A Novel Lytic Phage SZW_AS01 of Human Gut Bacteria Alistipes Shahii

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Abstract
Alisitipes phage SZW_AS01, a novel lytic phage that specifically infects Alistipes shahii, was isolated from wastewater samples in Shenzhen, China. The phage's genome consists of 45,392 bp, with a GC content of 47%. The genome encodes 56 putative open reading frames (ORFs) and 1 tRNA gene. Direct terminal repeats with a length of 55 bp are present at both ends of the genome. Phylogenetic analysis of the amino acid sequences of terminase large subunit shows that phage SZW_AS01 forms a distinct branch from the Siphoviridae family phages, but is far from the Podoviridae and Myoviridae family phages. Transmission electron microscopy confirmed that SZW_AS01 belongs to the Siphoviridae family. To the best of our knowledge, this is the first report of a lytic phage infecting bacteria in the Alistipes genus.

Introduction
Bacteriophages (phages) are the most abundant and diverse group of biological entities on earth[1]. They can be detected in various environments inhabited by bacteria. It is estimated that on average $10^{13}~14$ phages exist in the human gut, given by bioinformatics analysis of metagenomic sequencing data, yet we know little about them[2]. Alistipes shahii is a Gram-negative strictly anaerobic gut bacteria[3]. The strain (A. shahii) used in this study was isolated from a human gut fecal sample. It has been reported that this species is likely associated with some human diseases[4]. For example, the decrease in its abundance correlates to increased hepatic encephalopathy, one of the severe complications of decompensated liver cirrhosis[5]. Here, we characterize a novel phage named SZW_AS01, which was isolated from wastewater samples in Shenzhen, China, and can specifically infect the A.shahii strain. Whole genome sequencing analyses revealed that the genome of this phage is distinct from those of all other known phages, highlighting its genetic novelty. To our knowledge, this is the first representative lytic phage of A. shahii.

Results
Identification and characterization of phage SZW_AS01
Bacteriophage SZW_AS01 was isolated, using the A. shahii strain as the host bacterium, from wastewater samples. The genomic DNA was extracted from purified phage particles. Sequencing library was prepared by Illumina Nextera DNA Flex Library Prep kit and sequenced by Illumina MiSeq. The high-quality reads were assembled using SOAPdenovo[6]. Direct terminal repeats were found at both ends of phage SZW_AS01. Both repeats are 55 bp in length. Open reading frames (ORFs) were predicted using Prokka[7]. The functions of the ORFs were annotated against the NCBI nr (non-redundant protein sequence) database using the software BLASTP. The presence of tRNA genes was detected by tRNAscan-SE[8]. Phylogenetic analysis was performed using MEGA X software based on the amino acid sequence of the terminase large subunit[9].
Alistipes phage SZW_AS01’s genome is double-stranded DNA containing 45,392bp. The G+C content is 47%. 1 tRNA gene was identified. The genome was predicted to contain 56 open reading frames (ORF). Twenty-one of them (37.5% of the total gene number) was assigned as functional proteins based on blastp (TableS1).

The functional proteins could be classified into four categories: (i) DNA replication and metabolism module, (ii) DNA packing module, (iii) capsid and tail morphogenesis module, (iv) host lysis module. The remaining 35 ORF encode proteins showing similarities with hypothetical proteins. Because the genome of SZW_AS01 diverges from other available phage genomes, 5 protein functions could not be predicted with similarity searches, highlighting the phage's novelty. The whole-genome annotation of SZW_AS01 was visualized with CGView online[10] (Fig. 1). The DNA replication and metabolism module have twelve genes, ORF21, ORF32, ORF34, ORF38, ORF39, ORF41, ORF42, ORF43, ORF45, ORF47, ORF48, ORF49, which encode ribosomal large subunit pseudouridine synthase B, DNA-binding protein, putative RNaseH-like domain protein, AAA family ATPase, TIGR02757 family protein, DUF3820 family protein, 3'-5' exonuclease, putative DNA topoisomerase, putative HNH endonuclease, single-stranded DNA-binding protein, radical SAM protein, and ASCH domain-containing protein, respectively. The DNA packaging module is restricted to ORF3 and ORF6, whose protein product has the best sequence similarity to terminase large subunit of Bacteroides phage vB_BfrS_23 and portal protein of Parabacteroides phage PDS1. The capsid and tail morphogenesis module consists of five genes, ORF8, ORF9, ORF13, ORF14, ORF16, whose protein products have the best sequence similarity to the putative capsid associated protein of Parabacteroides phage PDS1, the major capsid protein of Parabacteroides phage PDS1, the putative tail assembly chaperone protein of Bacteroides phage Barc2635, the phage tail tape measure protein of Bacteroides salyersiae, and the putative phage tail fibre protein of Bacteroides phage B124-14, respectively. The lysis module has two genes, ORF18 and ORF19, which encode holing protein and N-acetylmuramoyl-L-alanine amidase. Furthermore, the remaining ORFs show various sequence similarities to hypothetical proteins in the NCBI database. Gene annotation of SZW_AS01 did not identify any known virulence genes or antibiotic resistance genes. Therefore, this phage has the potential to be used as an antibiotic agent.

The amino acid sequences of SZW_AS01 and references were aligned by Clustal W[11]. The phylogenetic tree was constructed using the Neighbor-Joining method and based on the Poisson model in MEGAX[9]. The phylogenetic tree based on the amino acid sequence of terminase large subunit indicates that SZW_AS01 is a new member of the Siphoviridae family (Fig. 2A). SZW_AS01 forms a distinct clade with other phage members, distant from other phages within the Siphoviridae family and is far away from Podoviridae and Myoviridae family.

To validate the taxonomic classification of this novel phage, we performed transmission electron microscopy (TEM). The sample was enriched in BHI medium and followed the density gradient centrifugation method by cesium chloride[12]. The suspension was fixed on the copper screen for about 10 min and stained for 3 min with 2% phosphotungstic acid. TEM indicates that the particle has a capsid of approximate 60.0 nm in diameter, and a tail length of approximate 159.7 nm (Fig. 2). Therefore, phage
SZW_AS01 belongs to *Siphoviridae* family in the order *Caudovirales*. To the best of our knowledge, SZW_AS01 is the first phage infecting the strain of *Alistipes* genus.

**Declarations**

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**Compliance with Ethical Standards:**

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

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

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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