNAScanSE [11] was used for detection of transfer RNA genes. The phage genome sequence was used to query the nr/nt database using MegaBLAST at NCBI. The deduced amino acid sequences of the large terminase subunit and DNA polymerase I of phage Phobos were aligned with those of related bacteriophages with sequences in the NCBI database, using the Clustal W algorithm, and the resulting alignment file was used to create phylogenetic trees by the maximum-likelihood method, applying a bootstrap test with 1000 replicates using Geneious Prime (Biomatters Ltd).

Results and discussion

Transmission electron microscopy revealed that Phobos has a hexagonal head of about 67 nm in diameter and a long non-contractile tail of about 226 nm and therefore belongs to the family *Siphoviridae* (Fig. 1). Host range analysis showed that 64.7% of the bacterial strains investigated were susceptible to phage Phobos (Supplemental Table 1). Genome sequencing resulted in a single contig with an average coverage of 264×. The sequencing results revealed that the genome of bacteriophage Phobos is a linear dsDNA molecule with a size of 56,734 bp and an average GC content of 63.3%, which is slightly higher than that exhibited by most *P. syringae* genomes published in the NCBI database (range between 58%-60%), and also higher than that of other characterized *Pseudomonas* phages for which the genome sequence is available. This result was surprising, given that the GC% of phage genomes generally correspond to the GC content of their bacterial hosts [12]. The biological implications of GC content in phages are not well understood, but it may be related to virus adaptation to growth in their host. The GC content of phages is considered a result of a complex interaction and long-term coevolution with their hosts [12].

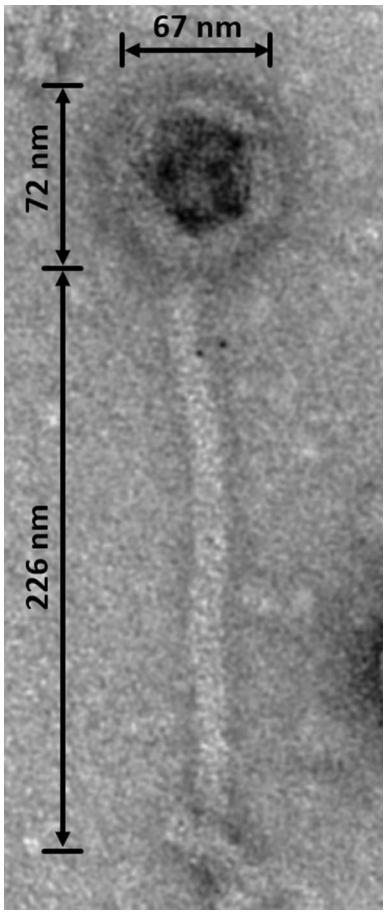

Fig. 1 Transmission electron microscopy image of phage Phobos negatively stained with 2% (w/v) uranyl acetate at a magnification of 100,000×

A total of 65 putative ORFs, six promoters, and 12 rho-independent transcriptional terminators were predicted in the phage genome (Fig. 2). Based on sequence similarities, putative functions could only be assigned to 26 of the predicted ORFs, 37 ORFs shares sequence similarity with

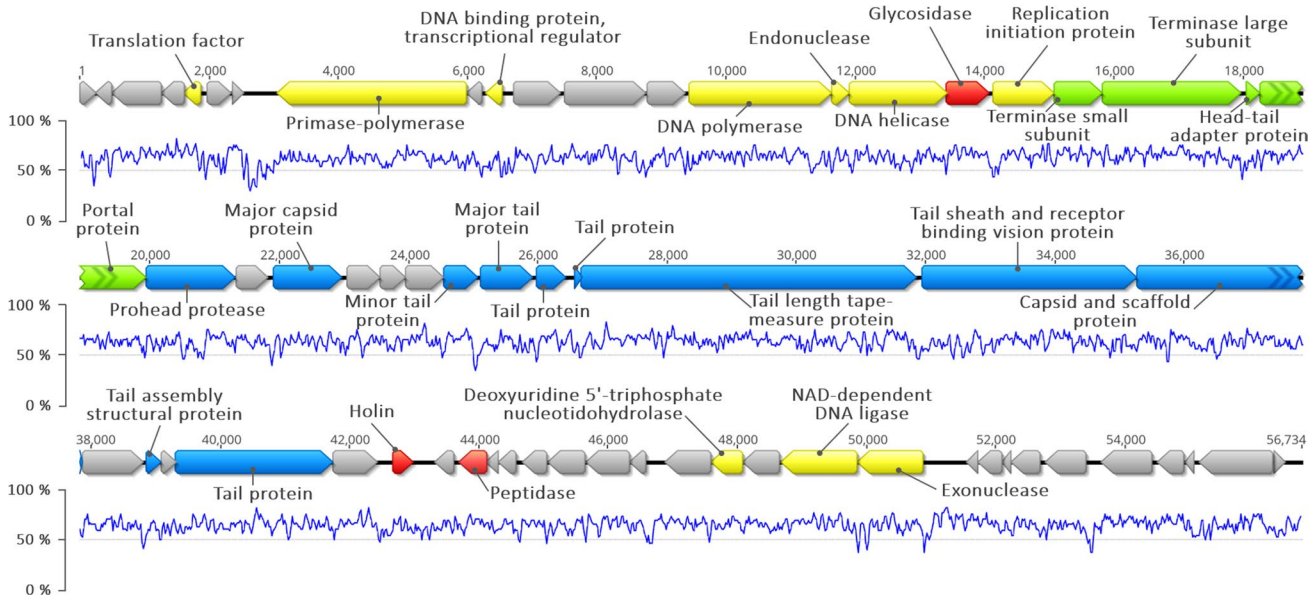

Fig. 2 Map of the genome organization of bacteriophage Phobos. The predicted ORFs are represented by arrows, the orientation of which shows the direction of transcription. Different colors represent the predicted molecular functions of the ORFs. DNA regulation module, yellow arrows; packaging module, green arrows; phage structural proteins, blue arrows; host lysis proteins, red arrows; hypothetical proteins, gray arrows

phage proteins of unknown function, while two of the predicted ORFs products had no homology to previously characterized proteins present in the NCBI database.

Like those of other tailed phages, the Phobos genome displays a modular organization, with each module containing ORF clusters with specific functions. These include viral morphogenesis, genome packaging, DNA replication and regulation, and host lysis (Supplemental Table 2). Genome analysis confirmed the strictly lytic nature. No integrase, repressor, or other genes related to lysogeny were identified in the genome. None of the predicted proteins encoded by Phobos exhibit homology to known virulence factors, antibiotic resistance factors, or potential immunoreactive allergens. Safety assessment of phages at the genome level is essential to evaluate their suitability for biocontrol applications [4].

Bacteriophage Phobos encodes two proteins (ORF40 and ORF43, the holin-endolysin system) involved in host cell lysis. This phage also encodes a possible lytic transglycosylase (ORF16) associated with the phage structure. We hypothesize that this enzyme is an additional component associated with the phage structure to breach the cell wall at the beginning of the infection process [13].

A MegaBLAST search of the complete genome sequence of phage Phobos showed similarity to another phage not yet assigned to a genus (PspYZU01, ID: KY971609.1), and these phages are unrelated to any other known phages. These phages had nucleotide sequence similarity (52% identity) that was too low for them to be considered members of the same species, since, according to the guidelines published by Adriaenssens and Brister [14], members of the same species of bacteriophage should have 95% whole-genome DNA sequence identity. Furthermore, phylogenetic analysis of large terminase subunit and DNA polymerase sequences showed that Phobos and PspYZU01 are phylogenetically related to each other, but significantly divergent from other known phages with sequences in the NCBI database (Supplemental Fig. 1). Based on these results, we suggest creating a new genus in the family *Siphoviridae* with two species, represented by the phages PspYZU01 and Phobos.

Nucleotide sequence accession number

The complete genome sequence of phage Phobos was deposited in the GenBank database under accession number MN478374.1.

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Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

Ethical approval This article does not contain studies with human participants or animals performed by any of the authors.

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