Characterization and Genomic Analysis of ϕ SHP3, a New Transposable Bacteriophage Infecting *Stenotrophomonas maltophilia*

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ABSTRACT This study describes a novel transposable bacteriophage, ϕ SHP3, continuously released by *Stenotrophomonas maltophilia* strain c31. Morphological observation and genomic analysis revealed that ϕ SHP3 is a siphovirus with a 37,611-bp genome that encodes 51 putative proteins. Genomic comparisons indicated that ϕ SHP3 is a B3-like transposable phage. Its genome configuration is similar to that of *Pseudomonas* phage B3, except for the DNA modification module. Similar to B3-like phages, the putative transposase B of ϕ SHP3 is a homolog of the type two secretion component ExeA, which is proposed to serve as a potential virulence factor. Moreover, most proteins of ϕ SHP3 have homologs in transposable phages, but only ϕ SHP3 carries an RdgC-like protein encoded by gene 3, which exhibits exonuclease activity *in vitro*. Two genes and their promoters coding for ϕ SHP3 regulatory proteins were identified and appear to control the lytic-lysogenic switch. One of the proteins represses one promoter activity and confers immunity to ϕ SHP3 superinfection *in vivo*. The short regulatory region, in addition to the canonical bacterial promoter sequences, displays one LexA and two CpxR recognition sequences. This suggests that LexA and the CpxR/CpxA two-component system might be involved in the control of the ϕ SHP3 genetic switch.

IMPORTANCE *S. maltophilia* is an emerging global pathogenic bacterium that displays genetic diversity in both environmental and clinical strains. Transposable phages have long been known to improve the genetic diversity of bacterial strains by transposition. More than a dozen phages of *S. maltophilia* have been characterized. However, no transposable phage infecting *S. maltophilia* has been reported to date. Characterization of the first transposable phage, ϕ SHP3, from *S. maltophilia* will contribute to our understanding of host-phage interactions and genetic diversity, especially the interchange of genetic materials among *S. maltophilia*.

KEYWORDS *S. maltophilia*, transposable phage, genome sequence, RdgC, lytic-lysogenic switch, *Stenotrophomonas*, siphovirus

Bacteriophages (phages) are the most abundant entities on earth, with 10 times more phages than the estimated number of bacteria in the biosphere. Phages are ubiquitous on earth and are found in a variety of external and internal environments, such as the human gut (1, 2). Temperate phages can enter either the lytic cycle or the lysogenic state. The synthesis and release of these phages are generally controlled by host signals and phage-encoded regulators (3–5). Temperate phages influence bacterial evolution and promote the emergence of novel pathogens by transferring virulence genes (6–7). One notable kind of temperate phage is the transposable phage, which replicates its genome by transposition (8). The first transposable phage, *Escherichia virus Mu*, was described in 1963 (9). Since then, many transposable phages have been identified in α , β , γ , and *Epsilonproteobacteria* and *Firmicutes* (10–12). Transposable phages belong to

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two families, *Myoviridae* and *Siphoviridae*, each of which includes phages with one of two main genomic organizations, Mu and B3-like (13). All genes are transcribed in the same direction except the lysogenic repressor gene in phage Mu (12). In phage B3, the approximately 8.5-kb left end is inverted with respect to that of Mu, and the *mor* gene is located at the leftmost end (14). BcepMu of *Burkholderia cepacia* and Mu are myophages, while the genome configuration of BcepMu is B3-like (15). *Pseudomonas aeruginosa* phages D3112 and B3 are siphophages, but the genome configuration of D3112 is the Mu type (16). In addition to conserved structural proteins, lysis systems, and DNA modification proteins present in tailed phages, all transposable phages have unique conserved proteins, such as Mor, GemA, and transposase A (TnpA) and B (TnpB) (13).

In transposable phages, the genome is a linear double-stranded DNA (dsDNA) molecule with a similar length (35 to 40 kb); the ends display a 2-bp 5'-TG-CA-3' inverted repeat and are flanked by heterogeneous segments of host DNA, with 100 to 150 bp on the left end and 1 to 1.5 kbp on the right end. The 5'-TG-CA-3' terminal base pairs of most transposable prophages are flanked by a 6-bp direct repeat of the host target sequence (8, 15, 17). It is well known that phages express their genes sequentially during the lytic cycle. Genes for genome replication are usually expressed early, and genes for phage particle morphogenesis and cell lysis are expressed late. The Mu genome consists of seven functional modules, which are expressed in the early, middle, and late stages (12, 18). One of the two early expressed modules synthesizes only the lysogenic repressor RepC, and the other synthesizes the lytic repressor Ner, the transposases MuA and MuB, GemA, Gam, Mor, and other dispensable proteins. Then, Mor activates the expression of the middle gene C. The C protein activates the expression of late genes, including four modules encoding the lysis system, head, tail, and DNA modification system. However, gene C is not present in all transposable phages (14, 19).

Similar to other temperate phages, transposable phages also benefit their hosts in various ways. However, transposable phages are unique in other ways, as they not only interfere with host gene expression but also destroy genes by means of random insertion of their DNA into the target gene, or by rearrangement of the bacterial chromosome due to transposition. Therefore, transposable phages promote the evolutionary divergence of bacterial populations and accelerate bacterial adaption to new environments (8, 20, 21). Furthermore, many useful genetic tools derived from Mu have been constructed for various gene manipulations, such as insertional mutagenesis, gene mapping, and DNA cloning (22). These mini-Mu derivatives have been used successfully in bacteria, yeast, and mammalian genomes (23–25). In addition to Mu, phage D3112 has been developed into a powerful tool for studying gene function and regulation in *P. aeruginosa* (26, 27).

Stenotrophomonas maltophilia is a multi-antibiotic-resistant Gram-negative bacterium that is ubiquitous in diverse environments, from soils and plants to hospital settings (28). In addition to their ecological niches, *S. maltophilia* strains are also diverse with respect to their phenotypes and genotypes. Although *S. maltophilia* has been useful in biotechnology as a biocontrol of plant pathogens and for bioremediation, it is becoming an emerging global opportunistic pathogen (29–30). Temperate phages have been proposed to play an important role in *S. maltophilia* pathogenicity, as in other pathogens. Of the *S. maltophilia* phages characterized to date, temperate phages have been reported to carry an antibiotic-resistance gene and potential virulence genes. The phage DLP4 carries the *folA* gene, which encodes the dihydrofolate reductase (DHFR, FolA) responsible for resistance to the antibiotic trimethoprim (31). Phage S1 encodes the GspM and VirE proteins (32), and phage DLP5 is predicted to have six potential virulence factors, including serine protease XkdF, SAM methyltransferase, rhomboid membrane protein, PIG-L family deacetylase, WecE, and an SPFH domain-containing protein (33).

In this work, a novel temperate phage was isolated and characterized in *S. maltophilia*. ϕ SHP3 belongs to the *Siphoviridae* family and is a B3-like transposable phage. To our knowledge, ϕ SHP3 is the only transposable phage isolated from *S. maltophilia* to date. To understand the genetic control of the lytic-lysogenic switch in ϕ SHP3, we

FIG 1 Transmission electron micrograph of ϕ SHP3 negatively stained with uranyl acetate. Scale bar represents 100 nm.

determined the repressor genes and defined the promoters. We also purified the product of gene 3 and identified its exonuclease activity *in vitro*.

RESULTS

Morphology and host range. The phage tail-like bacteriocin produced by *S. maltophilia* is named maltocin (34). During screening of maltocins, we found that *S. maltophilia* strain c31 simultaneously produced a maltocin and a phage designated maltocin c31 and ϕ SHP3. To facilitate the characterization of ϕ SHP3, the deletion mutant strain c31 Δ s was constructed by deleting the 1.2-kb tail sheath gene of maltocin c31. ϕ SHP3 was isolated from strain c31 Δ s to perform all subsequent experiments in this study. Transmission electron microscopy revealed that ϕ SHP3 had a flexible tail of 180.4 ± 4.3 nm in length and an icosahedral head of 58.8 ± 2.6 nm in diameter (Fig. 1), suggesting that ϕ SHP3 belongs to the *Siphoviridae* family.

ϕ SHP3 infected 20/83 *S. maltophilia* strains tested and did not infect other examined species. In this study, *S. maltophilia* strain S16 was used as the sensitive strain to investigate the characteristics of ϕ SHP3. ϕ SHP3 forms irregular plaques of different sizes on lawns of strain S16. Successful ϕ SHP3 infection and lysis of strain S16 were confirmed using PCR (data not shown).

General features of the ϕ SHP3 genome. As shown in Fig. 2, the ϕ SHP3 genome was insensitive to RNase A but susceptible to DNase I, indicating that its genetic material is DNA. Exonuclease EXOIII digestion confirmed that the genomic DNA of ϕ SHP3 is linear. After digestion of the ϕ SHP3 genome with restriction enzymes, several clear bands were observed. However, diffusion in the banding pattern was also observed. The genomic DNA fragments digested with BamHI or KpnI were cloned into pUC18 and sequenced. The gap sequences were filled using genome walking PCR. Genomic sequence assembly was performed with DNAMAN and NCBI BLASTn.

Similar to other transposable phages, the ends of the consensus sequence displayed a 2-bp 5'-TG-CA-3' inverted repeat. Therefore, the consensus sequence was designated the ϕ SHP3 genome, which was 37,611 bp in length and with a GC content of 65.3%. BLASTn searches revealed that the ϕ SHP3 genome has no similarity with the genomic sequences of Mu-like phages but shares significant sequence identity with two fragments of the *S. maltophilia* strain FDAARGOS_325 chromosome (GenBank no. [CP022053](#)). Its nucleotide (nt) identities with DNA fragments from nt 1108219 to 1070729 (37,491 bp) and nt

FIG 2 Analysis of ϕ SHP3 genomic DNA. Genomic DNA was digested by various enzymes at 37°C for 1 h. The electrophoresis samples were as follows: M, λ DNA/HindIII marker; 1, ϕ SHP3 genomic DNA (gDNA); 2, gDNA/DNase I; 3, gDNA/RNase A; 4, gDNA/EXOIII; 5, plasmid pUC18; 6, pUC18/EXOIII.

4010367 to 4049624 (39,258 bp) were 91.2% and 66.5%, respectively, suggesting that the sequences in strain FDAARGOS_325 might correspond to SHP3-like prophages. As a landmark of integration by conservative transposition, a 5-bp (Mu-like) or 6-bp (B3-like) direct repeat of host DNA flanking the ends has been detected in most transposable prophages (8, 17, 35). We detected the SHP3 prophage flanking sequence by TAIL-PCR with the chromosomal DNA of strain c31 as the template. The results indicated that the 6-bp direct repeat flanking the prophage ends was 5'-CGCAGC-3' in strain c31, different from 5'-GCTGGG-3' and 5'-GCCATC-3' repeats in strain FDAARGOS_325.

Fifty-one genes were discovered in the ϕ SHP3 genome via ORF finder and NCBI BLASTP (Table S1 in the supplemental material). These genes can be classified into five putative genetic modules: DNA replication, regulation, cell lysis, virion morphogenesis (including DNA packaging and head/tail morphogenesis), and DNA modification. The modular configuration of ϕ SHP3 was very similar to that of phage B3, except for the DNA modification module (Fig. 3A). Genes are located on two arms, where genes 1 to 19 are transcribed leftward and the other genes are transcribed rightward. In addition, most putative proteins of ϕ SHP3 are homologs of the corresponding proteins in phage B3. These similarities led us to propose that ϕ SHP3 has only two phases of transcription, early and late, similar to the B3 group phages.

Early genes. Genes 1 to 17 constitute the primary early operon of ϕ SHP3. Genes 1, 2, 15, and 16 encode Mor, GemA, TnpB, and TnpA, respectively, which are widely distributed throughout transposable phages (13). DNA replication module genes encode TnpA and TnpB (8, 18). TnpA, an integrase, binds to phage attachment sites and mediates integration of a DNA copy of the phage genome into the host chromosome (36, 37). TnpB, an AAA+ ATPase, is essential for the selection of insertion sites and is involved in transposition by allosterically activating TnpA (38). TnpA and TnpB of B3-like phages are apparently different from those of phage Mu. The gene 15 and 16 products (gp15 and gp16) are homologous to the TnpB and TnpA proteins of B3-like phages, respectively (Table S1). Gp15 is also an ExeA-like protein that is proposed to serve as a potential virulence factor for pathogenesis. The TnpA protein gp16 carries an rve integrase core domain (pfam00665) that is related to the *Staphylococcus aureus* transposon Tn552 (39). Sequence comparison showed that the nucleotides of the two ends among ϕ SHP3, B3, and BcepMu were quite consistent and exhibited imperfect direct repeats (Fig. 3B). The TnpA-binding regions in Tn522 are 23-nt direct repeats of the two ends (40). Thus, we speculate that the end direct repeats may be the TnpA-binding sites in B3-group phages.

FIG 3 Comparison of genomic structures (A) and ends (B) among ϕ SHP3, B3, and BcepMu. (A) The same color indicates proteins with similar or identical functions. The genomic sequences of B3 (AF232233) and BcepMu (AY539836) were extracted from GenBank. (B) The left and right ends of the genomes are shown aligned with each other. The asterisks indicate the consensus nucleotides in the TnpA-binding sites. All sequences are displayed from 5' to 3'.

Except for genes 1, 2, 15, and 16, the only other early gene that could be proposed to have a function was gene 3. The gene 3 product (gp3) was predicted as an exonuclease due to its RdgC superfamily conserved domain. In *Escherichia coli*, RdgC is an exonuclease required for growth in recombination-deficient exonuclease-depleted strains (41). To assess the function of gp3, gene 3 was cloned into the pET26b(+) plasmid and expressed in *E. coli* BL21(DE3). Purified gp3-His was incubated with plasmid pUC18 and the linear DNA fragment at 30°C (Fig. 4). As the reaction time increased, more DNA fragment degradation occurred until it was completely abolished at 60 min, indicating that gp3-His exhibits exonuclease activity *in vitro*. Therefore, we proposed that gp3 might play a role in recombination similar to RdgC in *E. coli* (42). Gp4 has a DUF2528 domain of unknown function, showing low similarity with the putative single-stranded DNA binding protein of *Escherichia* phage JLK-2012 and gp03 of phage BcepMu. Of the remaining predicted proteins in this region, eight (gp6, gp7, and from gp9 to gp14) had no detectable homologs, while the remaining three (gp5, 8, and 17) had phage homologs of unknown function.

Late genes. Genes 22 to 49 were rightward transcribed and proposed to be expressed late. The intergenic region between genes 21 and 22 was 202 nt and predicted to possess a typical sigma 70 promoter, suggesting that genes 21 and 22 should belong to two operons. Lysis genes are often positioned upstream of morphogenetic genes and generally feature a well-defined holin-endolysin system (43). According to the location of their encoding genes, the proteins from gp22 through gp24 seem to constitute the lysis system of ϕ SHP3. None of the endolysin-related conserved domains was found in gp22, but gp22 exhibits approximately 40% amino acid identity with putative endolysins of two *Salmonella* phages, SSU5 and Chi (AFL46954 and AFO71148). TMHMM prediction revealed two transmembrane domains in the C terminus, implying that gp22 might be a novel lytic enzyme. No significant matches to homologs were found for either gp23 or gp24. However, a transmembrane domain was present in the N terminus of gp23. A +1 translation frameshift of gp23 encodes an

FIG 4 The protein gp3 exhibits exonuclease activity *in vitro*. (A) SDS-PAGE of the purified gp3-His protein. (B) Agarose gel electrophoresis of gp3-His digested DNA samples. Purified gp3-His (1 μ M) was incubated with plasmid pUC18 (lanes 1 to 4, 0.25 μ g) and the linear DNA fragment (ORF3) amplified by PCR with the primers ORF3-F/NdeI and ORF3-R/XhoI (lanes 5 to 8, 0.45 μ g) at 30°C, respectively. The reaction time was marked in each lane as follows: 1 and 5, 0 min; 2 and 6, 20 min; 3 and 7, 40 min; 4 and 8, 60 min; M, DL5000 marker.

80-aa protein, which was named RZ1 with a P-rich (12P/60 amino acids [aa]) and 20-aa signal sequence. Correspondingly, the gp23 protein was predicted to be an RZ-like protein. RZ and RZ1 are spanins. In addition to holin-antiholin and endolysin, spanins have been shown to be required for outer membrane disruption for most phage release from Gram-negative hosts (44). Since no transmembrane domain was found in gp24, we proposed that gene 24 might not encode a holin. The putative holin gene may be located in another place within the genome or may not be necessary for ϕ SHP3 lysis.

Proteins gp25 through gp37 are involved in DNA packaging and head morphogenesis. In this module, the first identified products were gp27 and gp28, which were predicted to be the small and large subunits of terminase, with DUF3486 and Terminase_1 superfamily conserved domains, respectively. Otherwise, gp29 showed 57% identity with the portal protein of phage B3, which is an ortholog of Mu gp29. Similar to other tailed phages, gp30, following the portal protein, is a phage Mu gpF-like protein that is necessary for head morphogenesis in double-stranded DNA phages (12). Gp31 is an ortholog of Mu gpG, which is proposed to be involved in tail completion and stable head joining (45). Gp32 is a putative capsid protease, and its N-terminal sequence shares similarity with B3ORF36 (protease). Although gp33 has no homology with B3ORF36Z, it was predicted to be the scaffold protein of ϕ SHP3. Similar to B3ORF36Z and MuZ, gp33 is entirely embedded within a protease in the same reading frame. Gp34 contains a Phage_cap_E superfamily conserved domain and is the major capsid protein of ϕ SHP3.

The tail module of ϕ SHP3 consists of genes 38 through 48. In this module, the largest encoded protein is the gene 40 product (1,178 aa), which is the predicted tail tape measure protein. According to its gene location, the gp39 protein is an ortholog of phage λ gpG. The translation frameshift of gene 39 from 24468 to 25108 led to homology with gpGT (gp39'), which contained a potential slippery sequence, 24842 T TTT TTC 24848. However, proteins gp39 and gp39' shared very low homology with proteins gpG and gpGT in other tailed phages (46). Proteins gp35, gp36, and gp37 were hypothetical proteins. Whether they are involved in head or tail morphogenesis requires further analysis.

DNA modification gene. The 147-bp interspace between genes 48 and 49 suggested that gene 49 is likely transcribed separately. The product of gene 49 had a DNA adenine methylase (dam) conserved domain. However, ϕ SHP3 had no Com-like translational regulator, which is present in B3 and Mu. The DNA modification module of ϕ SHP3 only contained this single methyltransferase gene.

Lysogenic-lytic control switch. The lytic-lysogenic switch of ϕ SHP3 was proposed to be composed of two sets of genes that read in opposite directions. One set comprised genes 18 and 19, and the other set was composed of genes 20 and 21. Gene 20 encodes an HTH_XRE superfamily protein. The products of genes 18 and 19 contained the N-terminal regions of the HTH_IcLR and HTH_XRE conserved domains, respectively. They had no detectable homologs in GenBank except gp18, which had low homology with the hypothetical protein B3ORF14 ([AAQ13932](#)).

To discern whether they are effectors of the lytic-lysogenic control switch of ϕ SHP3, their coding sequences were cloned into the pBTP3 plasmid and transformed into the c31 Δ s strain. Experiments showed that overexpression of gene 19 improved the phage titer by approximately 100-fold, while overexpression of gene 20 resulted in a 100-fold reduction. However, the effects of gp18 and gp21 on the phage titer were not obvious (Fig. 5A). These results indicated that gp19 and gp20 are key elements in the lytic-lysogenic switch of ϕ SHP3, like Ner and Repc of Mu (47).

Start codon of gene 20. Two mutations were constructed to verify the translation start codon of gene 20. Based on the plasmid pBTP3-*orf* 20, pBTP3-*orf* 20-GTA was constructed by changing the predicted translation start codon GUG to GUA by a point mutant. In pBTP3-*orf* 20-ATG, gene 20 was translated from AUG after deletion of 27 nucleotides from its 5' terminus (Fig. 5B). Plasmids were transformed into strain c31 Δ s. The supernatant of various recombinant c31 Δ s isolates was spotted onto the lawn of strain S16 (Fig. 5C). Only strain c31 Δ s harboring pBTP3-*orf* 20 exhibited sharply reduced antibacterial activity. Both the point mutant and the deletion mutant displayed no difference from the control, indicating that gp20 markedly depressed the production of ϕ SHP3.

In Mu and D3112, the c repressor not only controls phage entry into the lysogenic state but is also an exclusion protein that resists superinfection (12, 48). To detect whether gp20 exerted immune functions, the above three plasmids were transformed into strain S16. Superinfection immunity assays were performed against various recombinant S16 strains using ϕ SHP3. As shown in Fig. 5C, ϕ SHP3 formed clear plaques on S16 strains with each of the pBTP3, pBTP3-*orf* 20-GTA, and pBTP3-*orf* 20-ATG plasmids, whereas no such plaques were formed on strain S16 with pBTP3-*orf* 20. This demonstrated that the expression of gene 20 in strain S16 could confer immunity to ϕ SHP3 infection. These results confirmed that gp20 is a lysogeny repressor, its translation start codon is the predicted GUG, and the first nine amino acids are required for its function.

The regulatory region between gene 19 and gene 20. Reverse transcriptase PCR (RT-PCR) showed that the first operon of rightward transcription consisted of genes 20 and 21 driven by promoter P_{20r} and the second operon was driven by promoter P_{22r}. Gene 17 is transcribed together with genes 18 and 19 and, seemingly, the leftward transcription genes compose one operon driven by promoter P₁₉ (Fig. 6). The untranslated region between genes 19 and 20 should represent an important regulatory region controlling both leftward transcription beginning with gene 19 and rightward transcription of gene 20. The transcription initiation site for both genes 19 and 20, as determined by the RNA ligase mediated-RACE (RLM-RACE) method, was C, located –55 and –35 bp upstream of their start codons, respectively (Fig. 7). Then, two typical bacterial promoters were predicted in opposite directions. One is a typical bacterial promoter for gene 19, with a –35 hexamer (TTGCAA) separated by 16 nt from a –10 hexamer (TTAGGA). The other typical bacterial promoter is oriented to initiate gene 20 transcription with a –35 hexamer (TGTAAG) located 16 bp upstream of a –10 hexamer (TCCGGA) (Fig. 7). The high similarity between these two promoters to consensus promoter sequences (–35 TTGACA, 16- to 18-nt spacer, and –10 TATAAT) suggests they are directly recognized by host RNA polymerase, efficiently driving leftward and rightward transcription of the operons (49).

In addition, two typical transcription factor binding boxes in two directions were identified by Softberry BPROM (Fig. 7), including a typical LexA box (AAACCACA) located upstream of the gene 19 promoter. At the rightward transcription, two CpxR

FIG 5 Detection of the roles of putative regulators. (A) Phage titration of each recombinant *S. maltophilia* c31Δs strain harboring the corresponding plasmid. Vector, pBTP3; gp18, pBTP3-*orf18*; gp19, pBTP3-*orf19*; gp20, pBTP3-*orf20*-GTA; gp21, pBTP3-*orf21*. Data are represented as the means $\pm$ SD with three replicates. All error bars represent standard deviation (SD). (B) The 5' terminal nucleotides of gene 20 and its mutants. (C, left) The supernatant of strain c31Δs with various recombinant plasmids was spotted with double dilution on the lawn of strain S16. (C, right) ϕ SHP3 ($\sim 10^{10}$ PFU/ml) was spotted by 10-fold dilution on the lawn of strain S16 with various recombinant plasmids. Abbreviations in the figure represent the following: *orf20*, pBTP3-*orf20*; *orf20* (GTA), pBTP3-*orf20*-GTA; *orf20* (ATG), pBTP3-*orf20*-ATG.

boxes (G/AGTGAAA) were found, one of which overlapped with the putative -35 hexamer region, while the other was near the transcriptional initiation site of gene 20. This suggests that LexA and the CpxR/CpxA two-component system should be involved in the control of the genetic switch in ϕ SHP3.

Interaction of the regulators and the promoters. Using the β -galactosidase gene (*lacZ*) as the reporter gene, the interaction of the four predicted transcriptional regulators (gp18 to gp21) and gp1 (Mor-like protein) on these three promoters P_{19} , P_{20} , and P_{22} was analyzed (Fig. 7). The promoters were cloned upstream of the *lacZ* gene, while various regulators were expressed in the same vector. The promoter activity was characterized by β -galactosidase activity. Remarkably, the activities of the three promoters differed significantly. The activity of P_{19} was approximately 130-fold stronger than that of the promoter P_{20} , while the activity of P_{22} was very low, just 8% of the P_{20} activity. The expression of gene 1 activated the promoter P_{22} by four fold. The expression of gene 19 caused the promoter activities of P_{19} and P_{20} to decrease by approximately 60%. Moreover, the expression of gene 20 caused the promoter activity of P_{19} to decrease sharply, such that only 1.59% of the original activity was retained. The results showed that the lysogenic repressor gp20 could repress P_{19} promoter activity, and the lytic repressor gp19 could repress the promoters P_{20} and P_{19} . However, gp18 and gp21 did not display any effects on any promoters tested.

FIG 6 The operon identification of genes 17 through 22 by the PCR method. Each lane 1 was the negative control with no template. The template in each lane 2 was *S. maltophilia* c31 genomic DNA, which was used as the positive control. The template in each lane 3 was the cDNA of *S. maltophilia* c31. The two primer sequences in each pair correspond to the sequences of two adjacent genes. The primer pairs used in each lane were as follows (see Table S2 in the supplemental material): TC17-18-F and TC17-18-R in lanes TC17-18; TC18-19-F and TC18-19-R in lanes TC18-19; TC20-21-F and TC20-21-R in lanes TC20-21; TC21-22-F and TC21-22-R in lanes TC21-22. M was the marker DL2000.

Phylogenetic analyses. Phylogenetic analyses were conducted based on the deduced amino acid sequences of the large subunit of terminase, portal, major capsid, and tape measure proteins (Fig. 8). The ϕ SHP3 genome, like other phages, has a mosaic structure. Tape measure proteins of transposable phages from the same

FIG 7 The interaction of regulators and promoters. (A) Diagram of the locations of putative regulator genes and promoters in the genome, as well as interregional sequence characteristics between gene 19 and gene 20. Boldface font marks the translation initiation codons, and +1 indicates the transcription start sites. The sequences in the blue and red boxes represent the predicted CpxR-box and LexA box sequences, respectively. (B) Schematic diagram of β -galactosidase reporter constructs. (C) The β -galactosidase activities of the recombinant S16 strains were detected when the *lacZ* gene was driven by various promoters and a predicted regulator was expressed simultaneously. The vectors represent plasmids pBCZIP19, pBCZIP20, and pBCZIP22, respectively, in which no regulator gene was cloned.

FIG 8 Phylogenetic analysis based on the amino acid sequences of the terminase large subunit (A), portal protein (B), major capsid protein (C), and tail tape measure protein (D). The neighbor-joining trees were constructed using MEGA version X with the phage λ sequence as the root. All reference sequences were collected from the NCBI database. The scale bar represents 0.2 substitutions per site. The bootstrap values were based on 10,000 replicates.

species were more likely to be clustered together, but ϕ SHP3 was an independent branch, as it is the only transposable phage of *Stenotrophomonas*. Although each of the other three protein phylogenetic trees could be divided into two groups, each phage was located on different branches, indicating that their encoding genes originated from more complex gene transfer events. Except for the tree based on the tape measure protein, ϕ SHP3 and B3 were clustered in the same group in the other three phylogenetic trees. In particular, the portal protein of ϕ SHP3 displayed high similarity to the corresponding protein of phage B3 (B3ORF32). Phylogenetic analyses showed that ϕ SHP3 was more highly related to phage B3 than to phage Mu.

DISCUSSION

The ϕ SHP3 genome. This study revealed the complete genomic sequence and comparative phylogeny of a novel B3-like transposable phage produced by *S. maltophilia* strain c31. The ϕ SHP3 genome encodes 51 putative proteins, 14 of which had no homologs in the database, while other proteins showed quite low but significant sequence similarity with other transposable phages. ϕ SHP3 and phage B3 exhibited the same genome organization, except for the DNA modification module, whereas their genomic sequences displayed no similarity. Moreover, some genes that are shared among transposable phages, such as *gam* and *com*, were not identified in the ϕ SHP3 genome. Gam is quite conserved in pathogens. Furthermore, it is an important factor for transposable phage Mu, as Gam protects linear double-stranded DNA ends from exonuclease degradation (50). However, no homolog with Mu Gam was detected

in B3-like genomes, whether in B3 or in BcepMu. Phage B3 has the corresponding *com-dam* modified gene cluster, located within the tail gene region approximately 12 kb from the right end (14). ϕ SHP3 has only a *dam* gene located at the extreme right end of the SHP3 genome, but no *com* gene. Moreover, some transposable phages, especially B3-like groups, did not have either of these two genes. This implied that methylation is not necessary in transposable phages.

RdgC protein. Compared to phage B3, the ϕ SHP3 genome harbored additional accessory genes, most of which are unique, including gene 3. The product of gene 3 (gp3) has an RdgC superfamily conserved domain and exhibits exonuclease activity, suggesting that gp3 is an RdgC-type exonuclease. In the *Vibrio* prophage Vp58.5 and several *Salmonella* phages, *rdgC*-like genes are interspersed among the virion assembly genes and are likely parts of evolutionarily mobile accessory genes (51, 52). However, their functions are unknown. In *E. coli*, RdgC regulates RecA function and plays a role in recombination and replication fork repair (42, 53). Gp4 is a putative single-stranded DNA binding protein. Genes 3 and 4 might correspond to *exo* and *bet* in phage λ to constitute the homologous recombination system.

Lytic-lysogenic switch regulation. Information on the lytic-lysogenic switch regulation of transposable phages is very limited. In phage Mu, the ratio of Repc and Ner repressors determines whether the phage enters the latent or lytic cycle. Most temperate phages have two master early regulators, one repressor for lysogeny and the other for the lytic cycle (54). Gp19 and gp20 were analogous to the Mu phage Ner and Repc repressors, but they showed no homology with any phage proteins. Interestingly, like Mu Repc, which blocks its own expression, the Ner-like protein gp19 in ϕ SHP3 represses its own promoter to reduce its own expression. Proteins gp18 and gp21 remain without assigned functions.

In the intergenic region between genes 19 and 20, sequence analysis revealed LexA and CpxR boxes. Moreover, ϕ SHP3 production could be induced by mitomycin C treatment, which will cause DNA damage and trigger the bacterial SOS response. The SOS system is an extensive stress response system in bacteria (55). LexA is the master repressor of the bacterial SOS response (56). Therefore, we speculate that the lytic-lysogenic switch of ϕ SHP3 should be regulated by the host SOS system. The Cpx pathway controls part of the envelope stress response system, type III secretion, pilus assembly, adherence, and biofilm development. Thus, some pathogenic bacteria require the Cpx pathway to invade their hosts (57, 58). This suggests that ϕ SHP3 may be an important effector for its host strain to exist in diverse environments, especially when invading humans and animals. Except for Mu, no information about switch regulation of transposable phages is known, although the regulatory region of the B3 switch contains two promoters driving rightward and leftward transcription. Softberry BPROM analysis revealed no binding sites for any known regulators. Therefore, the regulatory mechanism of the ϕ SHP3 lytic-lysogenic switch is distinct from that of Mu and B3. Further studies are required to reveal the molecular mechanism by which host signals and phage regulators control the lytic-lysogenic switch of ϕ SHP3 in various environments.

In conclusion, *S. maltophilia* is ubiquitous in nature and clinical environments. In the past decade, *S. maltophilia* has become an important opportunistic pathogen in humans (29, 30). To date, ϕ SHP3 is the only transposable phage that has been isolated from *Stenotrophomonas*, although only gp15 with an ExeA conserved domain was proposed to serve as a potential virulence factor for pathogenesis. Similar to other transposable phages, the lysogenic properties should potentially lead ϕ SHP3 to transfer more genes involved in bacterial pathogenicity.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Except for the *S. maltophilia* strains used to test the host spectrum of ϕ SHP3, the other bacterial strains used in this study are the same as those used for antimicrobial spectrum determination of endolysin P28 (59). *S. maltophilia* strains were cultivated at 30°C in LB medium, and antibiotics were added to LB medium as required. Primers used in this study are listed in Table S2. Using the chromosomal DNA of strain c31 as a template, a 1.2-kb DNA fragment was amplified with PCR primers orf17-F and orf17-R, which were designed according to the nucleotide sequences of

maltocin P28 (GenBank no. [KC787694](#)). The cloned DNA exhibited 97.8% nucleotide identity with the sheath gene of maltocin P28 (34). Sequence identity and conserved domain analysis indicated that the cloned DNA encoded the sheath protein of maltocin c31. Subsequently, two pairs of PCR primers (TC orf16-F and Sheath U-R, Sheath D-F and Sheath D-R) were designed according to sequences of the nucleotide sequences of maltocin P28 to clone the sequences upstream and downstream of the sheath gene. Then, a 2,607-bp DNA fragment was obtained and showed 90.5% nucleotide identity with the DNA fragment of the maltocin P28 genetic cluster from 12479 to 15084. Deletion of the tail sheath gene of maltocin c31 was performed as described previously (34). After the 1.2-kb tail sheath gene of maltocin c31 was knocked out, the deletion mutant strain c31Δs was constructed.

Phage isolation and purification. LB medium (100 ml) was inoculated with 1% *S. maltophilia* c31Δs and the culture was cultivated for approximately 3 h at 37°C. When the optical density at 600 nm (OD₆₀₀) value of the culture reached 0.5, mitomycin C (MMC) was added at a final concentration of 0.5 μg/ml. After cultivating for 20 h at 37°C, the culture was centrifuged at 6,000 × *g* for 10 min. The supernatant was collected, filtered through a 0.22-μm membrane, mixed with polyethylene glycol 8000 (7% [wt/vol] final concentration) and NaCl (3% [wt/vol] final concentration) and incubated overnight at 4°C. After centrifugation at 10,000 × *g* for 30 min at 4°C, the phage pellet was gently resuspended in 2 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and the phages were then purified on a CsCl gradient (1.35 g/ml) by ultracentrifugation at 200,000 × *g* for 18 h at 4°C (Optima L-100 XP Ultracentrifuge, Beckman Coulter). The phage band was collected and dialyzed twice against TE buffer.

Electron microscopy. Phage suspension (10 μl) was added dropwise onto a copper grid and negatively stained with 2% aqueous uranyl acetate for 10 min. Transmission electron micrographs were obtained using a JEM-2100HC transmission electron microscope (JEOL, Japan) at an accelerating voltage of 120 kV. Phage size was calculated from at least 20 particles.

Host range analysis. To investigate the host range of ϕSHP3, plaque assays were performed on various bacterial strains, including 83 *S. maltophilia* strains, three *Pseudomonas* strains, two *Aeromonas* strains, and one strain each of *Escherichia coli* and the genera *Klebsiella*, *Proteus*, *Salmonella*, *Shigella*, and *Xanthomonas* (59). The host range was determined by adding 2.5 μl of a diluted phage suspension (~10⁷ PFU/ml) dropwise onto the surface of double-layer agar plates inoculated with the bacterial strain of interest. Plates were incubated at 30°C for up to 2 days, and plaque formation was assessed repeatedly throughout this period. All assays were performed in triplicate.

Phage DNA extraction and genomic analysis. To remove free DNA and RNA, purified phage samples were digested at 37°C for 1 h in DNase I and RNase A (TaKaRa) at a final concentration of 50 μg/ml. Solutions were treated at 75°C for 15 min and then incubated with proteinase K at a final concentration of 40 μg/ml at 37°C for 1 h. After incubation, solutions were extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1), after which the solutions were precipitated using 0.3 M sodium acetate (pH 7.0) and two volumes of ice-cold 100% ethanol. After overnight incubation at -20°C, DNA was collected by centrifugation (Eppendorf Centrifuge 5424R), successively washed twice with 70% ethanol, and then air-dried. The genomic DNA of ϕSHP3 was resuspended in TE buffer and stored at -20°C.

Phage genomes were digested by various enzymes. The BamHI- and KpnI-digested fragments were cloned into pUC18 and sequenced. The obtained sequences were analyzed and assembled primarily using the BLASTn, BLASTp and DNAMAN programs (60). Furthermore, the ORF Finder online server (<https://www.ncbi.nlm.nih.gov/orffinder/>) was used to identify putative open reading frames (ORFs) in the assembly sequence. Putative gene functions were assigned using BLAST searches, and pairwise comparison with hidden Markov models provided by the HHPred server TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) analyzing the number of transmembrane helices and relevant statistics (61, 62). Promoter analysis was performed using Softberry BPRM (<http://www.softberry.com/>).

The amino acid sequences of terminase large subunits, portal, major capsid, and tape measure proteins from ϕSHP3 and other transposable phages were used to construct neighbor-joining phylogenetic trees with MEGA X and 1,000 bootstrap replications. For use in the phylogenetic analysis, the amino acid sequences of these four proteins from closely related phages were retrieved from GenBank.

TAIL-PCR method. To ascertain the flanking sequences of prophage in host chromosome, TAIL-PCR was performed as previously described (63). TAIL-PCR is commonly composed of three nested amplifications. The primers used in each amplification reaction consisted of left or right end specific (SP) primers, corresponding to the border sequence of the phage genome, and arbitrary degenerate (AD) primers (Table S2). All TAIL-PCR products were separated on agarose gels and cloned into the pMD19-T vector for sequencing.

Lytic-lysogenic switch analysis. The pBTP3 plasmid was constructed based on pBBR1MCS (64), in which *rrnB* terminators and the *orf3* core promoter (P_{orf3}) of maltocin P28 replaced the *lac* promoter. First, genes 18, 19, 20, and 21 were amplified by PCR and cloned into pBTP3 under the control of P_{orf3}. Then, the recombinant plasmids pBTP3-*orf18*, pBTP3-*orf19*, pBTP3-*orf20*, and pBTP3-*orf21* were transformed into strain c31Δs. Each recombinant strain was incubated at 30°C for 12 h. Following centrifugation, the supernatant was diluted to the appropriate concentration. One hundred microliters of 10-fold diluted supernatant was mixed with 50 μl of overnight strain S16 culture and immediately plated for phage titration using the double-layer agar plate method. All assays were performed in triplicate.

Determination of the start codon of gene 20. To verify the start codon of gene 20, two mutants were constructed based on pBTP3-*orf20*, one where the predicted initiation codon GUG was changed to GUA (pBTP3-*orf20*-GTA) and the other where the coding sequences for the first nine amino acids of gp20 were deleted, which initiated translation at AUG (pBTP3-*orf20*-ATG). Similar to pBTP3-*orf20*, these

two plasmids and the vector pBTP3 were transformed into strain c31Δs. After incubation at 30°C for 8 h, 2.5 μl of 2-fold diluted supernatant was placed dropwise onto the surface of double-layer agar plates inoculated with strain S16. Plates were incubated at 30°C for up to 2 days.

Furthermore, the plasmids pBTP3-*orf20*, pBTP3-*orf20*-GTA, and pBTP3-*orf20*-ATG were also transformed into strain S16. To determine whether the expression of gene 20 conferred superinfection exclusion, 2.5 μl of a diluted ϕSHP3 suspension (~10¹⁰ PFU/ml) was added dropwise onto the surface of double-layer agar plates inoculated with the recombinant S16 strains harboring the different plasmids. Plates were incubated at 30°C for up to 2 days. All assays were performed in triplicate.

Identification of operons and transcription start sites. Overnight cultures were diluted 1:100 in 50 ml fresh LB medium and then cultivated to the mid-exponential phase. The bacterial precipitate was collected, and the total RNA was extracted by the TRIzol method. Then, total RNA was purified and reverse transcribed into cDNA. Using cDNA as the template, PCR was performed with a pair of primers designed according to two different gene sequences. If the sequences of the PCR-amplified DNA fragment were consistent with the corresponding sequence in the ϕSHP3 genome, we proposed that these two genes were in the same operon.

The transcription start sites of genes 19 and 20 were analyzed by the RNA ligase mediated-RACE (RLM-RACE) approach (65). The above total RNA was ligated with T4 RNA ligase for 16 h followed by phenol-chloroform extraction to remove proteins. The circular form of the RNA was subjected to reverse transcription reactions to obtain circular cDNA. Subsequently, outer and inner PCRs were performed using two pairs of suitable nested PCR primers. The products obtained in the inner PCR were separated on agarose gels and cloned into the pMD19-T vector for sequencing.

Promoter activity assay. Plasmid pBCZI was also constructed based on pBBR1MCS (64). First, the *lacZ* gene was cloned into pBBR1MCS by HindIII and XbaI, and *rrnB* terminators (Ter1) were inserted by KpnI and XhoI. Then plasmid pBTP3 was used as a template, *rrnB* terminators (Ter2) and P_{orf3} were amplified to replace the *lac* promoter (P_{lacZ}) with the overlap PCR method. The PCR fragment was cloned into the recombinant plasmid by NcoI and KpnI. In addition, several restriction endonuclease sites (BglII, SmaI, and BamHI) were designed for convenient cloning of target genes. The promoters could insert the upstream *lacZ* gene, and the target genes could clone the downstream promoter P_{orf3} in pBCZI. First, the promoters P₁₉ (101 bp), P₂₀ (128 bp), and P₂₂ (426 bp) were amplified by PCR and inserted upstream of the *lacZ* gene in pBCZI through BglII and KpnI to construct the recombinant plasmids pBCZIP19, pBCZIP20, and pBCZIP22, respectively. Subsequently, five regulator genes, gene 1, gene 18, gene 19, gene 20, and gene 21, amplified by PCR were cloned into these recombinant plasmids through XhoI and HindIII to obtain a series of promoter and protein interaction plasmids. Finally, these constructed plasmids were transformed into *S. maltophilia* S16. The promoter activity was assessed through the measurement of β-galactosidase activity as previously described (66). The assays were performed in triplicate.

Exonuclease activity analysis. Gene 3 (*rdgC*-like gene) was amplified from the genomic DNA of ϕSHP3 by PCR with the primers ORF3-F/NdeI and ORF3-R/XhoI and cloned into pET26b(+) using NdeI and XhoI. The constructed plasmid pET26-*orf3* was transformed into *E. coli* strain BL21(DE3). Then, the gp3-His protein was expressed and purified according to a previously described protocol (59). Subsequently, purified gp3-His was incubated with plasmid pUC18 (0.25 μg), and the above gene 3 DNA fragment (0.45 μg) was amplified by PCR in a 20-μl total volume. The working concentration of gp3-His was adjusted to 1 μM. After incubation at 30°C for different times, the mixtures were separated on agarose gels.

Data availability. The genome sequence of ϕSHP3 was deposited in the GenBank database under accession number [MT872956](https://www.ncbi.nlm.nih.gov/nuclseq/MT872956).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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REFERENCES

1. Hatfull GF. 2015. Dark matter of the biosphere: the amazing world of bacteriophage diversity. *J Virol* 89:8107–8110. <https://doi.org/10.1128/JVI.01340-15>.
2. Manrique P, Dills M, Young MJ. 2017. The human gut phage community and its implications for health and disease. *Viruses* 9:141. <https://doi.org/10.3390/v9060141>.
3. Veasler D, Cambillau C. 2011. A common evolutionary origin for tailed bacteriophage functional modules and bacterial machineries. *Microbiol Mol Biol Rev* 75:423–433. <https://doi.org/10.1128/MMBR.00014-11>.
4. Engelberg-Kulka H, Kumar S. 2015. Yet another way that phage λ manipulates its *Escherichia coli* host: λrexB is involved in the lysogenic-lytic switch. *Mol Microbiol* 96:689–693. <https://doi.org/10.1111/mmi.12969>.
5. Shao Q, Trinh JT, Zeng L. 2019. High-resolution studies of lysis-lysogeny decision-making in bacteriophage lambda. *J Biol Chem* 294:3343–3349. <https://doi.org/10.1074/jbc.TM118.003209>.
6. Obeng N, Pratama AA, Elsas JDV. 2016. The significance of mutualistic phages for bacterial ecology and evolution. *Trends Microbiol* 24:440–449. <https://doi.org/10.1016/j.tim.2015.12.009>.

7. Davies EV, Winstanley C, Fothergill JL, James CE. 2016. The role of temperate bacteriophages in bacterial infection. *FEMS Microbiol Lett* 363:fnw015. <https://doi.org/10.1093/femsle/fnw015>.
8. Toussaint A, Rice PA. 2017. Transposable phages, DNA reorganization and transfer. *Curr Opin Microbiol* 38:88–94. <https://doi.org/10.1016/j.mib.2017.04.009>.
9. Taylor AL. 1963. Bacteriophage-induced mutation in *Escherichia coli*. *Proc Natl Acad Sci U S A* 50:1043–1051. <https://doi.org/10.1073/pnas.50.6.1043>.
10. Toussaint A. 2013. Transposable Mu-like phages in Firmicutes: new instances of divergence generating retroelements. *Res Microbiol* 164:281–287. <https://doi.org/10.1016/j.resmic.2013.01.008>.
11. Cazares A, Mendoza-Hernández G, Guarneros G. 2014. Core and accessory genome architecture in a group of *Pseudomonas aeruginosa* Mu-like phages. *BMC Genomics* 15:1146. <https://doi.org/10.1186/1471-2164-15-1146>.
12. Morgan GJ, Hatfull GF, Casjens S, Hendrix RW. 2002. Bacteriophage Mu genome sequence: analysis and comparison with Mu-like prophages in *Haemophilus*, *Neisseria* and *Deinococcus*. *J Mol Biol* 317:337–359. <https://doi.org/10.1006/jmbi.2002.5437>.
13. Hulo C, Masson P, Le Mercier P, Toussaint A. 2015. A structured annotation frame for the transposable phages: a new proposed family "Saltoviridae" within the *Caudovirales*. *Virology* 477:155–163. <https://doi.org/10.1016/j.virol.2014.10.009>.
14. Braid MD, Silhavy JL, Kitts CL, Cano RJ, Howe MM. 2004. Complete genomic sequence of bacteriophage B3, a Mu-like phage of *Pseudomonas aeruginosa*. *J Bacteriol* 186:6560–6574. <https://doi.org/10.1128/JB.186.19.6560-6574.2004>.
15. Summer EJ, Gonzalez CF, Carlisle T, Mebane LM, Cass AM, Savva CG, LiPuma J, Young R. 2004. *Burkholderia cenocepacia* phage BcepMu and a family of Mu-like phages encoding potential pathogenesis factors. *J Mol Biol* 340:49–65. <https://doi.org/10.1016/j.jmb.2004.04.053>.
16. Wang PW, Chu L, Guttman DS. 2004. Complete sequence and evolutionary genomic analysis of the *Pseudomonas aeruginosa* transposable bacteriophage D3112. *J Bacteriol* 186:400–410. <https://doi.org/10.1128/jb.186.2.400-410.2004>.
17. Toussaint A, Van Gijsegem F. 2018. Extension of the transposable bacterial virus family: two genomic organisations among phages and prophages with a Tn552-related transposase. *Res Microbiol* 169:495–499. <https://doi.org/10.1016/j.resmic.2017.11.002>.
18. Harshey RM. 2014. Transposable phage mu. *Microbiol Spectr* 2:1100602544. <https://doi.org/10.1128/microbiolspec.MDNA3-0007-2014>.
19. Carballo-Ontiveros MA, Cazares A, Vinuesa P, Kameyama L, Guarneros G. 2020. The concerted action of two B3-like prophage genes excludes superinfecting bacteriophages by blocking DNA entry into *Pseudomonas aeruginosa*. *J Virol* 94:e00953-20. <https://doi.org/10.1128/JVI.00953-20>.
20. Davies EV, James CE, Williams D, O'Brien S, Fothergill JL, Haldenby S, Paterson S, Winstanley C, Brockhurst MA. 2016. Temperate phages both mediate and drive adaptive evolution in pathogen biofilms. *Proc Natl Acad Sci U S A* 113:8266–8271. <https://doi.org/10.1073/pnas.1520056113>.
21. O'Brien S, Kümmerli R, Paterson S, Winstanley C, Brockhurst MA. 2019. Transposable temperate phages promote the evolution of divergent social strategies in *Pseudomonas aeruginosa* populations. *Proc Biol Sci* 286:20191794. <https://doi.org/10.1098/rspb.2019.1794>.
22. Toussaint A. 2018. Transposable bacteriophages as genetic tools. *Methods Mol Biol* 1681:263–278. https://doi.org/10.1007/978-1-4939-7343-9_19.
23. Akhverdyan VZ, Gak ER, Tokmakova IL, Stoyanova NV, Yomantas YA, Mashko SV. 2011. Application of the bacteriophage Mu-driven system for the integration/amplification of target genes in the chromosomes of engineered Gram-negative bacteria. *Appl Microbiol Biotechnol* 91:857–871. <https://doi.org/10.1007/s00253-011-3416-y>.
24. Schagen FH, Rademaker HJ, Cramer SJ, van Ormondt H, van der Eb AJ, van de Putte P, Hoebe RC. 2000. Towards integrating vectors for gene therapy: expression of functional bacteriophage MuA and MuB proteins in mammalian cells. *Nucleic Acids Res* 28:e104. <https://doi.org/10.1093/nar/28.23.e104>.
25. Paatero AO, Turakainen H, Happonen LJ, Olsson C, Palomaki T, Pajunen MI, Meng X, Otonkoski T, Tuuri T, Berry C, Malani N, Frilander MJ, Bushman FD, Savilahti H. 2008. Bacteriophage Mu integration in yeast and mammalian genomes. *Nucleic Acids Res* 36:e148. <https://doi.org/10.1093/nar/gkn801>.
26. Darzins A, Casadaban MJ. 1989. Mini-D3112 bacteriophage transposable elements for genetic analysis of *Pseudomonas aeruginosa*. *J Bacteriol* 171:3909–3916. <https://doi.org/10.1128/jb.171.7.3909-3916.1989>.
27. Darzins A, Casadaban MJ. 1989. In vivo cloning of *Pseudomonas aeruginosa* genes with mini-D3112 transposable bacteriophage. *J Bacteriol* 171:3917–3925. <https://doi.org/10.1128/jb.171.7.3917-3925.1989>.
28. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM. 2009. The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nat Rev Microbiol* 7:514–525. <https://doi.org/10.1038/nrmicro2163>.
29. Brooke JS. 2012. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. *Clin Microbiol Rev* 25:2–41. <https://doi.org/10.1128/CMR.00019-11>.
30. Lira F, Berg G, Martínez JL. 2017. Double-face meets the bacterial world: the opportunistic pathogen *Stenotrophomonas maltophilia*. *Front Microbiol* 8:2190. <https://doi.org/10.3389/fmicb.2017.02190>.
31. Peters DL, McCutcheon JG, Stothard P, Dennis JJ. 2019. Novel *Stenotrophomonas maltophilia* temperate phage DLP4 is capable of lysogenic conversion. *BMC Genomics* 20:300. <https://doi.org/10.1186/s12864-019-5674-5>.
32. García P, Monjardín C, Martín R, Madera C, Soberón N, García E, Meana A, Suárez JE. 2008. Isolation of new *Stenotrophomonas* bacteriophages and genomic characterization of temperate phage S1. *Appl Environ Microbiol* 74:7552–7560. <https://doi.org/10.1128/AEM.01709-08>.
33. Peters DL, Dennis JJ. 2018. Complete genome sequence of temperate *Stenotrophomonas maltophilia* bacteriophage DLP5. *Genome Announc* 6:e00073-18. <https://doi.org/10.1128/genomeA.00073-18>.
34. Liu J, Chen P, Zheng C, Huang YP. 2013. Characterization of maltocin P28, a novel phage tail-like bacteriocin from *Stenotrophomonas maltophilia*. *Appl Environ Microbiol* 79:5593–5600. <https://doi.org/10.1128/AEM.01648-13>.
35. Haapa-Paananen S, Rita H, Savilahti H. 2002. DNA transposition of bacteriophage mu. A quantitative analysis of target site selection in vitro. *J Biol Chem* 277:2843–2851. <https://doi.org/10.1074/jbc.M108044200>.
36. Mizuuchi K. 1992. Transpositional recombination: mechanistic insights from studies of mu and other elements. *Annu Rev Biochem* 61:1011–1051. <https://doi.org/10.1146/annurev.bi.61.070192.005051>.
37. Savilahti H, Mizuuchi K. 1996. Mu transpositional recombination: donor DNA cleavage and strand transfer in trans by the Mu transposase. *Cell* 85:271–280. [https://doi.org/10.1016/S0092-8674\(00\)81103-4](https://doi.org/10.1016/S0092-8674(00)81103-4).
38. Mizuno N, Dramicanin M, Mizuuchi M, Adam J, Wang Y, Han YW, Yang W, Steven AC, Mizuuchi K, Ramon-Maiques S. 2013. MuB is an AAA+ ATPase that forms helical filaments to control target selection for DNA transposition. *Proc Natl Acad Sci U S A* 110:E2441–E2450. <https://doi.org/10.1073/pnas.1309499110>.
39. Rowland SJ, Dyke KG. 1990. Tn552, a novel transposable element from *Staphylococcus aureus*. *Mol Microbiol* 4:961–975. <https://doi.org/10.1111/j.1365-2958.1990.tb00669.x>.
40. Rowland SJ, Sherratt DJ, Stark WM, Boocock MR. 1995. Tn552 transposase purification and in vitro activities. *EMBO J* 14:196–205. <https://doi.org/10.1002/j.1460-2075.1995.tb06990.x>.
41. Ryder L, Sharples GJ, Lloyd RG. 1996. Recombination-dependent growth in exonuclease-depleted recBC sbcBC strains of *Escherichia coli* K-12. *Genetics* 143:1101–1114. <https://doi.org/10.1093/genetics/143.3.1101>.
42. Briggs GS, McEwan PA, Yu J, Moore T, Emsley J, Lloyd RG. 2007. Ring structure of the *Escherichia coli* DNA-binding protein RdgC associated with recombination and replication fork repair. *J Biol Chem* 282:12353–12357. <https://doi.org/10.1074/jbc.C700023200>.
43. Fernandes S, São-José C. 2018. Enzymes and mechanisms employed by tailed bacteriophages to breach the bacterial cell barriers. *Viruses* 10:396. <https://doi.org/10.3390/v10080396>.
44. Cahill J, Young R. 2019. Phage lysis: multiple genes for multiple barriers. *Adv Virus Res* 103:33–70. <https://doi.org/10.1016/bs.aivir.2018.09.003>.
45. Grundy FJ, Howe MM. 1985. Morphogenetic structures present in lysates of amber mutants of bacteriophage Mu. *Virology* 143:485–504. [https://doi.org/10.1016/0042-6822\(85\)90388-5](https://doi.org/10.1016/0042-6822(85)90388-5).
46. Xu J, Hendrix RW, Duda RL. 2004. Conserved translational frameshift in dsDNA bacteriophage tail assembly genes. *Mol Cell* 16:11–21. <https://doi.org/10.1016/j.molcel.2004.09.006>.
47. Ranquet C, Toussaint A, de Jong H, Maenhaut-Michel G, Geiselmann J. 2005. Control of bacteriophage mu lysogenic repression. *J Mol Biol* 353:186–195. <https://doi.org/10.1016/j.jmb.2005.08.015>.
48. Salmon KA, Freedman O, Ritchings BW, DuBow MS. 2000. Characterization of the lysogenic repressor (c) gene of the *Pseudomonas aeruginosa* transposable bacteriophage D3112. *Virology* 272:85–97. <https://doi.org/10.1006/viro.2000.0341>.

49. Lonetto M, Gribskov M, Gross CA. 1992. The sigma 70 family: sequence conservation and evolutionary relationships. *J Bacteriol* 174:3843–3849. <https://doi.org/10.1128/jb.174.12.3843-3849.1992>.
50. Bhattacharyya S, Soniat MM, Walker D, Jang S, Finkelstein IJ, Harshey RM. 2018. Phage Mu Gam protein promotes NHEJ in concert with *Escherichia coli* ligase. *Proc Natl Acad Sci U S A* 115:E11614–E11622. <https://doi.org/10.1073/pnas.1816606115>.
51. Zabala B, Hammerl JA, Espejo RT, Hertwig S. 2009. The linear plasmid prophage Vp58.5 of *Vibrio parahaemolyticus* is closely related to the integrating phage VHML and constitutes a new incompatibility group of telomere phages. *J Virol* 83:9313–9320. <https://doi.org/10.1128/JVI.00672-09>.
52. Moreno Switt AI, Orsi RH, den Bakker HC, Vongkamjan K, Altier C, Wiedmann M. 2013. Genomic characterization provides new insight into *Salmonella* phage diversity. *BMC Genomics* 14:481. <https://doi.org/10.1186/1471-2164-14-481>.
53. Drees JC, Chittani-Pattu S, McCaslin DR, Inman RB, Cox MM. 2006. Inhibition of RecA protein function by the RdgC protein from *Escherichia coli*. *J Biol Chem* 281:4708–4717. <https://doi.org/10.1074/jbc.M513592200>.
54. Little JW, Shepley DP, Wert DW. 1999. Robustness of a gene regulatory circuit. *EMBO J* 18:4299–4307. <https://doi.org/10.1093/emboj/18.15.4299>.
55. Maslowska KH, Makiela-Dzibenska K, Fijalkowska IJ. 2019. The SOS system: a complex and tightly regulated response to DNA damage. *Environ Mol Mutagen* 60:368–384. <https://doi.org/10.1002/em.22267>.
56. Butala M, Zgur-Bertok D, Busby SJ. 2009. The bacterial LexA transcriptional repressor. *Cell Mol Life Sci* 66:82–93. <https://doi.org/10.1007/s00018-008-8378-6>.
57. Debnath I, Norton JP, Barber AE, Ott EM, Dhakal BK, Kulesus RR, Mulvey MA. 2013. The Cpx stress response system potentiates the fitness and virulence of uropathogenic *Escherichia coli*. *Infect Immun* 81:1450–1459. <https://doi.org/10.1128/IAI.01213-12>.
58. Acosta N, Pukatzki S, Raivio TL. 2015. The Cpx system regulates virulence gene expression in *Vibrio cholerae*. *Infect Immun* 83:2396–2408. <https://doi.org/10.1128/IAI.03056-14>.
59. Dong H, Zhu C, Chen J, Ye X, Huang Y-P. 2015. Antibacterial activity of *Stenotrophomonas maltophilia* endolysin P28 against both Gram-positive and Gram-negative bacteria. *Front Microbiol* 6:1299. <https://doi.org/10.3389/fmicb.2015.01299>.
60. Altschul SF, Lipman DJ. 1990. Protein database searches for multiple alignments. *Proc Natl Acad Sci U S A* 87:5509–5513. <https://doi.org/10.1073/pnas.87.14.5509>.
61. Sonnhammer EL, von Heijne G, Krogh A. 1998. A hidden Markov model for predicting transmembrane helices in protein sequences. *Proc Int Conf Intell Syst Mol Biol* 6:175–182.
62. Moller S, Croning MDR, Apweiler R. 2001. Evaluation of methods for the prediction of membrane spanning regions. *Bioinformatics* 17:646–653. <https://doi.org/10.1093/bioinformatics/17.7.646>.
63. Liu YG, Whittier RF. 1995. Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. *Genomics* 25:674–681. [https://doi.org/10.1016/0888-7543\(95\)80010-J](https://doi.org/10.1016/0888-7543(95)80010-J).
64. Kovach ME, Phillips RW, Elzer PH, Roop RM, Jr., Peterson KM. 1994. pBBR1MCS: a broad-host-range cloning vector. *Biotechniques* 16:800–802.
65. Kuhn J, Binder S. 2002. RT-PCR analysis of 5' to 3'-end-ligated mRNAs identifies the extremities of cox2 transcripts in pea mitochondria. *Nucleic Acids Res* 30:439–446. <https://doi.org/10.1093/nar/30.2.439>.
66. Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1995. Short protocols in molecular biology: a compendium of methods from Current Protocols in Molecular Biology, 3rd ed. Wiley, New York, NY.