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Characterization of a Novel *Pseudomonas Aeruginosa* Phage Species: PaYy-2

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ABSTRACT

A novel *Pseudomonas aeruginosa* phage, designated as PaYy-2, has been isolated from a lake in China and its genome meticulously sequenced and characterized. The PaYy-2 phage possesses a 92,348 base pair double-stranded DNA genome that encodes for 168 proteins, exhibiting a 68% similarity to the genome of the known Pseudomonas phage YS35. Phylogenetic analysis reveals that the putative RNA polymerase of PaYy-2 shares a striking 94% similarity with that of Pseudomonas phage SRT6, while the putative terminase large subunit bears a 96% resemblance to the phage YS35, both of which are classified under the Pakpunavirus genus. Morphological examination via transmission electron microscopy (TEM) has provided insights into the phage's tail and particle morphology. These collective findings underscore the classification of PaYy-2 as a distinct species within the Pakpunavirus genus of the family Myoviridae.

INTRODUCTION

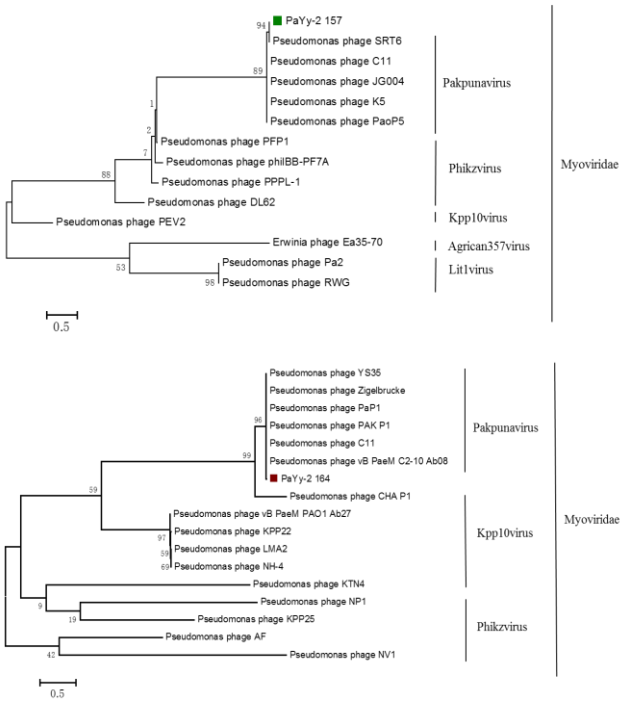
Pseudomonas aeruginosa is a formidable opportunistic pathogen that typically resides in soil and aquatic environments Cao et al. (2023). Infections caused by this bacterium can elicit a robust neutrophil response, leading to substantial tissue damage and, in extreme cases, mortality, compounded by its resistance to antibiotics Muggeo et al. (2023). Phage therapy has emerged as a promising strategy for combating P. aeruginosa infections. In this study, we present the isolation and characterization of a novel bacteriophage, PaYy-2, which appears to specifically target Pseudomonas aeruginosa.

METHODS AND RESULTS

The bacteriophage PaYy-2 was isolated from a freshwater lake in Yueyang city, Hunan province, following methods outlined in previous research Oliveira et al. (2017). Subsequent large-scale phage preparation was conducted using 1 liter of liquid culture, as described by Boulanger et al. (2009). The phage genome was extracted in accordance with the Lambda Bacteriophage Genomic DNA Rapid Extraction Kit (DN22, Aidlab, China) and sequenced using Illumina HiSeq1500 technology. Clean reads were assembled into a contiguous genome sequence with the SOAP denovo software Sun et al. (2023), while open reading frames (ORFs) were predicted using GeneMark.hmm Gabriel et al. (2023). The functions of the ORFs were inferred via BlastP against the NCBI non-redundant protein database Dai et al. (2023).

Phylogenetic analysis was performed with MEGA 6, employing the neighbor-joining method Tzeng et al. (2023). tRNA coding regions were predicted using the tRNAScan-SE server Wu et al. (2023). Genomic comparisons were facilitated by Easy figure 1.2.3 software Huang et al. (2022).

Figure 1: Phylogenetic analyses were conducted on the putative RNA Polymerase



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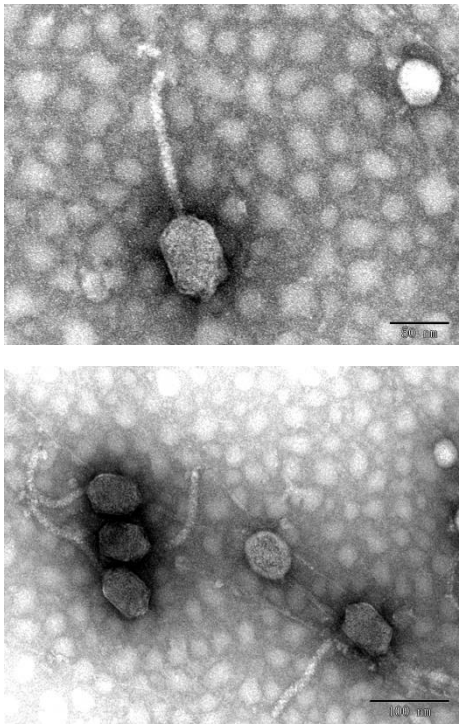
(a) and terminase large subunit (b) protein sequences using the neighbor-joining method. Amino acid sequences were aligned using ClustalW, and evolutionary distances were calculated employing the p-distance method. The analysis was performed utilizing MEGA6 software to elucidate the phylogenetic relationships.

Transmission electron microscopy (TEM) images (Figure. 2) reveal that PaYy-2 possesses a capsid measuring approximately 47.9 nm in width and a tail length of 144 nm. The phage's 92,348 bp double-stranded DNA genome encodes 168 ORFs, with the encoded proteins showing varying degrees of sequence similarity to those of Pakpunavirus genus Pseudomonas phages (ranging from 34% to 100%) and a G+C content of 49.33%.

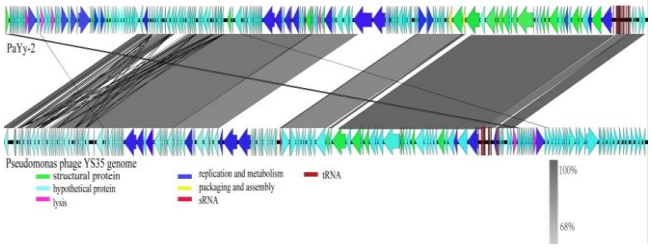
Figure 2: Transmission electron microscopy (TEM) images of the bacteriophage PaYy-2, providing a detailed visual representation of the phage's morphology.



We identified 15 tRNAs within the genome (Figure. 3), surpassing the count in Pseudomonas phage YS35 by three, suggesting that the new phage may encode tRNAs to overcome codon usage bias and enhance adaptability Gaur et al. (2023).

BLASTP homology scanning Geier et al. (2021) revealed 20 structure-related proteins in the PaYy-2 genome, including unique proteins such as the tail sheath protein and head-tail joining protein, as well as two tail fiber proteins, two baseplate proteins, one tape measure protein, and one major capsid protein. Additionally, the new phage contains several specific structural proteins, including a putative protease subunit, a macro domain protein, a toxic anion resistance protein, a SprT-like family protein, a constituent protein, a portal protein, and three unknown structural proteins. The phage also harbors four lysis-related proteins, including a putative endolysin, which likely plays a crucial role in hydrolyzing the host cell wall, and three other common proteins—a CMP/dCMP deaminase with a zinc-binding domain, a cell wall hydrolase, and a putative phosphoesterase also found in the reference phage YS35. Moreover, the new phage carries 22 genes encoding replication and metabolism proteins, which are predicted to facilitate the phage's autonomous replication and metabolic pathways.

Figure 3: Comparative genomic analysis of bacteriophage PaYy-2 and its closely related phage YS35.



Distinct arrows represent the predicted proteins for structural proteins (denoted in green), hypothetical proteins (marked in blue), lysis-related proteins (shaded in purple), as well as proteins associated with replication and metabolism (colored in deep blue), packaging and assembly (highlighted in yellow), small RNA (sRNA, depicted in deep red), and transfer RNA (tRNA, represented as vertical bars). The degree of amino acid sequence similarity between the two phages is visually represented by the areas of gray shading.

These proteins include an RNA ligase/tail attachment protein, a phosphoribosylpyrophosphate synthetase, a nictotinate phosphoribosyltransferase, two DNA recombination-mediator proteins, a ribonucleoside-diphosphate reductase alpha chain, a ribonucleoside-diphosphate reductase beta subunit, a 3'-phosphatase, a 5'-polynucleotide kinase, a nucleotide pyrophosphohydrolase, an RNA ligase, a pyrophosphatase, and a putative RNA polymerase, in addition to nine common proteins found in both phages (Supplemental Table S1, S2). The genome annotation also identified two packaging and assembly-associated proteins, including a peptidoglycan binding protein and a tail fiber assembly protein, which are unique to the PaYy-2 phage.

CONCLUSION

The PaYy-2 phage genome sequence shares a 68% similarity with that of Pseudomonas phage YS35 (Figure 1), with a total of 23 predicted functional proteins shared between the two phages (ranging from 85% to 99% similarity).

For phage species classification, phylogenetic analysis was based on the RNA polymerase protein and the terminase large subunit protein sequence, which are considered conserved elements in phage genomes. The RNA polymerase protein of PaYy-2 is 94% homologous to those of Pseudomonas phages SRT6, suggesting that PaYy-2 is a new member of the Pakpunavirus genus within the Myoviridae family. The terminase large subunit protein exhibits homology with those of Pakpunavirus genus phages, including Pseudomonas phages PAK P1, PAK P2, PAP1, and YS35, with an identity of 96%, indicating that PaYy-2 may represent a novel species within the Pakpunavirus genus.

DECLARATIONS

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Nucleotide sequence accession numbers: The annotated genome sequence of the phage has been submitted to the NCBI nucleotide database under the accession number GenBank: MH725810.1.

Conflict of interest: The authors declare that they have no conflict of interest.

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

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