Genomic sequence of temperate phage Smp131 of *Stenotrophomonas maltophilia* that has similar prophages in xanthomonads

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Abstract

**Background:** *Stenotrophomonas maltophilia* is a ubiquitous Gram-negative bacterium previously named as *Xanthomonas maltophilia*. This organism is an important nosocomial pathogen associated with infections in immunocompromised patients. Clinical isolates of *S. maltophilia* are mostly resistant to multiple antibiotics and treatment of its infections is becoming problematic. Several virulent bacteriophages, but not temperate phage, of *S. maltophilia* have been characterized.

**Results:** In this study, a temperate myophage of *S. maltophilia* (Smp131) was isolated and characterized. Sequence analysis showed that its genome is 33,525-bp long with 47 open reading frames (ORFs). Its similarity to P2-like phages and prophages in *S. maltophilia* and several *Xanthomonas* pathovars includes genomic organization, arrangement of several operons, and possession of a slippery sequence T7G for translational frameshifting in tail assembly genes. Smp131 encodes a tyrosine family integrase that shares low degrees of similarity with those of other phages and a lysin belonging to family 19 chitinase that is observed in plants and some bacteria, although not in phages. tRNA are the preferred sites for host integration of Smp131 and the related phages: tRNA-Thr for Smp131 and prophage of *S. maltophilia* K279a; tRNA-Lys for prophages of *X. campestris* pv. campestris ATCC33913, *X. oryzae* pv. oryzae strains MAF311018, and KACC10331; and tRNA-Asn for prophage of *X. oryzae* pv. oryzae PXO99A and remnant of *X. axonopodis* pv. citri 306. Regions flanking the prophages are varied highly in nucleotide sequence and rich in transposase genes, suggesting that frequent insertion/excision had occurred.

**Conclusions:** Prevalence of closely related prophages in *Stenotrophomonas* and *Xanthomonads* may have contributed to the diversity of these closely related species owing to possible horizontal gene transfer mediated by the phages.

**Keywords:** Genomic sequence, Integration, Prophage, *Stenotrophomonas*, Temperate phage, *Xanthomonas*

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**Background**

*Stenotrophomonas maltophilia*, previously named as *Pseudomonas maltophilia* and then *Xanthomonas maltophilia* [1], is an aerobic, Gram-negative, rod-shaped bacterium common in different environments. *S. maltophilia* can cause various types of nosocomial infections, resulting in high morbidity and mortality in severely immunocompromised and debilitated patients [2,3]. This organism is increasingly prevalent in hospitals worldwide; in Taiwan, it is ranked one of the highest occurring nosocomial infections [4]. In addition, isolates obtained from hospitalized patients show significant genetic diversity, suggesting that they can be derived from various sources [5]. Recently, treatment of *S. maltophilia* infections has become more difficult because of the high prevalence of multiple resistance to antibiotics of this organism [6].

Phage therapy has attracted significant attention for its effectiveness in treating bacterial infections [7]. Some *S. maltophilia* phages have been reported including i) two lytic phages (phiSMA5 and Smp14) from our laboratory that resemble members of *Myoviridae* in morphology with a genome of approximately 250 and 160 kb, respectively [4,8], ii) a T7-like phage lytic to pan-resistant *S. maltophilia* and a phage that has large burst
size and unique plaque polymorphism, with their genomes being sequenced [9,10], iii) a phage remnant in S. maltophilia strain P28 that is capable of producing a novel phage tail-like bacteriocin, designated as maltocin P28 [11], iv) detection of a phage genome carrying a zonula occludens like toxin gene [12], and v) three filamentous phages [13,14]. In addition, we have described a novel lysozyme encoded by a Xanthomonas oryzae phage, phiXo411, that is active against both Xanthomonas and Stenotrophomonas [15]. Although the lytic phages, the lysozyme and the maltocin P28 are potentially useful in treating S. maltophilia infection, feasible testing has yet been reported. In spite of the above mentioned efforts in phage study, no temperate phage of S. maltophilia has been reported. In this study, we isolated a temperate phage of S. maltophilia and designated as Smp131. Since acquisition of external DNA by horizontal gene transfer and gene loss are major driving-forces of bacterial genome evolution and integration and excision of temperate bacteriophages contribute actively to such evolution [16], we deemed it worthy to study this phage. The phage genome was sequenced and sequence analysis revealed that Smp131 is similar to phage P2 and shares high degrees of identity with prophages of Stenotrophomonas and xanthomonads.

Results and discussion
Phage Smp131 is a temperate myophage infecting S. maltophilia
In this study, temperate phages were detected by spotting culture supernatants from 86 clinical isolates of S. maltophilia onto lawns formed separately by all other isolates. The culture supernatant from S. maltophilia strain T13 was observed to cause clearing zones on 3 of the samples (ATCC 13637, BCRC 11901, and T16). Following 3 rounds of single plaque isolation, Smp131 was obtained and used for further study. Less turbid plaques were formed on lawns of strain T16; therefore, this strain was used as the host for phage propagation and indicator host in titering the phage.

Cultures of S. maltophilia T13 released from 1 × 10^4 to 1 × 10^6 PFU/ml of Smp131 and treatment by adding mitomycin C (1 μg/ml) into the cultures produced titters of approximately 7 × 10^8 PFU/ml. Electron microscopy showed that Smp131 has an icosahedral head approximately 60 nm in diameter and a contractile tail 100–120 nm in length and 20–30 nm in width (Figure 1), resembling members of Myoviridae phages.

In SDS-polyacrylamide gel (10%) electrophoresis, phage particles purified by CsCl ultracentrifugation displayed more than 15 distinct protein bands, with molecular masses ranging from 16 to 120 kDa, upon staining the gel with Coomassie brilliant blue. Four bands, with molecular masses of 44, 39.5, 38, and 21 kDa, were more abundant than the others. The 38-kDa protein was the most abundant and is likely the major capsid protein.

Host range testing showed that only the three S. maltophilia strains, ATCC 13637, BCRC 11901, and T16, were sensitive to Smp131 as indicated by the formation of single plaques. Several reasons are possible for the phage resistance, including immunity, impaired adsorption and block at later stages during phage infection, and further study is needed to test these possibilities. With such a narrow host range, Smp131 apparently has limited use in control of S. maltophilia infection. Spot tests and plaque assays were also tested on bacteria other than S. maltophilia strains, including Escherichia coli (n = 14, with n being the number of isolates), Serratia marcescens (n = 33), Enterobacter cloacae (n = 12), Klebsiella pneumoniae (n = 10), Proteus mirabilis (n = 11), Pseudomonas aeruginosa (n = 7), Xanthomonas campestris pv. campestris (n = 7), X. axonopodis pv. citri (n = 1), X. axonopodis pv. dieffenbachiae (n = 1), X. axonopodis pv. glycines (n = 1), X. axonopodis pv. phaseoli (n = 1), X. axonopodis pv. vesicatoria (n = 46), and X. oryzae pv. oryzae (n = 2). None of these bacteria were sensitive to Smp131, indicating that this phage has a narrow host range. This is different from phage P2 that can infect several enteric bacterial species [17].

The circular Smp131 genome has a cohesive region conserved in P2-like phages
Restriction endonucleases AvaI, EcoRI, EcoRV, HincII, KpnI, NcoI, NotI, PstI, PvuII, and SphI were tested and
found to be capable of cutting the Smp131 genomic DNA into distinct fragments. Sequencing of the Smp131 genome showed 33,525 bp, and 47 ORFs were identified (Additional file 1: Table S1). Nucleotide sequence comparison revealed that Smp131 had a region similar to the 55-bp cos region conserved in P2 and the related phages required for phage packaging [18]; GC-rich 19-nt 5′-extruding cohesive ends (5′-GGCGTGCGGAAAGCAACGAG-3′) similar to those of P2-related phages (5′-GG CGAGCGGAAAGCAACGAGC-3′) were observed in the cos region of Smp131 (Figure 2) [19]. By analogy to the P2 case, the extruding regions were set as the ends of the Smp131 genome.

The circularity of the Smp131 genome was demonstrated as follows. As shown in Additional file 2: Figure S1A, when displayed in a circular form, the left- and right-hand 19-nt extruding ends of the Smp131 genome would be paired. The genome had 6 EcoRI and 12 EcoRV sites, which were numbered from E1 to E6 and V1 to V12, respectively. Based on this predicted map, we isolated and sequenced a 2.5 kb EcoRI fragment (V12-V1). As anticipated, a 4.7-kb fragment found to be capable of cutting the Smp131 genomic DNA into distinct fragments. Sequencing of the Smp131 genome showed 33,525 bp, and 47 ORFs were identified (Additional file 1: Table S1). Nucleotide sequence comparison revealed that Smp131 had a region similar to the 55-bp cos region conserved in P2 and the related phages required for phage packaging [18]; GC-rich 19-nt 5′-extruding cohesive ends (5′-GGCGTGCGGAAAGCAACGAG-3′) similar to those of P2-related phages (5′-GG CGAGCGGAAAGCAACGAGC-3′) were observed in the cos region of Smp131 (Figure 2) [19]. By analogy to the P2 case, the extruding regions were set as the ends of the Smp131 genome.

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**Figure 2** Smp131 cos region deduced by analogy to those of P2-related phage. The Smp131 sequence is aligned with the known cos regions of Enterobacteria phages P2 (GenBank:NC_001895) and P4 (GenBank:NC_001609), with arrowheads indicating cos cleavage sites [12]. Also shown are corresponding regions from Enterobacteria phages 186 (GenBank:U32222) and PSP3 (GenBank:NC_005340), and Pseudomonas phage phiCTX (GenBank:NC_003278). CLUSTAL X1.83 was used for alignment. Letters with black and grey backgrounds are nucleotides identical in all and four or more sequences, respectively.

**Smp131 is similar to prophages in Stenotrophomonas and Xanthomonas**

Sequence analysis shows that Smp131 shares similarity with several prophages in genome organization and encoded proteins. They included 1) a 27-kb prophage remnant in X. axonopodis pv. citri strain 306; 2) a prophage each in X. campestris pv. campestris strain ATCC33913 (37 kb) and X. oryzae pv. oryzae strains ATCC10331 (40 kb), MAFF311018 (37 kb) and PXO99A (42 kb); and 3) a 35-kb prophage in S. maltophilia K279a (Figure 3, Additional file 3: Table S2). Additionally, most Smp131-encoded proteins were similar to those encoded by several P2-like temperate phages (see below).

Similarity between Smp131 and prophages in Xanthomonas and Stenotrophomonas can be summarized as follows (Additional file 3: Table S2). First, genomes of these prophages (defined as the regions flanked by attL and attR, see below) were slightly larger than that of Smp131 (Figure 3), suggesting that some insertions in these prophages (Figure 3, numbered in red) and deletions (in/del) from Smp131 had occurred during evolution. Most of these in/dels encode hypothetical proteins. It is apparent that those absent from Smp131 are nonessential genes. Second, some Smp131 genes (orf01, 02, 03, 05, 22, 29, 36, 38, 41, 44, 45, and 46) were absent from one or more of the other prophages (remnant in X. axonopodis pv. citri strain 306 lacked orf 01, 02, 03, 25–40, and orf 44–46). Third, there were transposase genes associated with the Xanthomonas prophages and remnant (Figure 3): 1) two in the upstream region and three in the downstream flanking region of the remnant, 2) four in the downstream flanking region of X. oryzae pv. oryzae KACC10331 prophage, 3) one in the upstream flanking region and three in the upstream of X. oryzae pv. oryzae PXO99A prophage, and 4) five in the downstream flanking region of X. oryzae pv. oryzae MAFF311018 prophage. Fourth, identity in amino acid sequence between corresponding proteins of Smp131 and these prophages ranged between 30% and 94%, with the majority falling above 50% (Additional file 3: Table S2). However, because none of their encoded proteins had been characterized, sequence comparison with...
proteins of these prophages did not lead to the identification of Smp131 gene functions.

Among the prophage harboring strains of *Stenotrophomonas* and *Xanthomonas*, *X. campestris* pv. *campestris* ATCC33913 was the only strain available to us. Spot test showed that the culture supernatant from *X. campestris* pv. *campestris* ATCC33913 did not form lysis zones on lawns of *X. campestris* pv. *campestris* strains Xc11 and Xc17, indicating that this strain may not release phage particles.

The majority of Smp131-encoded proteins are similar to those of P2-like phages.

No homologues were identified for proteins encoded by *orf1*, *orf2*, and *orf3* in the database, whereas *orf4* and *orf5* encoded a site-specific DNA methyltransferase and a hypothetical protein, respectively. Cluster *orf6* to *orf11* encoding capsid and packaging proteins was organized in the same order as P2 genes QPONML; *orf12* was similar to P2 gene X, annotated as tail protein (Additional file 1: Table S1, Figure 3).

Proteins encoded by *orf13* and *orf14* possessed three transmembrane domains similar to Class I holins [20]. The product of *orf13* had a highly charged C terminus, which is characteristic of members of Class I, whereas ORF14 contained a slightly lower charged C terminus. *Orf15* was assigned as the endolysin gene. Rather than sharing similarity with phage lysozymes, the *orf15* product had a motif (aa 114–127) highly conserved in members of the GH19 chitinases family, [FHY]-G-R-G-[AP]-X-Q-[IL]-[ST]-[FHYW]-[HN]-[FY]-NY, that forms the substrate binding region [21] (Figure 4). Moreover, Glu50/Glu59 of ORF15 were similar to Glu68/Glu77 of *Streptomyces coelicolor* chitinase G experimentally identified as the active sites [22]. Family GH19 chitinases have long been identified in plants [23] and recently in bacteria [22,24-27], although not in phages; this Smp131 enzyme appears to be the first reported for phages.

Proteins encoded by *orf17* and *orf18* were homologous to R and S of P2, the tail completion proteins essential for stable head joining [28]. Proteins encoded by *orf19*, *orf20*, *orf23*, and *orf24* were homologous to that of the P2 J, I, V, and W (clustered with H and G as VWJIHG), respectively, whereas the position of *orf21* and *orf22* is similar to that of P2 H and G. Among the P2 genes, VWJI code for baseplate assembly proteins; H for putative tail fiber protein; and G for probable tail fiber
assembly protein [29,30]. The difference in gene order suggests that rearrangement of these genes had occurred during evolution.

Orf25 to orfB1, except orf29 that encoded a possible membrane protein, encoded tail proteins, whereas orf32 encoded a late gene control protein. These genes corresponded to the P2 operon FIFIIEE'TUD (Figure 3, Additional file 1: Table S1; [31]). In P2, E' overlaps the start of gene T, lacks a potential ribosome binding site, and extends 37 nt back into E in the −1 reading frame. A run of 6 T residues (T6G slippery sequence) was located 20 nt upstream of the possible GUG start of E' and an extension of gene E following a −1 translational frameshift has been designated as E+E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33]. Near the 3′-end of orf27, there is a T2G similar to the conserved T6G slippery sequence [31], nt 288–295 relative to the orf27 start codon. Thus, by analogy, a −1 translational frameshift has been designated as E + E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33]. Near the 3′-end of orf27, there is a T2G similar to the conserved T6G slippery sequence [31], nt 288–295 relative to the orf27 start codon. Thus, by analogy, a −1 translational frameshift has been designated as E + E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33]. Near the 3′-end of orf27, there is a T2G similar to the conserved T6G slippery sequence [31], nt 288–295 relative to the orf27 start codon. Thus, by analogy, a −1 translational frameshift has been designated as E + E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33]. Near the 3′-end of orf27, there is a T2G similar to the conserved T6G slippery sequence [31], nt 288–295 relative to the orf27 start codon. Thus, by analogy, a −1 translational frameshift has been designated as E + E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33]. Near the 3′-end of orf27, there is a T2G similar to the conserved T6G slippery sequence [31], nt 288–295 relative to the orf27 start codon. Thus, by analogy, a −1 translational frameshift has been designated as E + E' [31]. The arrangement of E and E' genes within the tail gene cluster and their coupling through a translational frameshift is conserved among P2-related phages as well as in several other phages such as lambda although they share no similarity in amino acid sequence [31-33].
Smp131 late genes may be regulated in a manner similar to that in P2

P2 has four late promoters, \( P_b \), \( P_O \), \( P_V \), and \( P_b \), possessing the consensus sequence TGT-N\(_{12}\)-ACA and controlling PQ, ONMLKRS, VWJHIG, and F\(_{\text{Fg}}\)EEPUTD operons, respectively [36,37]. Transcription of these operons depends on the Ogr protein, a zinc-finger containing transcriptional activator with a conserved cysteine motif, CX\(_2\)CX\(_{22}\)CX\(_4\)C, where a zinc atom coordinates with four cysteine residues [38,39]. In Smp131, four putative late promoters were observed with sequences similar to TGT-N\(_{12}\)-ACA, which were designated as \( P_P \), \( P_O \), \( P_P \), and \( P_P \), located at nt 4398–4381, 4381–4398, 10,964–10,981, and 14,928–14,946 in the genome, respectively (Figure 3). Operons presumably controlled by \( P_P \) and \( P_O \) were analogous to those by P2 \( P_b \) and \( P_O \), respectively, but those by \( P_P \) and \( P_O \) had some exchanged members due to gene rearrangement, that is, VWJHIG and F\(_{\text{Fg}}\)EEPUTD in P2 versus orf19-orf22 (homologous to JIHG) and orf23-orf32 in Smp131 (Figure 3). Additionally, the protein encoded by Smp131 orf34, which had a relative position similar to that of the P2 Ogr gene, had a conserved CX\(_2\)CX\(_{22}\)CX\(_4\)C motif, although overall similarity shared by the two proteins was low. Thus, similarity in genome organization, promoter sequence, and a regulatory protein suggests that Smp131 late genes are regulated in a manner similar to that in P2.

tRNA genes are the preferred sites for integration of P2-like prophages of Xanthomonas and Stenotrophomonas

It is known that in E. coli i) P2 can integrate at over 10 different loci, with loci (attB site containing the core sequence, 5′-AAAAAATAAGCCCGTGTAAGGGAGATT-3′, which is identical to the attP sequence) being preferred over any other sites in E. coli C, ii) this site is occupied by a remnant of a P2 prophage in E. coli K-12 and P2 therefore will integrate into secondary sites, and iii) P2 integrase accepts at least up to 37% mismatches within the core sequence [40]. Searching for a region similar to the P2 attP site in Smp131 genome revealed no such region. We then turned to identify putative attR and attL at the ends of prophage sequences from Stenotrophomonas and Xanthomonas and observed a 46-nucleotide perfect direct repeat at the extremities of the integrated prophage of S. maltophilia K279a, apparently representing attL and attR of the prophage (GenBank:NC_010943, Figure 3 and Additional file 7: Table S4). This 46-nucleotide sequence corresponded to the 3′-end of an intact tRNA-Thr gene. Nucleotide sequence comparison showed that a region identical to the att regions of the S. maltophilia K279a prophage was present in bp 30,738-30,783 (orf43/orf44 intergenic region) of the Smp131 genome (Additional file 7: Table S4). This region, situated downstream of the integrase gene and similar in location to those in P2-like prophages (phiCTX, GenBank:NC_003278; 186, GenBank:U32222), was thus predicted to be the attP site for Smp131 (Figure 3).

Based on the position of attP, we predicted that upon integration via attP, orf44 and orf43 would become flanked by attL and attR, respectively. In addition, an Nael and a HincII restriction sites were located 644 bp and 667 bp relative to the orf43 and orf44 start codons, respectively, in the Smp131 genome (Additional file 8: Figure S4). Sequencing revealed that the amplicons were 1,092 bp and 704 bp containing attL and attR, respectively, which had a sequence identical to that of the Smp131 attP. To verify the att-flanking sequences, primers L3/L4 and R2/R3 were used to amplify the junctions of attL and attR regions, respectively (Additional file 8: Figure S4). Sequencing of these 2 replicons confirmed that our inverse PCR reactions had faithfully amplified the targeted regions. The result revealed that a segment of a possible defective integrase gene (480 bp) downstream of the attL was similar to that of Burkholderia thailandensis E264 (GenBank:YP_441483), whereas a 177-bp long host chromosomal region upstream of the attR was highly similar to the sequence adjacent to the tRNA-Thr of S. maltophilia strains (K279a and R551-3). These results suggest that upstream regions of tRNA-Thr are conserved in different strains of S. maltophilia, whereas the downstream regions are not. It was also noticed that upon integration, an intact tRNA-Thr that included the attR was regained, similar to the target site duplication observed by Rocco et al. [41].

In addition to S. maltophilia strain K279a (GenBank:NC_010943), the genome sequence has been determined for strain R551-3 (GenBank:NC_011071) [42,43]; they each had only one copy of tRNA-Thr located near one o’clock relative to the origin of chromosome replication (ori), as identified by containing DnaA boxes and genes involved in the initiation of bacterial chromosome replication [44]. Therefore, it is highly probable that this tRNA-Thr is the preferred site for Smp131 integration. Sequence analysis of junctions of integrated Xanthomonas prophage suggests that 1) prophages of X. campes-tris pv. campestris strain ATCC33913, and X. oryzae pv. oryzae strains MAF311018 and KACC10331 integrated into a 45-bp region corresponding to 3′-end of a tRNA-Lys gene (GenBank:XCC3013, GenBank:XOXO_r26, GenBank:XOO4676), 2) prophage of X. oryzae pv. oryzae PXO99A integrated into a 46-bp region corresponding to the 3′-end of a tRNA-Asn (GenBank:PXO_rna33), and 3) prophage remnant of X. axonopodis pv. citri 306 used the same sequence (GenBank:XAC2627) as that of X. oryzae pv. oryzae PXO99A prophage for integration, except that only attL was retained (Figure 3, and Additional file 7: Table S4). All identified attB sites for Xanthomonas are also located near one o’clock on the bacterial chromosomes.
Host integration of P2-like phages involves binding of integrase to the two arm-binding sites flanking the imperfect repeat, each having two direct repeats [45]. Careful examination of the Smp131 sequence revealed a pair of perfect direct repeats (5′-AATTTTACCGG-3′, bp 30635–30645 and bp 30647–30657) and an inverted repeat (5′-AAAAAGGCAGGCACCGCCTGGCCTT TTT-3′, bp 30665–30695) in the upstream of attP (after the integrase gene, orf43), but no such sequences were found between attP and orf44. By analogy, it is possible that these repeats are involved in recognition by Smp131 integrase for host integration. However, lack of conserved repeats in the downstream suggests that the Smp131 integrase may be less demanding for sequence conservation in the downstream region for the function.

Conclusions
This study is the first to isolate a temperate phage of S. maltophilia, Smp131. It is identified as a P2-like phage based on similarities to P2 in amino acid sequences of the encoded proteins, genomic organization, arrangement of several operons, and possession of a slippery sequence T-G for translational frameshifting in tail assembly genes. Smp131 is able to infect only S. maltophilia, different from phage P2 that can infect several enteric bacterial species. Several P2-like prophages in S. maltophilia and xanthomonads are also identified by bioinformatic analyses. In contrast to P2 that can integrate into several loci of the host chromosome, with certain loci being favoured and none of them being t-RNA gene, single t-RNA genes are found to be the locus for integration of these Stenotrophomonas and xanthomonads prophages. In addition, the regions flanking the prophages are rich in transposase-like genes, suggesting frequent exchange of genes during evolution. Existence of closely related prophages in Stenotrophomonas and xanthomonads is consistent with the close relatedness of these bacteria and the previous classification including Stenotrophomonas in genus Xanthomonas. Prevalence of the phages may have contributed to diversity of these closely related species owing to possible horizontal gene transfer mediated by the phages. With a narrow host range, the value to use Smp131 for controlling S. maltophilia infection is apparently limited.

Methods
Bacterial strains and growth conditions
Bacterial strains used in this study have been described previously [4]. S. maltophilia strains ATCC13637, BCRC 11901 and BCRC 15678 were used as reference strains [4]. Strain T16 was the host for propagation of phage Smp131 and as the indicator host in plaque assay. Luria-Bertani (LB) broth and LB agar plate were the general-purpose media [46] used to cultivate S. maltophilia (30°C), Escherichia coli (37°C), Serratia marcescens (37°C), Enterobacter cloacae (37°C), Klebsiella pneumoniae (37°C), Proteus mirabilis (37°C), Pseudomonas aeruginosa (37°C), and Xanthomonas strains (28°C).

Spot test, isolation of bacteriophage and plaque assay
To detect the presence of phage in the culture supernatants and the phage sensitivity of a bacterium, spot tests were performed as described previously [4], except that LB broth and LB agar plates were used. The top agar containing the clearing zones was picked and soaked for 30 min in 100 μl of LB broth. Following appropriate dilution, the suspensions were plated for single plaque formation. Two more rounds of single-plaque isolation were performed to obtain the pure phage culture. To determine the phage titers, double-layered bioassays were performed on LB agar plates in which the top and bottom layers contained 0.75% and 1.5% agar, respectively. One-tenth of a milliliter each of a phage suspension after serial dilutions and cells of S. maltophilia strain from an overnight culture were mixed with 3 ml of molten soft agar and poured onto the bottom solidified agar (12 ml). Numbers of plaques were counted after the plates were incubated overnight. The same method was used to confirm phage susceptibility with the cells of different bacteria as the indicator hosts.

Purification of phage particles
High-titer lysates of Smp131 (400 ml, approximately 1.0 × 10¹⁰ PFU/ml) were centrifuged (10,000 × g, 20 min at 4°C). The supernatants were passed through a membrane filter (0.45 μm pore size) and then centrifuged (15,000 × g at 4°C) for 2 hr. The phage pellets were suspended in 1.0 ml of the SM buffer (50 mM Tris–HCl, pH 7.5, containing 100 mM NaCl, 10 mM MgSO₄, and 0.01% gelatin) and loaded on the block gradient of CsCl (1.2, 1.35, 1.45, 1.50, and 1.70 g/ml), followed by ultracentrifugation (28,000 rpm for 2 h at 4°C) with rotor TH641 (Sorvall OTD Combi) [15]. The phage particles concentrated into a zone were recovered and dialyzed against the SM buffer.

DNA techniques
Phage particles purified following ultracentrifugation were treated with sodium dodecyl sulfate (SDS, 1%) and 20 U of proteinase K (Sigma P-2308) at 58°C for 1 h. An equal volume of phenol/chloroform (1:1) was then added to remove the proteinaceous materials. Phenol/chloroform extraction was repeated twice and the DNA was precipitated as described previously [47]. Restriction enzyme digestion of the phage DNA was performed in accordance with supplier instructions. DNA fragments were separated in 0.7% agarose gels in a TAE buffer (40 mM Tris acetate, pH, 8.0, containing 2 mM EDTA).
Isolation of DNA fragments from agarose gel was performed using commercial kits (Qiagen). Standard protocols were followed for blotting DNA fragments onto the membrane (NEN catalog number NEF988), preparation of probes by labeling with [α-32P] dCTP (Du Pont. NEN), and Southern hybridization.

**DNA sequencing and bioinformatics**

Processes for purification and shearing of phage DNA, cloning the DNA fragments into pBluescript II SK, and determination of the nucleotide sequence were performed as detailed in previous research [48]. Gaps were closed by primer walking with PCR-amplification on Smp131 genomic DNA as the template using primers designed according to available sequences. Programs used for DNA sequence analysis and similarity search based on domain architecture were selected according to previous research [49]. Possible ORFs were searched in 6 reading frames on both strands of the Smp131 genomic DNA, which used ATG or GTG as the start codon, consisted of longer than 50 amino acid residues, and had a putative ribosomal binding site in the upstream region.

The 33,525-bp DNA sequence determined in this study for phage Smp131 has been deposited in GenBank under accession number JQ809663.

**Cloning of the attL and attR regions flanking the Smp131 prophage**

To clone the junction regions containing attL and attR, an inverse PCR-based strategy was employed. The chromosome prepared from *S. maltophilia* T13, the Smp131 lyogenic strain, was cleaved with Nael and HincII separately and self-ligated to circularize the DNA molecules. Inverse PCR was performed using the circularized HincII and Nael fragments as the templates with primer pairs L1/L2 (for amplification of the attL-containing region) and R1/R2 (for amplification of the attR-containing region), respectively. The amplicons obtained were sequenced for comparison.

**Separation of virion proteins by SDS-polyacrylamide gel electrophoresis**

Following dialysis, phage particles (approximately 1 × 10^8 PFU) purified by ultracentrifugation were boiled in a loading buffer for 3 min and separated in SDS-PAGE (10% polyacrylamide and 0.1% SDS). Protein bands were visualized by staining the gel with Coomassie brilliant blue (Bio-Rad) [47].

**Electron microscopy**

Phage Smp131 was examined by electron microscopy of negatively stained preparations as described previously [4] using a JEM-1200 EX II transmission electron microscope (JEOL, Peabody, Mass) operated at 120 kV.

**Additional files**

**Additional file 1:** Table S1. Assignment of Smp131 genes.

**Additional file 2:** Figure S1. Strategy employed to test whether Smp131 has a circular form of genome. Lines: 1, restriction map deduced from the Smp131 sequence determined in this study; 2, fragments E1-3 (2.5 kb) and ES1 (0.7 kb) used as probes for Southern hybridization; 3 and 4, 4.7-kb Aval fragment (A1) and 4.7-kb EcoRV fragment (B5), respectively, that would hybridize to probes E1-3 and ES1 should the genome be circular. (B) Southern hybridization of Aval and EcoRV digests from Smp131 genome using E1-3 and ES1 separately as probes.

**Additional file 3:** Table S2. Comparison of proteins deduced from prophages or remnants in *Xanthomonas* and *Stenotrophomonas*.

**Additional file 4:** Figure S2. Predicted T7G translational frameshift sites in Smp131 and closely related prophages from *Xanthomonas* and *Stenotrophomonas*. (A) T7G (enclosed by a rectangle) and the surrounding regions including genes p27, p27.1 and p28 of Smp131. Stop codons are denoted by three dots after the amino acids. Predicted start codon ATG of p27.1 is underlined, whereas ribosomal binding site AGAGG for gene p28 is in gray background. (B) DNA sequence alignment of the regions surrounding T7G translational frameshift sites (enclosed in rectangles) from Smp131 and the related prophages from *X. campestris* pv. campesiris 33913, *X. oryzae* pv. oryzae strains KACC10331, MAFF311018 and PKO99A. An asterisk indicates identical nucleotides in all phages.

**Additional file 5:** Figure S3. Comparison of tyrosine integrase of Smp131 and its homologues. Identical residues found in more than 3 residues are highlighted. Active sites determined for XerD are indicated by downward arrowhead and the RIKRH pentad conserved residues are indicated above. The α-helix (empty rectangle) and β-sheet (empty arrow) structural motifs under the alignments are based on the crystal structure of E. coli XerD. Abbreviations: Smp131, integrase deduced from Smp131 orf43; P2, integrase of *Enterobacter* phage P2 (GenBank: P36992); 186, integrase of *Enterobacteria* phage 186 (GenBank: P366723); XerD, site-specific recombine of E. coli (GenBank: 1AOP_A).

**Additional file 6:** Table S3. Identities of amino acid sequence shared between the proteins deduced from Smp131 and those from *bacteriophages*.

**Additional file 7:** Table S4. Positions and sequences of att sites and tRNA of Smp131 and prophages in *Xanthomonas* and *Stenotrophomonas*.

**Additional file 8:** Figure S4. Strategy for cloning the host-prophage junctions from Smp131-lysogenized *S. maltophilia* T13. (A) Sketch depicting the circular Smp131 genome and genes near the predicted attP site. Arrows represent the genes and predicted attP site (B) Sketch showing the host *S. maltophilia* T13 chromosome and its attL site. (C) Map showing relative positions of genes after Smp131 integration into host *S. maltophilia* T13. Primers used in PCR were: L1; 5′-TGAAGTTGCTGATGA CCAACAGG-3′; L2, 5′-GCGTTGCCAAGGTCAGATCGG-3′; R1, 5′-CTCGTAGGAATGAA-3′; R2, 5′-GATATGATTAACCTGTAAGG-3′; R3, 5′-AGGCCAACGGCAAC-3′; L3, 5′-GGCAATTG-3′; L4, 5′-AATACGACAA CTCCTGAGTG-3′; R1, 5′-ACGCGTTCCTCCTGGCCTGC-3′; R2, 5′-TGATAGCCCTATTTTCAAGGGC-3′; R3, 5′-AGGCCAACGGCAAC-3′; L4, 5′-TGCCCTTGCCGCGACCT-3′. *S. maltophilia* T13 chromosome containing prophage Smp131 was digested with HincII and Nael. The fragments were self-ligated and the circularized DNA was then used as the templates for inverse PCR. Amplicons obtained were sequenced for comparison.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

SFW designed the experiments. CNL and HCC carried out the wet lab. TTT and CNL performed bioinformatic analyses. WL and TTT edited the manuscript. All authors read and approved the final manuscript.

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