Biological Characteristics And Genomic Analysis of A Novel Bacteriophage BUCT609 Infecting *Stenotrophomonas Maltophilia*

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Abstract

*Stenotrophomonas maltophilia* is widely distributed in nature and has a high isolation rate in nosocomial infections. Due to its potential application and important role in clinical practice, the relevant studies of *S. maltophilia* have received much attention. *S.maltophilia* phage BUCT609 (GenBank: MW960043) was isolated from hospital sewage with *S.maltophilia* strain No. 3015 as a host. Morphologically, it can be inferred as *Podoviridae* phage from the result of transmission electron microscopy (TEM). The electron microscopy also shows that the phage has an isometric capsid ~50 nm in diameter. The one-step growth curve demonstrated that the incubation period of 10 min and the burst size is 382 pfu/cell when its optimal multiplicity of infection (MOI) is 0.01. Phage BUCT609 had a high survival rate at pH 3 to 10 and tolerant temperature from 4 ℃ to 55 ℃. Next-Generation Sequencing (NGS) results demonstrated that its complete genome is linear double-stranded DNA of 43145 bp in length, and the GC content is 58 %. It has very little resemblance to other phages. The BlastN analysis shows that the genome of phage BUCT609 shares 22 % homology with *S.maltophilia* phage Ponderosa (GenBank: MK903280.1), and it encodes 56 putative proteins, of which only 25 have annotated function. Phage BUCT609 with a relatively large burst size and excellent survival ability in a wide pH and temperature range, suggests BUCT609 is a potential alternative for multi-drug resistance *S.maltophilia* therapy.

Highlights

· A novel *Stenotrophomonas maltophilia* phage BUCT609, which was successfully isolated and characterized, has only 22 % homology with known *Stenotrophomonas maltophilia* phages.

· BUCT609 is a typical T7-Like phage with a short incubation period and burst size is 382 pfu/cell when its optimal multiplicity of infection (MOI) is 0.01.

· BUCT609 had a high survival rate at a relatively wide range of temperature and pH.

Introduction

*Stenotrophomonas maltophilia* is a kind of Gram-negative bacteria commonly widely exists in nature, and it can also reside in the human respiratory tract and intestinal tract. The isolation rate of *S.maltophilia* was third only to *Acinetobacter* and *Pseudomonas aeruginosa* among non-fermented Glycogram negative bacilli [1]. As a conditioned pathogen, stenotrophomonas maltophilia is a major pathogenic bacterium of iatrogenic infection. The bacteria can cause many diseases, such as infections of the respiratory tract, urinary tract, and wounds. In recent years, with the extensive use of broad-spectrum antibiotics, *S. maltophilia* has become one of the most important pathogens of nosocomial infection [2], which brings great difficulties to clinical treatment.

Bacteriophages are considered a potential alternative to antibiotics for the treatment of multi-resistant bacteria because of their host specificity. In this context, humans have once again turned their attention to phage therapy [3]. Bacteriophages are widespread in the environment and organisms and are specific for infection and lysis of host bacteria. Using this feature to kill bacteria with phage is called phage therapy [4]. In recent years, many countries have made exciting achievements in phage therapy [5], and these successful cases demonstrate the unique advantages of phage therapy and greatly promote the further research of phage therapy. This also indicates that phage therapy has a very bright application prospect [6].

In this study, the phage BUCT609 was isolated from hospital sewage, which biological characteristics and genomics were analyzed respectively, which not only enriched the bacteriophage species of *S.maltophilia*, but also provided the basis for bacteriophage therapy.

Materials And Methods

**Identification of the host bacteria by sequencing the 16S rRNA gene**

The *S.maltophilia* No. 3015 was isolated from sewage samples collected from the Clinical Laboratory center, Taian City Central Hospital. Microbial diversity sequencing is based on a second-generation high-throughput technology to sequence the 16S rRNA genes[7]. Primers were used to amplify the 16S rRNA genes, and the purified PCR product could be sequenced with an Illumina MiSeq
sequencer (San Diego, CA, USA). The strain was identified by using the BlastN in NCBI (National Center for Biotechnology Information)(Table 1).

Isolation and purification of BUCT609

The samples were collected from the China-Japanese Friendship hospital. 2 mL of samples were centrifuged at 12000 g for 5 min, and then the impurities were then removed by membrane filtration with a pore diameter of 0.22 µm [8]. The host bacteria were cultured to the exponential phase (OD$_{600}$ = 0.7) at 37 °C, 500 µL host bacteria and 100 µL phages were mixed evenly, then 5 mL LB liquid medium was added into the mixture, followed by incubation at 37 °C for 5 h. The phage stock was obtained after centrifuged and filtered. And then the phage was serially diluted by PBS buffer for 8 gradients ($10^1$~$10^8$). The 100 µL liquid of each gradient was mixed with 500 µL host bacteria and incubated at room temperature for 10 min. The activity of phage was assessed by standard double-layer agar methods. And then the single plaque was picked and purified at least three times to obtain a relatively pure phage solution [9].

Electron microscope observation

Add 32 mL of phage solution to the centrifuge tube, and then add 8 mL of 30 % sucrose solution, which is located in the lower part of the centrifuge tube due to density. Centrifugation at 30000 g at 4 °C for 2 h. The centrifuge tube was inverted for 5 h to dry, and 200 µL PBS was added to re-suspend the phage to obtain high purity phage solution.

The morphology of the phage was observed by phosphotungstic acid negative staining. the phage BUCT609 was stained with 2 % phosphotungstic acid (PTA) and the dyeing time was about 10 min. After the samples were dried at room temperature for 5 hours, morphological characteristics of BUCT609 were observed by a transmission electron microscope (JEM-1200EX, Japan) at 80 kV [10].

The optimal multiplicity of infection and one-step growth curve

The multiplicity of infection is the ratio of the number of bacteriophages to the infected host bacteria, and the optimal multiplicity of infection is the multiplicity of infection when the bacteriophage can achieve the optimal growth state [11].

The phages and host bacteria were incubated with MOI = 100, 10, 1, 0.1, and 0.01 respectively for 20 min, and then added into fresh sterile LB liquid medium for 5 h at 37 °C. The supernatant phages were obtained by centrifugation and filtration. 100 µL phages were taken for gradient dilution with PBS buffer solution. Each gradient phage was mixed with 100 µL and 500 µL of host bacteria and incubated at room temperature for 10 min. The plaque of the double-layer plate was observed and the phage titer was calculated after 5 h incubation. The MOI with the highest titer was the best. This experiment was repeated three times.

Some necessary information about bacteriophages can be obtained by studying the one-step growth curve of bacteriophages, such as the growth rule of bacteriophages, such as outbreak time, outbreak size, etc [12].

The host strain No. 3015 was cultured to the exponential phase (OD$_{600}$ = 0.7). Phage and host bacteria were mixed up to the optimal MOI. After 20 min incubation at 37 °C, the supernatant was removed by centrifugation and the sediment was resuspended with 20 mL LB liquid medium. Samples were collected every 10 min during the incubation at 37 °C. Three samples were taken at one time point and the average phage titer was calculated after centrifugation and filtration.

Thermal Stability and pH Sensitivity

To determination of the thermal stability of phage BUCT609, the phages were incubated at different temperatures (4 °C, 37 °C, 45°C 55°C 65°C and 75 °C) for an hour, the plaque of the double-layer plate was observed and the phage titer was calculated after 5 h incubation [13]. Similarly, to explore the effect of pH on phages, the phages were incubated in PBS buffers with different pH values (pH 1 ~ pH 12) at 37 °C for 1 h, then the bacteriophage titer was calculated. This experiment was repeated three times.

Multilocus sequence typing (MLST) and the host spectrum determination

Multilocus sequence typing (MLST) is a bacterial typing method based on nucleic acid sequence determination [14]. In this method, multiple internal fragments of housekeeping genes were amplified by PCR and sequenced to analyze the variation of strains. Using housekeeping gene primers of *S.maltophilia* (*atpD, gapA, guaA, mutM, nuoD, ppsA, recA*) for PCR amplification (Table 1), and the amplified products were sequenced and analyzed on the MLST database.
13 strains of *S. maltophilia* were cultured to the exponential phase (OD$_{600}$ = 0.7), and 500 µL of each sample were added into 5 mL upper LB semi-solid medium. The mixture was immediately poured into the lower solid medium. After solidification, 2 µL phages were added to a double-layer plate, and PBS was added on the other side as a control. And then put the plates at 37 °C. 9 hours later, the formation of plaque was observed and recorded.

**Drug sensitive experiment**

In order to quickly and effectively detect the sensitivity of pathogenic bacteria to various antibiotics and guide rational drug use in the clinic, the drug sensitivity test of *S. maltophilia* strain No. 3015 was carried out. Disc diffusion method is a common method for drug sensitivity testing. The host bacteria were cultured to the exponential phase (OD$_{600}$ = 0.7) at 37 °C, 500 µL host bacteria were mixed with 5 mL LB liquid medium evenly, followed by incubation at 37 °C for 5 h. 500 µL bacteria was added into 5 mL upper LB semi-solid medium. The mixture was immediately poured into the lower solid medium. After solidification, the plates covered by the paper containing the care and exposure upside down in the incubator at 37 °C overnight. The formation of the inhibitory zone was observed in next day. This experiment was repeated three times.

**Phage DNA preparation**

Phage genomic DNA was extracted by the Proteinase K/SDS method [15]. 600 µL purified phage were incubated with DNase I and RNase A overnight at 37°C, and then the nucleases were inactivated at 80 °C for 15 min. 24 µL EDTA (20 mM), 1.5 µl proteinase K (50 µg/ml) and 30 µL SDS (0.5%) were added to the mixture, which was then incubated at 56°C for 1 h. Phage lysate was extracted with an equal volume of phenol and phenol: chloroform: isoamyl alcohol (25:24:1) in turn. Then, Phage was again extracted with an equal volume of chloroform two times. 400 µl of isopropanol was added to the upper aqueous layer and then the sample was incubated at −70°C for more than 1 h. Then, 75 % ethanol was added to rinse the precipitate. The deionized water was used to dissolve the nucleic acids which then was stored at −20°C.

**Whole genome sequencing and bioinformatics analysis**

The whole genome was sequenced using Illumina's MiSeq sequencing platform (Thermo Fisher Scientific, Waltham, MA, USA). and the complete genomic sequence of BUCT609 was assembled by Newbler V3.0 software (Roche 454) and CLC software (CLC Bio).
Then the low-quality sequences were filtered by Trimmomatic (V0.32) program. Using the online tools RAST and InterPro to annotate the DNA sequencing result. Sequence similarity analyses and comparisons were performed using the NCBI BLAST algorithm. Evolutionary phylogenetic analysis of phage BUCT609 was conducted in MEGA7 using the maximum likelihood ratio method [16].

Results

Identification of the host bacteria by sequencing the 16S rRNA gene

The result of BLAST analysis showed that the target sequence had high homology with *S. maltophilia*.

Morphology of phage BUCT609

A lytic phage, named BUCT609, was successfully isolated from untreated sewage in the hospital using *S. maltophilia* (strain No. 3015) as the indicator bacterium. BUCT609 can form transparent plaque with a diameter of 1 ~ 2 mm on a double-layer plate containing 500 µL of indicator bacteria and 100 µL of phages. Scanning electron microscope photos showed that the head diameter of the phage was about 50 nm, and the tail length was about 10 nm, which can be inferred as *Podoviridae* phage from the result of the transmission electron microscopy (TEM) (Fig. 1).

The optimal multiplicity of infection and one-step growth curve of BUCT609

Phage and host bacteria were added to the culture for 5 h at the MOI ratio as shown in Table 2, and the average titer of each phage was measured respectively. As can be seen from the table, when MOI = 0.01, the titer of phage was the highest, so the best infection number multiplicity of phage was 0.01. As can be seen from the table, when MOI = 0.01, the titer of phage was the highest, so the optimal infection number multiplicity of phage was 0.01.

| Number(No.) | Bacterial concentration (CFU/mL) | Phage concentration (PFU/mL) | Multiplicity of infection (MOI) | 5-h titer (PFU/mL) |
|-------------|---------------------------------|-----------------------------|-------------------------------|-------------------|
| 1           | 10⁸                             | 10¹⁰                        | 100                           | 3.4×10⁸           |
| 2           | 10⁸                             | 10⁹                         | 10                            | 5.2×10⁸           |
| 3           | 10⁸                             | 10⁸                         | 1                             | 9.1×10⁸           |
| 4           | 10⁸                             | 10⁷                         | 0.1                           | 1.7×10⁹           |
| 5           | 10⁸                             | 10⁶                         | 0.01                          | 3.1×10⁹           |

A one-step growth assay was conducted as described previously. As can be seen from Fig. 2, the incubation period of phages was 10 min, and the number of phages didn't change significantly during this period. The burst period last for 60 min and the burst size is 382 pfu/cell.

Thermal Stability and pH Sensitivity

Regarding temperature stability. Figure 3 shows that the phage BUCT609 is high-temperature sensitive. It still had high activity at 55 °C for 1 h. After 65 °C, the titer began to decline, and at 75 °C, the phage was almost completely inactive. For pH tolerance, BUCT609 exhibited a strong tolerance from pH 3 to 10. When exposed to pH 2 or lower, BUCT609 was significantly or completely inactivated, respectively. At pH 12 for 1 h, phages were basically inactivated.

MLST and the host spectrum

The analysis of the host range results showed that phage BUCT609 could lyse not only *S. maltophilia* strains No. 3015, but also the other 4 *S. maltophilia* strains (No. 118, No. 548, No. 992 and No. 1207). BUCT609 has a strong broad-spectrum lytic ability (Table 3).
Table 3
MLST typing results of bacterium and the host range of phage BUCT609

| Stenotrophomonas maltophilia No. | Lytic ability | ST  | Isolation time | Provider       |
|----------------------------------|---------------|-----|----------------|----------------|
| 118                              | +             | ST4 | 2012/8/24      | 307 hospital   |
| 209                              | -             | ST463| 2012/9/25      | 307 hospital   |
| 532                              | -             | ST296| 2013/5/14      | 307 hospital   |
| 548                              | +             | ST190| 2013/5/22      | 307 hospital   |
| 690                              | -             | ST115| 2013/8/9       | 307 hospital   |
| 824                              | -             | ST413| 2013/10/23     | 307 hospital   |
| 826                              | -             | ST378| 2013/10/24     | 307 hospital   |
| 992                              | +             | ST8 | 2014/3/13      | 307 hospital   |
| 1207                             | +             | ST502| 2014/7/19      | 307 hospital   |
| 1209                             | -             | ST7 | 2014/4/25      | 210 hospital   |
| 1785                             | -             | ST31 | 2015/11/11     | 210 hospital   |
| 1786                             | -             | ST362| 2015/11/11     | 210 hospital   |
| 3015                             | +             | ST95 | 2021/4/13      | Taian City Central Hospital |

Symbols: (+) have plaques and (-) no plaques after infection with phage.

Drug sensitive experiment

Drug susceptibility tests confirmed that No. 3015 strains of Streptococcus maltophilia was resistant to a variety of antibiotics but sensitive to only a few antibiotics, such as ceftriaxone, tetracycline, trimethoprim, minocycline, and levofloxacin (Table 4).

Table 4 Sensibility of S. maltophilia strain No.3015 to 12 kinds of antibiotics

| Antibiotics    | Zone of inhibition (mm) | Sensibility |
|----------------|-------------------------|-------------|
| cefalexin      | -                       | R           |
| erythromycin   | -                       | R           |
| roxithromycin  | -                       | R           |
| ceftriaxone    | 11                      | S           |
| cefazolin      | -                       | R           |
| cephalothin    | -                       | R           |
| tetracycline   | 28                      | S           |
| cefaclor       | -                       | R           |
| Norfloxacin    | -                       | R           |
| Trimethoprim   | 27                      | S           |
| Minocycline    | 26                      | S           |
| Levofloxacin   | 26                      | S           |

Symbols: (−) no zone of inhibition after infection.

Characterization of phage BUCT609 genome
The complete sequence of bacteriophage BUCT609 was 43145 bp, and its complete genome sequence had been submitted to the NCBI database with the accession number MW960043. The CG content of the genome was 58 %. The online comparison tool BlastN showed that it had 22 % homology with *S.maltophilia* phage Ponderosa (MK903280.1). The results of RAST online annotation showed that BUCT609 had 56 open reading frames (ORFs), of which 25 had known functions, and the rest were hypothetical protein sequences. The majority of ORFs presented an ATG start codon (87.5 %), while 2 started with TTG, 5 with GTG. There is no tRNA in the whole genome and these functional proteins are shown in different colors on the whole genome map (Fig. 4).

**Functional ORF analysis**

The phage BUCT609 has the same modular genomic structure as most dsDNA phages, such as DNA replication, regulation, phage packaging, structural proteins, and host lytic (Table 5).
| ORFs  | Start | Stop | Strand | Predicted Function                        | Best-match BLASTp Result                 | Accession number         | E-values | Cover | Identity |
|-------|-------|------|--------|-------------------------------------------|------------------------------------------|--------------------------|----------|-------|-----------|
| ORF1  | 897   | 130  | R      | hypothetical protein                      | Xylella phage Paz                        | YP_008858922.1           | 1.00E-68 | 96%   | 53.36%    |
| ORF2  | 1366  | 911  | R      | hypothetical protein                      | Xanthomonas phage Xaa_vB_phi31           | QOI69551.1               | 1.00E-40 | 99%   | 50.65%    |
| ORF3  | 3165  | 1375 | R      | terminase large subunit                   | Stenotrophomonas phage Ponderosa        | QEG09767.1               | 0        | 99%   | 69.26%    |
| ORF4  | 3448  | 3149 | R      | terminase small subunit                   | Xanthomonas phage Xaa_vB_phi31           | QOI69549.1               | 3.00E-09 | 100%  | 39.00%    |
| ORF5  | 3626  | 3438 | R      | holin class II                            | Stenotrophomonas phage Ponderosa        | QEG09765.1               | 1.00E-16 | 98%   | 59.68%    |
| ORF6  | 4206  | 3628 | R      | tail fiber protein                        | Bacteriophage Titan-X                   | QGH45075.1               | 1.00E-36 | 99%   | 38.78%    |
| ORF7  | 5216  | 4203 | R      | hypothetical protein                      | Bacteriophage Titan-X                   | QGH45074.1               | 8.00E-58 | 100%  | 38.76%    |
| ORF8  | 6424  | 5213 | R      | tail fiber protein                        | Stenotrophomonas phage Ponderosa        | QEG09762.1               | 1.00E-134 | 70%   | 66.67%    |
| ORF9  | 7540  | 6425 | R      | tail fiber protein                        | Stenotrophomonas phage Ponderosa        | QEG09761.1               | 0        | 100%  | 80.65%    |
| ORF10 | 11683 | 7601 | R      | transglycosylase                          | Stenotrophomonas phage Ponderosa        | QEG09760.1               | 0        | 100%  | 69.15%    |
| ORF11 | 14140 | 11696| R      | internal virion protein                   | Stenotrophomonas phage Ponderosa        | QEG09759.1               | 0        | 97%   | 49.69%    |
| ORF12 | 14979 | 14149| R      | internal virion protein                   | Stenotrophomonas phage Ponderosa        | QEG09758.1               | 7.00E-94 | 99%   | 51.99%    |
| ORF13 | 17519 | 14979| R      | tail tubular protein B                    | Stenotrophomonas phage Ponderosa        | QEG09757.1               | 0        | 100%  | 73.32%    |
| ORF14 | 18149 | 17529| R      | tail tubular protein A                    | Stenotrophomonas phage Ponderosa        | QEG09756.1               | 7.00E-91 | 99%   | 62.98%    |
| ORF15 | 18418 | 18203| R      | hypothetical protein                      | Xanthomonas phage Xaa_vB_phi31           | QOI69538.1               | 4.00E-14 | 100%  | 55.56%    |
| ORF16 | 19464 | 18463| R      | major capsid protein                      | Stenotrophomonas phage Ponderosa        | QEG09754.1               | 2.00E-169| 99%   | 72.62%    |
| ORF17 | 20248 | 19493| R      | scaffold protein                          | Xylella phage Paz                        | YP_008858906.1           | 2.00E-54 | 96%   | 44.94%    |
| ORF18 | 21762 | 20245| R      | portal protein                            | Stenotrophomonas phage Ponderosa        | QEG09752.1               | 0        | 98%   | 71.20%    |
| ORF19 | 22094 | 21759| R      | hypothetical protein                      | Stenotrophomonas phage Ponderosa        | QEG09751.1               | 4.00E-34 | 91%   | 72.55%    |
| ORF20 | 22384 | 22205| R      | hypothetical protein                      | Xylella phage Paz                        | YP_008858903.1           | 6.00E-15 | 100%  | 57.63%    |
| ORF21 | 24947 | 22548| R      | RNA polymerase                            | Stenotrophomonas phage Ponderosa        | QEG09749.1               | 0        | 100%  | 71.62%    |
| ORF22 | 25189 | 24953| R      | not hits                                  |                                          |                          |          |       |           |
| ORFs | Start | Stop  | Strand | Predicted Function | Best-match BLASTp Result | Accession number | E-values | Cover | Identity |
|------|-------|-------|--------|-------------------|--------------------------|-------------------|---------|-------|----------|
| ORF23 | 26232 | 25186 | R      | DNA ligase        | Stenotrophomonas phage Ponderosa | QEG09747.1     | 1.00E-75 | 99%   | 42.42%   |
| ORF24 | 26592 | 26323 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa | QEG09745.1     | 4.00E-15 | 96%   | 50.00%   |
| ORF25 | 27173 | 26589 | R      | hypothetical protein | Xanthomonas phage Xaa_vB_phi31 | QOI69527.1     | 1.00E-46 | 100%  | 41.59%   |
| ORF26 | 28025 | 27183 | R      | Phage exonuclease | Xylella phage Cota | CAB1282933.1   | 6.00E-83 | 98%   | 44.40%   |
| ORF27 | 28264 | 28022 | R      | DNA exonuclease   | Xanthomonas phage XAJ24 | YP_009785928.1 | 2.00E-27 | 100%  | 65.00%   |
| ORF28 | 29363 | 28413 | R      | exonuclease       | Xanthomonas phage Xaa_vB_phi31 | QOI69524.1     | 4.00E-139 | 95%   | 65.12%   |
| ORF29 | 30246 | 29365 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa | QEG09740.1     | 2.00E-88 | 100%  | 51.01%   |
| ORF30 | 32597 | 30243 | R      | DNA polymerase    | Stenotrophomonas phage Ponderosa | QEG09739.1     | 0       | 99%   | 69.95%   |
| ORF31 | 32926 | 32606 | R      | not hits         |                          |                  |         |       |          |
| ORF32 | 33146 | 32943 | R      | hypothetical protein | Xylella phage Prado | YP_008859401.1 | 6.00E-23 | 92%   | 69.35%   |
| ORF33 | 34456 | 33149 | R      | DNA helicase      | Stenotrophomonas phage Ponderosa | QEG09736.1     | 0       | 100%  | 63.53%   |
| ORF34 | 34996 | 34457 | R      | not hits         |                          |                  |         |       |          |
| ORF35 | 35826 | 34981 | R      | DNA primase      | Xanthomonas phage Xaa_vB_phi31 | QOI69516.1     | 1.00E-131 | 99%   | 63.44%   |
| ORF36 | 36032 | 35823 | R      | hypothetical protein | Xanthomonas phage Xaa_vB_phi31 | QOI69515.1     | 5.00E-14 | 100%  | 66.67%   |
| ORF37 | 36339 | 36034 | R      | hypothetical protein | Xanthomonas phage Xaa_vB_phi31 | QOI69514.1     | 8.00E-08 | 89%   | 32.22%   |
| ORF38 | 36521 | 36339 | R      | not hits         |                          |                  |         |       |          |
| ORF39 | 37135 | 36659 | R      | hypothetical protein | Xylella phage Paz | YP_008858885.1 | 3.00E-47 | 100%  | 57.86%   |
| ORF40 | 37736 | 37296 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa | QEG09730.1     | 9.00E-15 | 97%   | 34.23%   |
| ORF41 | 38149 | 37736 | R      | not hits         |                          |                  |         |       |          |
| ORF42 | 38710 | 38153 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa | QEG09728.1     | 4.00E-07 | 98%   | 35.64%   |
| ORF43 | 38964 | 38707 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa | QEG09727.1     | 1.00E-04 | 96%   | 33.72%   |
| ORF44 | 39141 | 39007 | R      | not hits         |                          |                  |         |       |          |
| ORF45 | 39284 | 39144 | R      | not hits         |                          |                  |         |       |          |
| ORF46 | 39423 | 39277 | R      | not hits         |                          |                  |         |       |          |
| ORFs  | Start  | Stop  | Strand | Predicted Function | Best-match BLASTp Result                  | Accession number  | E-values | Cover | Identity   |
|-------|--------|-------|--------|-------------------|------------------------------------------|-------------------|----------|-------|------------|
| ORF47 | 39590  | 39420 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa        | QEG09722.1        | 5.00E-12 | 87%   | 66.04%     |
| ORF48 | 39799  | 39668 | R      | not hits          |                                          |                   |          |       |            |
| ORF49 | 40074  | 39796 | R      | not hits          |                                          |                   |          |       |            |
| ORF50 | 40205  | 40071 | R      | not hits          |                                          |                   |          |       |            |
| ORF51 | 40767  | 40255 | R      | hypothetical protein | Stenotrophomonas phage Ponderosa        | QEG09719.1        | 2.00E-68 | 92%   | 68.15%     |
| ORF52 | 40947  | 40792 | R      | hypothetical protein | Xanthomonas phage Suba                  | CAA2409834.1      | 0.011    | 96%   | 35.19%     |
| ORF53 | 41505  | 41386 | R      | not hits          |                                          |                   |          |       |            |
| ORF54 | 42342  | 42193 | R      | o-spanin          | Stenotrophomonas phage Ponderosa        | QEG09771.1        | 2.00E-13 | 100%  | 57.14%     |
| ORF55 | 42658  | 42374 | R      | i-spanin          | Xylella phage Paz                       | YP_00885924.1     | 7.00E-06 | 100%  | 30.85%     |
| ORF56 | 43145  | 42642 | R      | endolysin         | Stenotrophomonas phage Ponderosa        | QEG09769.1        | 4.00E-66 | 99%   | 65.06%     |

The structural proteins of phage BUCT609 are mainly distributed at the front end of the gene sequence. According to previous data analysis, ORF6, ORF8, ORF9, ORF13, ORF14 were predicted to be tail proteins and tail associated proteins. ORF14 encodes caudate protein A. Tail tubular protein A (TTPA) is a structural tail protein of *S. maltophilia* bacteriophage BUCT609 and is responsible for adhering the bacteriophage to host cells [17]. ORF14 had 99 % homology to *S. maltophilia* phage Ponderosa. A major capsid protein encoded by ORF16 and has 99 % homology with *S. maltophilia* phage Ponderosa.

Terminating enzymes are the main components of DNA packaging genes, including large and small subunits. Generally, the large and small subunits of terminal enzymes are adjacent [18], which is involved in the splicing and packaging of DNA. Small subunits can bind and cleave specifically near the initial package site [19]. Large subunits are responsible for ATP-driven DNA translocations, while small subunits interact with large subunits and initiate packaging [20]. The small terminase unit specifically recognizes viral DNA, while the large terminase subunit plays an important role in ATP recognition and hydrolysis [21]. As is known from the chart, ORF3 encodes the terminase large and has 99 % homologies with *S. maltophilia* phage Ponderosa. ORF4 is predicted to be the terminase small subunit, and exhibited 100 % identity to the Xanthomonas phage Xaa_vb_phi31. The portal control protein is encoded by ORF18 and forms a channel at the phage tail attachment site through which the phage can inject its own genome into the host cell [22].

Replication is a complex process involving a variety of proteins and enzymes. In the initiation phase, the DNA helicase encoded by ORF33 can unlock double-stranded DNA by hydrolyzing ATP for energy. ORF33 has 100 % homology with *S. maltophilia* phage Ponderosa. DNA primer enzyme encoded by ORF35 has 99 % homology with Xanthomonas phage XAA_VB_Phi31, which can catalyze the synthesis of RNA primer. Different DNA polymerases play different roles in DNA replication [23]. The DNA polymerase encoded by ORF30 is mainly used for DNA replication and repair and exhibited 99 % identity to the *S. maltophilia* phage Ponderosa. Exonuclease is a class of enzymes that degrade nucleotides one by one from the end of a polynucleotide chain [24]. ORF26, ORF27, and ORF28 encode three different exonuclease enzymes. The homology of ORF26 to the phage exonuclease of Xylella phage Cota was 98 %, and that of ORF27 to the DNA exonuclease of Xanthomonas phage Xaj24 was 100 %. ORF28 showed 95 % homology with the exonuclease of Xanthomonas phage XAA_VB_phi31. The DNA ligase encoded by ORF23 is an important enzyme in organisms. The reaction catalyzed by ORF23 plays an important role in the process of DNA replication and repair, that is, connecting two adjacent bases [25]. The catalysis of ligases requires the consumption of ATP. By comparison, ORF23 has 99 % homology with *S. maltophilia* phage Ponderosa. ORF21 encodes RNA polymerase, which the main function is to use DNA chain or RNA as a template, triphosphate ribonucleoside as a substrate, through the phosphate diester bond synthesis of RNA. Because RNA is involved in the transcription of genetic information about DNA and genes within a cell, it is also called a transcriptase [26]. The RNA polymerase of ORF21 and *S. maltophilia* phage Ponderosa were significantly similar (100 % identical).
For the lytic mechanism, the phage cleaves the host bacteria under the combined action of holin, lyase, and spanin. Holin encoded by OFR5 plays a crucial role in the penetration of phages after they successfully attach to host bacteria. Holin forms pores in the cell membrane and begins the process of osmosis and dissolution [27]. By the BlastN comparison, the holin class predicted by OFR5 has 98 % homology with *S. maltophilia* phage Ponderosa. Spanin has been shown to be necessary for outer membrane destruction. Most phages produce a two-component protein complex consisting of outer membrane lipoprotein (o-spanin) and inner membrane protein (i-spanin) [28]. The phage BUCT609 had a o-spanin (ORF54) to *S. maltophilia* phage Ponderosa, with a 100 % homology. Similarly, ORF55 encoded i-spanin has 100 % homology with Xylella phage Paz. ORF56 was predicted to be an endolysin, which showed 99 % identity to *S. maltophilia* phage Ponderosa, this protein synthesized by host bacteria will result in bacteria death by inducing the lysis of bacteria cell walls specifically and effectively [29].

**Phylogenetic analysis and comparative genome result of BUCT609**

In previous BlastN sequence alignment, BUCT609 was a relatively new phage, which has low homology to known phages in NCBI. In order to directly understand the evolutionary process of BUCT609 and its relationship with other phages, the major capsid protein (ORF3) and terminase large subunit (ORF16) amino acid sequence with conserved genes and evolutionary significance was selected, and the homologous phylogenetic tree was constructed by the Neighbor-joining method of MEGA software. Phylogenetic tree results figure that phage BUCT609 is closely related to *S. maltophilia* phage Ponderosa and *S. maltophilia* phage BUCT598 (Fig. 5). Combining the results of the phylogenetic trees, comparative genomic analysis was performed phage on BUCT609, *S. maltophilia* phage Ponderosa and BUCT598. And Figure.6 shows the high similarity of the three phages in packaging, regulation, and replication.

**Discussion**

*S. maltophilia* is one of the most frequently found Gram-negative bacilli in the airways of cystic fibrosis patients, its separation rate showed an increasing trend year by year. Lytic phages are natural killers of infected bacteria because of their host specificity. Phage therapy has a very bright application prospect, which requires mature and reliable methods for phage screening and identification. In this study, a rapid and reliable method for the isolation of Streptococcus maltophilia phages was developed.

Bacteriophage was successfully isolated from hospital sewage using multi-drug resistant *S. maltophilia* strain No. 3015 as host and named as BUCT609. T7 phages are considered to be of potential clinical application because of their genome only about 40 kb, rapid replication, and strong ability of lytic.

The host spectrum test showed that 5 of 13 strains of *S. maltophilia* could be lysed by BUCT609, which indicated that BUCT609 had the broad-spectrum bactericidal ability. Other biological characteristics experiments showed that BUCT609 had a short incubation period and burst size is 382 pfu/cell when its optimal multiplicity of infection (MOI). It was stable to temperature and had a wide tolerance to pH. Meanwhile, in the drug sensitivity test, *S. maltophilia* strain No. 3015 was not sensitive to most antibiotics and had strong drug resistance. BUCT609 can lysis No. 3015 in a very short time, which shows great potential to solve the problem of clinical multi-drug resistant bacterial infection. In dealing with the problem of phage pollution, high temperature, or strong acid and strong alkali can be used to kill phage.

The whole genome of BUCT609 was analyzed by the high-throughput sequencing method. Bioinformatics software was used to assemble and analyze the sequencing data. The full-length genome of BUCT609 was 43145 bp, and the GC content is 58 %. It has very little resemblance to other phages. The BlastN analysis shows that the phage BUCT609 shares 22 % homology with *S. maltophilia* phage Ponderosa (GenBank: MK903280.1). The function of each ORF was predicted by RAST online gene annotation tool, and it encodes 56 putative proteins, of which only 25 have annotated function. Genome-wide annotation provides information for the future application of known functional genes, including lyase genes, DNA polymerase genes, etc. It provides information for the study of unknown gene function of phage.

**Conclusion**

Due to the overuse of antibiotics, most bacteria have become drug-resistant, and there is an urgent need to find a new approach to solve the disease caused by *S. maltophilia*. Phage therapy has become a new possibility to prevent bacteria from harming humans, and understanding the diversity of phages in nature may help in the future development of phage-based therapies.
characteristics and genomic analysis of *S. maltophilia* phage BUCT609 are of great significance for the exploration of phage-host interaction and coevolution and for the supplement of the viral reference database.

**Declarations**

**Data Availability Statement**

The sequence of phage BUCT609 used for the study is available in Genbank under the accession number MW960043.

**Author Contributions**

Ke Han carried out experiments, analyzed the data, and wrote the manuscript. Huahao Fan, Fei Li, and Yigang Tong designed the experiment and revised the manuscript.

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**Conflicts of Interest**

All the authors declare no competing interests.

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Figures

Figure 1

(a) Transmission electron micrograph of S. maltophilia BUCT609. Scale bar, 100 nm. (b) Plaques formed by BUCT609
Figure 2

One-step growth curve of phage BUCT609. The incubation period of the phage is only 10 min, and the lysis period is about 60 min, the burst size is about 382 pfu/cell when its optimal multiplicity of infection (MOI) is 0.01.

Figure 3

Thermal Stability and pH Sensitivity.
Figure 4

The whole genetic map of phage BUCT609. Colors distinguish different functional genes, arrows represent ORF directions.
A phylogenetic tree was constructed by Neighbor-joining method of MEGA software based on the amino acid sequence of the major capsid protein and terminase large subunit, showing the evolutionary relationship between the BUCT609 and other phages. The bar graph shows the evolution distance.

Figure 5

A major capsid protein

B terminase large subunit
Figure 6

Genome-wide comparison of S.maltophilia phage BUCT609\hspace{1pt}S.maltophilia phage Ponderosa and S.maltophilia phage BUCT598. Colors distinguish different functional genes, arrows represent ORF directions, and the level of identity is indicated by the gray shading.