Complete Genome Sequence of *Proteus mirabilis* Phage pPM_01 Isolated from Raw Sewage

Ira Aryani Wirjon^{a, b} Nyok-Sean Lau^b Yahya Mat Arip^a

^aSchool of Biological Sciences and ^bCentre for Chemical Biology, University Sains Malaysia, Sains@USM, Bayan Lepas, Penang, Malaysia

Keywords

Proteus mirabilis · Lytic phage pPM_01 · Genome · Urinary tract infection

Abstract

Objectives: Phage pPM_01 was previously isolated from a raw sewage treatment facility located in Batu Maung, Penang, Malaysia, and it was highly lytic against *Proteus mirabilis*, which causes urinary tract infections in humans. In this paper, we characterize the biology and complete genome sequence of the phage. **Methods and Results:** Transmission electron microscopy revealed phage pPM_01 to be a siphovirus (the first reported virus to infect *P. mirabilis*), with its complete genome sequence successfully determined. The genome was sequenced using Illumina technology and the reads obtained were assembled using CLC Genomic Workbench v.7.0.3. The whole genome contains a total of 58,546 bp of linear double-stranded DNA with a G+C content of 46.9%. Seventy putative genes were identified and annotated using various bioinformatics tools including RAST, Geneious v.R7, National Center for Biotechnology Information (NCBI) BLAST, and tRNAscan-SE-v1.3 Search. Functional clusters of related potential genes were defined (structural, lytic,

packaging, replication, modification, and modulatory). The whole genome sequence showed a low similarity to known phages (i.e., *Enterobacter* phage Enc34 and *Enterobacteria* phage Chi). Host range determination and SDS-PAGE analysis were also performed. **Conclusions:** The inability to lysogenize a host, the absence of endotoxin genes in the annotated genome, and the lytic behavior suggest phage pPM_01 as a possible safe biological candidate to control *P. mirabilis* infection.

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Introduction

Bacterial viruses or phages belong to a group of viruses that specifically infect bacterial cells and utilize them as hosts to propagate [1]. Phages are environmentally ubiquitous, with an estimated 10^{31} particles on earth [1, 2]. Metagenomic analyses have revealed that they are immensely diverse and present in almost all niches where bacterial cells are present [3, 4]. Phages have the capability to kill specific bacteria during exponential bacterial growth. This is a desirable trait in phage therapy, which is one of the treatments for bacterial infectious diseases.

Phage therapy involves the employment of phages or their products as biological control agents against diseases initiated by pathogenic bacteria [5]. The Republic of Georgia has employed phage therapy primarily for standard medical practices, and phages have been routinely used in many hospitals and clinics [1, 6]. The Southwest Regional Wound Care Centre (Lubbock, TX, USA) has applied phages in conjunction with other methods to treat venous leg ulcers instigated by multidrug-resistant *Pseudomonas*. The antibiotic treatments proved to be unsuccessful in promoting recovery for more than a year even though conservative wound care practices were implemented. However, when treated with phage therapy, the infected wound responded rapidly to form healthy skin [7].

According to the International Committee on Taxonomy of Virus, most phages used in phage therapy belong to the order *Caudovirales*. These phages are tailed phages and could be further classified into the *Myoviridae*, *Siphoviridae*, or *Podoviridae* families [8, 9]. Two types of phages are found in nature according to their life cycle: virulent (lytic) and temperate (lysogenic). Generally, only lytic phages are qualified for therapeutic purposes [10]. Lytic phages destroy or kill their host cells, while temperate phages infect the host cell and directly incorporate its genome into the host genome (prophage) or exist as an episomal element in the bacteria [11]. The lysogenic cycle would eventually lead to a permanent association between the prophage and the host cell, as well as all the progenies [12]. The host cells that harbor these prophages are termed lysogenic bacteria. Prophage plays an important role in the introduction of gene clusters, which contributes to pathogen virulence. Temperate phages encoding a virulence gene or a potential toxin could convert the bacterial host from a nonpathogenic strain to a pathogenic strain or to a strain with increased virulence [13]. Due to a different replication pathway, infection by a temperate phage would not necessarily end with the formation of plaques, despite the ability of a temperate phage to perform the lytic cycle. Additionally, this type of phage is seldom utilized in phage typing, which is another phage-related application used in hospitals to define the origin and course of diseases including nosocomial infection [14]. Phage typing specifies which phage a bacterium is susceptible to and it is frequently applied to identify methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Salmonella typhi*, and others [15].

P. mirabilis is a highly motile bacterium that belongs to the *Enterobacteriaceae* family [16–18]. This bacterium

is part of the human gastrointestinal tract microflora, as well as an opportunistic pathogen that could harm the host. In an immunocompromised human, *P. mirabilis* has the potential to evoke infections in various parts of the body [19]. The *Proteus* species is considered one of the uropathogens in humans and it is also the most common species reported in hospital patients associated with urolithiasis or indwelling urinary catheters (~80% of infections) [20–24]. As a matter of fact, *P. mirabilis* is commonly isolated from the biofilms that are formed on urinary catheters [16, 18]. Compared to *Escherichia coli* infections, reports of urinary tract infections (UTI) involving *P. mirabilis* are fewer. However, *P. mirabilis* infections are likely to be chronic and relatively persistent, as well as difficult to cure. They could lead to serious complications, threatening the health of patients compared to those infected by *E. coli* [19, 20]. The *P. mirabilis* strain has been demonstrated to be resistant to various antibiotics [23, 25] and it has produced extended-spectrum class A β -lactamases [25]. Recently, the mortality rate due to antibiotic resistance of pathogenic bacteria has increased to 25,000 people per year in the USA and Europe [26]. The rise of multidrug-resistant bacteria poses serious public health and socioeconomic risks [27].

A promising alternative to antibiotics is the use of phage therapy. In this study, we present the biological characterization and complete genetic components of a lytic *P. mirabilis* phage designated pPM_01. To our knowledge, this is the first report of a complete genome sequence analysis for a lytic *Siphoviridae* phage infecting *P. mirabilis*. In addition, phage pPM_01 is free from any virulent or toxic genes and is hence classified as a potentially safe biological agent against diseases caused by *P. mirabilis*.

Materials and Methods

Bacterial Strains and Culture Media

The bacterial strains used in this study and their sources are listed in Table 1. *P. mirabilis* was used as a primary host for isolation, propagation, and characterization of phage pPM_01. The strain used was a clinical isolate obtained from the Microbiology Collections, School of Biological Sciences, Universiti Sains Malaysia. The bacterium was propagated at 37°C in lysogeny broth (LB) medium.

Agar Layer (Overlay) Method

The overlay method was performed according to the standard protocols of Adams [28] and Carey-Smith et al. [29] with minor modifications. To prepare the LB (Oxoid, UK) base plates, agar at a concentration of 1.5% was used while soft agar overlays were prepared at 0.5%. Exponential phase host culture (OD₆₀₀ ~0.5) and

Table 1. Bacterial strains used and their susceptibility to phage pPM_01

Bacterial strain	Susceptibility
<i>Escherichia coli</i> ATCC 13706 ^a	–
<i>Escherichia coli</i> ATCC 11303 ^a	–
<i>Escherichia coli</i> O157:H7 ^b	–
<i>Proteus mirabilis</i> ^c	+
<i>Pseudomonas aeruginosa</i> ^c	–
<i>Klebsiella</i> sp. ^c	–
<i>Enterobacter</i> sp. ^c	–
<i>Salmonella</i> sp. ^c	–
<i>Bacillus subtilis</i> ^c	–
<i>Micrococcus</i> sp. ^c	–

+, lysis; –, no lysis. ^a Purchased from the American Type Culture Collection (ATCC). ^b Purchased from the National Public Health Laboratory, Ministry of Health, Malaysia. ^c Obtained from the Microbiology Collections, School of Biological Sciences, Universiti Sains Malaysia.

phage were added to the base plate. A volume of 3 mL of soft agar (45°C) was poured onto each base plate, which was swirled several times to properly mix and spread the mixture into a uniform top layer. The plate was incubated at 37°C for 24 h, after which plaque (clear zone) formation in the bacterial lawn was observed.

Sewage Sample Collection and Treatment

The sample was collected from a raw sewage facility treatment located in Batu Maung, Penang, Malaysia (GPS: 5.2833° N, 100.2833° E). The samples were transported to the laboratory at ambient temperature (~30°C), stored at 4°C, and processed within 24 h. The raw sewage sample was passed through filter paper (Whatman No. 1) to remove cellular materials and solid particles. The filtrate was then immediately used for phage isolation.

Phage Isolation

Ten milliliter of overnight *P. mirabilis* culture (~10⁸ CFU/mL) was mixed with an equal amount of filtrate. The mixture was incubated by shaking at 37°C overnight. Following the incubation, the bacteria cells were removed by centrifugation at 12,000 g for 10 min at 4°C and the supernatant was filtered using a sterile syringe filter with a pore size of 0.45 µm (Sartorius AG, Germany). The presence of an infectious phage was screened using the standard agar layer method [28, 29]. Subsequently, 100 µL of the filtrate, 100 µL of the bacterial culture (OD₆₀₀~0.6), and the overlay agar were poured on the base plate. Plaques were identified after an overnight incubation at 37°C.

Phage Purification and Titration

A single plaque on the bacterial lawn was purified using 3 consecutive single-plaque isolations to obtain a pure phage stock [30]. The overlay method was performed to check the consistency of the plaque morphologies [28, 29]. After an overnight incubation, 5 mL of the SM buffer was added to the plate and left at room temperature for 2 h to elute the phage. The SM buffer was removed and

centrifuged at 4,500 g for 15 min, which was sufficient to decant any remaining agar. The supernatant was filtered using a 0.45-µm syringe filter (Sartorius AG, Germany) and ultracentrifuged (Beckman Coulter, US) for 2 h at 100,000 g at a temperature of 4°C to concentrate the phage. The supernatant was discarded, and 200 µL SM buffer was then added. The pellet was resuspended gently, and the phage titer was determined via the overlay method [28, 29].

Phage Propagation

The propagation of phage pPM_01 was performed based on a modified protocol of Sambrook and Russell [31] using *P. mirabilis* as a host. Briefly, a single colony of bacteria on the LB agar plate was grown in 10 mL of LB (10.0 g/L NaCl, 10.0 g/L tryptone, and 5.0 g/L yeast extract) and incubated on an orbital shaker at 180 rpm and 37°C for 3–5 h (OD₆₀₀ ~0.6; ~10⁸ CFU/mL) [32]. The phage stock (10¹⁰ PFU/mL) was mixed with the cultured host at a multiplicity of infection of 1 and incubated again overnight. The infected culture was centrifuged at 12,000 g for 20 min at 4°C, and the collected supernatant was filter sterilized using 0.45-µm syringe filters. Chloroform (1% v/v) was added into the phage stock and kept at a temperature of 4°C.

Transmission Electron Microscopy

A drop of the phage stock containing a high titer of 10¹⁰ PFU/mL was spotted onto a film-coated carbon grid for 3 min [33, 34]. One drop of the negative stain solution (2% methylamine tungstate, pH 6.5) was instantaneously dropped on the surface of the film-coated carbon grid and visualized using TEM (Philips CM12 equipped with an analysis system; Philips Electron Optics) at the Microscopy Unit, School of Biological Sciences, Universiti Sains Malaysia.

Host Range Analysis

Phage specificity was examined using a spot test assay against several bacterial strains (Table 1) on LB agar plates as described by Jun et al. [35], with modifications. In each test, a volume of 100 µL (OD₆₀₀~0.5) of bacteria culture and 3 mL of molten soft agar were added to the base plate. Subsequently, the plate containing the mixtures was swirled and left to solidify for 30 min. After that, 10 µL of the phage stock (10¹⁰ PFU/mL) was used to spot a bacterial lawn on a plate and the absorption was left for 30 min. The lytic activities of the isolated phage were inspected following an overnight incubation at 37°C and the plaque formations were differentiated on a scale as follows: + (lysis) and – (no lysis). Each test was performed in triplicate. Following that, a plaque assay was conducted to confirm that the plaques produced were not due to bacteriocin or any other effects. The phage stock was serially diluted to 10² PFU/mL and the presence of the infectious phage was screened using the standard agar layer method [28, 29].

Genome Extraction

The DNA genome was extracted from a concentrated phage stock (10¹⁰ PFU/mL) following the modified phenol-chloroform extraction protocols described by Ausubel et al. [36]. The extracted DNA was then precipitated with ice-cold 100% ethanol supplemented with sodium acetate (pH 4.8).

Genome Sequencing and Assembly

The whole phage genome was sequenced using the Illumina MiSeq platform (MiSeq reagent nano kit v2, 300 cycles, MiSeq

Fig. 1. Electron micrographs of phage pPM_01 after being negatively stained with 2% methylamine tungstate.

Desktop Sequencer) [37]. An amount of 1 µg of the purified phage DNA was used. The sequencing DNA library was prepared using a Nextera XT DNA sample preparation kit (Illumina, USA) following the manufacturer's protocol. The genome was sequenced using 2 × 150 bp paired-end reads to a depth of 100× coverage. The base pair reads were then de novo assembled using into a single sequence CLC Genomics Workbench (Qiagen) [38]. The DNA size and GC content of the isolated phage were determined. The presence of tRNA was scanned through tRNAscan-SE Search (tRNAscan v1.3) [39].

ORF Prediction and Genome Annotation

A whole genome sequence of phage pPM_01 was compared using BLAST search (blastn) against the National Center for Biotechnology Information (NCBI) GenBank database to find the sequence homology [40]. Open reading frames (ORF) were automatically identified employing Rapid Annotation using Subsystem Technology (RAST) (<http://rast.nmpdr.org/>) [41] and Geneious software (Biomatters Limited). Additional protein homology searches of nucleotide sequences and manual annotation of putative translated ORF products were carried out using blastx at NCBI (<http://blast.ncbi.nlm.nih.gov>) against a nonredundant protein sequence database (E value cut-off $<10^{-4}$) [3] to compare the homologies with known protein sequences. Finally, the genome annotation was mapped using Geneious software.

SDS-PAGE Analysis

The structural proteins of phage pPM_01 were analyzed using SDS-PAGE [36]. The standard protocols for the SDS-PAGE analysis were modified from the gel method of Laemmli [42]. Polyacrylamide gel electrophoresis was cast as 10% of the resolving gel

and topped with 4% of the stacking gel. The standard protocol for using the electrophoresis apparatus was followed as described in the Mini-PROTEAN® Tetra Cell (Bio-Rad, USA) manual. The phage particles were concentrated using ultracentrifugation (Beckman Coulter) at 100 000 g and 4°C for 2 h and the pellet was resuspended in 200 µL of LB. Fifteen microliters of the phage sample was solubilized with 5 µL of 4× sample loading buffer and the denatured samples were cooled on ice for several minutes. Electrophoresis was carried out as in the protocol and the gel was stained with Coomassie brilliant blue (Amresco, USA).

Nucleotide Sequence Accession Number

The full phage pPM_01 DNA genome sequence has been deposited in the NCBI GenBank database under the accession number KP063118.

Results

Electron Microscopy

TEM revealed phage pPM_01 as a tailed phage in the family of *Siphoviridae* based on its icosahedral head diameter (63 nm) and its noncontractile tail length and diameter (263 and 11 nm, respectively; Fig. 1). The head size is relatively similar to the general size of *Siphoviridae* phages, which possesses an icosahedral head size ranging from 55 to 60 nm [43].

Fig. 2. Map of phage pPM_01 genome. The direction of the arrow reflects the transcription orientation. HP, hypothetical protein. The scale unit is base pairs and the scale bars mark the genome positions at 500-bp intervals.

Host Range Analysis

The host range of phage pPM_01 was analyzed against several bacteria (Table 1) that have the potential to cause UTI. In this assay, the phage was lytic to *P. mirabilis* only as no lytic activity was observed on the other tested bacteria. Single plaques were observed after the plaque assay indicated that the clear zones were produced by infectious phages and not due to bacteriocin or other effects. The host range analysis investigated the virulence spectrum limitation of the phage towards different bacteria, which must be understood in order to enable successful in vivo testing [44]. Narrow host range may be undesirable, but phage pPM_01 can be applied as part of the phage cocktails in phage therapy due to its efficacy in restraining the *P. mirabilis* host.

Genomic Analysis

The paired-end reads are assembled into a complete genome sequence of 58,546 bp in length (Fig. 2) with a GC content of 46.9%. The shortest ORF encodes 38 amino acids while the highest encodes 1,446 amino acids. The

predominant initiation codons are ATG followed by 6 ORF beginning with TTG and 5 with GTG. Meanwhile, UAA (42 ORF) and UGA (17 ORF) are the predominant termination codons. A description of the predicted amino acid sequences is presented in Table 2, with no tRNA genes detected. When the complete genome sequence of phage pPM_01 was compared with those of the other phages for which data are available in the NCBI GenBank, it was found that phage pPM_01 shares some degree of similarity with *Enterobacter* phage Enc34 (Genbank accession No. JQ340774.1) and *Enterobacteria* phage Chi (JX094499). The *Enterobacter* phage Enc34 genome consists of 60,364 bp with a GC content of 51.07%, which is 1,818 bp larger than the phage pPM_01 genome [45].

Prediction of the Gene Function

The assembled complete genome indicates the presence of 70 potential ORF (Fig. 2). Based on the ORF analysis, 23 (32.9%) of the 70 putative ORF were assigned functions according to their known homologous sequences. The predicted ORF were classified into the fol-

Table 2. Annotated open reading frames of phage pPM_01

Open reading frame	Strand	Start	Stop	Size, amino acids	Predicted function	E value/aa identity, %	GenBank Accession No.
1	+	154	1,920	589	putative primase (<i>Salmonella</i> phage iEPS5)	3E-97/35	YP_008240303.1
2	–	3,590	3,366	75	hypothetical protein	–/–	–
3	–	4,180	3,602	193	hypothetical protein (<i>Providencia alcalifaciens</i> F90-2004)	3E-16/40	ETT05661.1
4	–	4,389	4,177	71	hypothetical protein	–/–	–
5	–	5,092	4,403	230	hypothetical protein	–/–	–
6	–	5,936	5,145	264	hypothetical protein	–/–	–
7	–	6,319	6,098	74	hypothetical protein	–/–	–
8	–	6,953	6,303	217	hypothetical protein (<i>Listeria</i> phage LP-114)	2E-70/54	YP_009045137.1
9	–	7,697	6,957	247	hypothetical protein	–/–	–
10	–	8,611	8,246	122	hypothetical protein	–/–	–
11	–	9,173	8,604	190	hypothetical protein	–/–	–
12	–	9,850	9,662	63	hypothetical protein	–/–	–
13	–	10,082	9,840	81	hypothetical protein	–/–	–
14	–	10,420	10,094	109	hypothetical protein	–/–	–
15	+	10,565	10,693	43	hypothetical protein	–/–	–
16	+	10,892	11,323	144	hypothetical protein (<i>Enterobacter</i> phage Enc34)	1E-6/31	YP_007007063.1
17	+	11,405	11,716	104	hypothetical protein	–/–	–
18	+	11,722	12,042	107	hypothetical protein (<i>Salmonella</i> phage FSL SP-124)	5E-5/29	AGF87997.1
19	+	12,036	12,383	116	hypothetical protein	–/–	–
20	+	12,370	13,458	363	putative recombinase RdgC (<i>Pseudomonas</i>)	4E-13/30	WP_003442690.1
21	+	13,461	13,661	67	hypothetical protein (<i>Enterobacter</i> phage Enc34)	1E-11/57	YP_007007058.1
22	+	13,728	14,546	273	hypothetical protein	–/–	–
23	+	14,543	15,310	256	hypothetical protein	–/–	–
24	+	15,307	15,753	149	hypothetical protein	–/–	–
25	+	15,746	16,378	211	hypothetical protein	–/–	–
26	+	16,378	17,115	246	putative DNA methyltransferase (<i>Providencia</i> phage Redjac)	1E-72/49	YP_006905998.1
27	–	17,267	17,142	42	hypothetical protein	–/–	–
28	+	17,417	17,950	178	hypothetical protein	–/–	–
29	+	17,943	18,194	84	hypothetical protein	–/–	–
30	+	18,196	19,065	290	hypothetical protein	–/–	–
31	+	19,062	19,526	155	hypothetical protein (<i>Salmonella</i> phage iEPS5)	2E-22/42	YP_008240264.1
32	–	20,030	19,776	85	hypothetical protein	–/–	–
33	–	20,734	20,030	235	putative lysis protein A (<i>Salmonella</i> phage FSL SP-099)	1E-81/57	AGF89680.1
34	–	21,059	20,745	105	putative lysis protein B (<i>Salmonella</i> phage iEPS5)	9E-13/33	YP_008240268.1
35	–	22,908	21,070	613	hypothetical protein (<i>Staphylococcus</i> phage SA1)	1E-29/29	ACZ55502.1
36	–	24,000	23,059	314	hypothetical protein	–/–	–
37	+	24,224	24,382	53	hypothetical protein	–/–	–
38	–	25,713	24,697	339	hypothetical protein (<i>Salmonella</i> phage iEPS5)	2E-152/60	YP_008240271.1
39	–	26,706	25,726	327	hypothetical protein (<i>Enterobacteria</i> phage Chi)	3E-94/46	YP_008058190.1

Table 2 (continued)

Open reading frame	Strand	Start	Stop	Size, amino acids	Predicted function	E value/aa identity, %	GenBank Accession No.
40	–	27,640	26,720	307	hypothetical protein (<i>Salmonella</i> phage FSL SP-088)	1E-41/34	YP_008239940.1
41	–	28,383	27,640	248	putative tail fiber protein (<i>Providencia</i> phage Redjac)	3E-83/50	YP_006905987.1
42	–	32,207	28,383	1,275	putative tail assembly protein (<i>Providencia</i> phage Redjac)	0/44	YP_006905986.1
43	–	32,409	32,200	70	putative tail assembly protein (<i>Enterobacter</i> phage Enc34)	4E-8/37	YP_007007028.1
44	–	32,645	32,409	79	Putative tail assembly protein 1 (<i>Salmonella</i> phage SPN19)	7E-24/67	YP_006990293.1
45	–	33,473	32,658	272	putative tail assembly protein (<i>Enterobacter</i> phage Enc34)	7E-100/55	YP_007007026.1
46	–	35,182	33,494	563	putative tail assembly protein (<i>Enterobacter</i> phage Enc34)	2E-124/40	YP_007007025.1
47	–	39,525	35,188	1,446	putative tape measure protein (<i>Salmonella</i> phage FSL SP-088)	0/44	YP_008239947.1
48	–	39,673	39,515	53	hypothetical protein	–/–	–
49	–	40,218	39,766	151	hypothetical protein (<i>Enterobacteria</i> phage Chi)	6E-30/40	YP_008058180.1
50	–	41,544	40,357	396	phage structural protein (<i>Enterobacter</i> phage Enc34)	3E-98/52	YP_007007020.1
51	–	42,060	41,548	171	hypothetical protein (<i>Salmonella</i> phage SPN19)	2E-31/46	YP_006990300.1
52	–	42,667	42,050	206	hypothetical protein (<i>Providencia</i> phage Redjac)	2E-66/56	YP_006906022.1
53	–	43,023	42,670	118	hypothetical protein (<i>Enterobacter</i> phage Enc34)	4E-25/46	YP_007007017.1
54	–	43,370	43,023	116	hypothetical protein (<i>Salmonella</i> phage FSL SP-030)	1E-5/47	YP_008239848.1
55	–	44,501	43,434	356	putative capsid protein E (<i>Salmonella</i> phage FSL SP-030)	7E-155/60	YP_008239847.1
56	–	44,930	44,514	139	putative decorator protein D (<i>Salmonella</i> phage FSL SP-030)	7E-24/42	YP_008239846.1
57	–	46,221	44,944	426	putative prohead protease ClpP (<i>Burkholderia</i> phage BcepNazgul)	2E-82/46	NP_918994.2
58	–	47,861	46,218	548	putative phage portal protein, lambda family (<i>Enterobacteria</i> phage Chi)	0/60	YP_008058171.1
59	–	48,136	47,861	92	putative head-to-tail joining protein (<i>Enterobacter</i> phage Enc34)	6E-14/49	YP_007007011.1
60	–	50,215	48,149	689	putative terminase large subunit (<i>Enterobacteria</i> phage Chi)	0/62	YP_008058169.1
61	–	50,792	50,208	195	putative terminase small subunit (<i>Enterobacter</i> phage Enc34)	2E-67/57	YP_007007009.1
62	–	52,318	50,795	508	putative SNF2 domain-containing protein (<i>Salmonella</i> phage SPN19)	1E-140/46	YP_006990311.1
63	–	52,565	52,278	96	putative VRR-NUC domain-containing protein (<i>Salmonella</i> phage SPN19)	6E-28/58	YP_006990312.1
64	–	54,598	52,565	678	putative DNA polymerase I (<i>Providencia</i> phage Redjac)	0/56	YP_006906013.1
65	–	55,268	54,663	202	hypothetical protein (<i>Salmonella</i> phage FSL SP-099)	4E-40/48	AGF89722.1
66	–	56,628	55,300	443	hypothetical protein (<i>Salmonella</i> phage FSL SP-124)	5E-103/39	AGF88050.1

Table 2 (continued)

Open reading frame	Strand	Start	Stop	Size, amino acids	Predicted function	E value/aa identity, %	GenBank Accession No.
67	–	57,171	56,689	161	hypothetical protein	–/–	–
68	+	57,201	57,344	48	hypothetical protein	–/–	–
69	–	57,517	57,404	38	hypothetical protein	–/–	–
70	+	57,524	57,808	95	hypothetical protein	–/–	–

Fig. 3. SDS-PAGE analysis of phage pPM_01 structural proteins on 10% gel staining with Coomassie brilliant blue. M, standard marker of molecular weight (kDa). Lane 1: phage pPM_01 protein profile.

lowing 6 functional groups: tail structural components and assembly, head structural components and assembly, DNA packaging, host lysis, DNA replication and gene expression modulation, and DNA recombination/modification. Seventeen (24.3%) of them were putative proteins, which matched the hypothetical proteins in the nonredundant and phage protein databases of NCBI. Thirty (42.9%) of the predicted ORF were lacking in identity to any known proteins, yet they were assigned to the hypothetical proteins.

The terminase subunits (ORF60 and ORF61) of phage pPM_01 are putative proteins that are involved in DNA packaging. Phage pPM_01 ORF60 encodes 689 amino acids with 62% similarities to the known large terminase subunit of phage chi, which implies a similar function in DNA packaging. The terminase enzymes producing the phage genome ends are relatively varied, but the genes are conserved among tailed phages. The presence of terminase_GpA superfamily in ORF60 may be involved in the late stages of DNA packaging as in λ phage [46]. To study the genome end of phage pPM_01, an additional phylogenetic analysis was conducted based on the terminase large subunit [47]. This comparative analysis suggested that the end of the genome for phage pPM_01 likely belongs to the “ λ -like 5'-extended COS ends” group. Phages belonging to this group usually have linear DNA genomes that circularize once released inside the infected host.

Based on the amino acid sequence analysis (Table 2), a clear lifestyle prediction for phage pPM_01 is likely impossible, probably due to the very low amino acid sequence identities of the predicted ORF in phage pPM_01 to those of other known phages. Most of the annotated genes encode unknown proteins, indicating that further genomic study of phage pPM_01 is required to elucidate its infection mechanism, the stage of the replication cycle, the presence of possible virulence genes, and much more.

SDS-PAGE Analysis

The predominant polypeptide band is generally proposed as a major capsid protein due to the high copy number of capsid proteins [48]. Lane 1 of the gel (Fig. 3) clearly showed distinct protein bands with a molecular weight ranging approximately from 11 to 130 kDa. Two predominant polypeptide bands were observed approximately between 35–48 kDa and 48–63 kDa. Mostly, the detected molecular masses correlated well to the theoretical molecular size deduced from the corresponding ORF. Thus, the theoretical molecular size of phage pPM_01 further suggests that the protein band with a weight from 35 to 48 kDa is the capsid protein E of phage pPM_01. Besides that, the estimated molecular weights of the phage pPM_01 capsid protein E (ORF 55) and its homolog in *Salmonella* phage FSL SP-030 are almost the same, i.e., 42.6 kDa and 39.9 kDa, respectively [49].

Discussion

Phage pPM_01 is the first lytic phage specific to host *P. mirabilis* whose complete genomic sequence has been determined. Phage pPM_01 genes are organized tightly in the genome as some of the stop codons are overlapped to the start codons of the following gene (Fig. 2) [50]. Significant portions of the ORF genes are located on the left arm of the genome and the encoding proteins, which means that no functional assignment could be made at the time of reporting.

The gene order revealed that most of the genes in phage pPM_01 were found on the reverse strand [51]. Schematic representation of the phage pPM_01 genome showed a very high similarity with the genome organization of phage [52]. The exception was the primase protein located at the right arm end of the phage iEPS5 genome instead of the left arm end, as in the pPM_01 genome. Four of the functional groups of the pPM_01 genome classified as the tail structure components and assembly, head structural and assembly, DNA packaging, and host lysis were on the reverse strand. Likewise, all of the genes encoded for the DNA replication and gene expression modulation were also found on the reverse strand, except for the primase protein located on the forward strand. Meanwhile, the genes encoded for DNA recombination/modification were also on the forward strand.

Based on genomic analyses, no genomic markers demonstrating a temperate lifestyle were found in the phage pPM_01 genome. This revealed phage pPM_01 as an appropriate candidate for phage therapy application in

treating diseases caused by *P. mirabilis*. In fact, in vitro study of phage pPM_01 showed that pPM_01 was highly lytic and virulent against *P. mirabilis* (data not shown). The presence of putative lysis proteins A and B (ORF 33 and ORF 34) would indicate that phage pPM_01 is a lytic phage [53]. A previous study by O'Flynn et al. [54] reported that an isolated phage of the *Myoviridae* family had acquired an *eaeA* virulence factor from the host genome. In contrast, neither virulence factors nor potentially known toxins were identified in the phage pPM_01 genome, thus indicating that phage pPM_01 might be safe for phage therapy. The phage pPM_01 genome was also unlikely to transfer genes to other bacteria since no prophage characteristics were found. Avoiding temperate phages for phage therapy is necessary since most prophages would integrate into the host genome and somehow acquire virulence genes upon excision from the host genome [54–56].

The phage pPM_01 genome organization was successfully constructed and analysis of the annotated genes provided insight into its genetic identity and relations with other phages. The data leaned toward identifying phage pPM_01 as a new addition to the phage database, specifically phages infecting *P. mirabilis*. In addition to the expected genes such as capsid and tail, other genes commonly present in a phage were annotated as well. The presence of putative proteins and uncharacterized proteins was typically anticipated for a new phage not previously isolated and characterized. Additionally, the absence of virulence and toxin genes in the genome of phage pPM_01 provides hope for this phage to be further developed for phage therapy.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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