Characterization of two novel bacteriophages infecting clinical isolates of *Serratia* spp. and therapeutic efficacy in a *Galleria mellonella* model of infection

Mia Horton[1]†, Yashwani Malik[1]†, Caitlin Wildsmith[1], Mohamed Eladawy[1,2], Lesley Hoyles[1], David Negus[1]*

[1] Nottingham Trent University, Department of Biosciences, Clifton Campus, Clifton Lane, Nottingham, NG11 8NS

[2] Microbiology and Immunology Department, Faculty of Pharmacy, Mansoura University, Egypt

† These authors contributed equally to the publication

* **Corresponding author:** David Negus, Department of Biosciences, Nottingham Trent University, Clifton Campus, Clifton Lane, Nottingham NG11 8NS, UK. E-mail david.negus@ntu.ac.uk;

Abbreviations: BLAST, basic local alignment search tool; CFU, colony forming units**;** EOP, efficiency of plating; MDR, multidrug-resistant; PEG, polyethylene glycol; PFU, plaque forming units; SME, *Serratia marcescens* carbapenemase; TEM, transmission electron microscopy; TSA, tryptone soy agar; TSB, tryptone soy broth; VC, viral cluster

Keywords: Wax moth, multidrug-resistant, antimicrobial resistance, phage, Queuovirinae

Running title: *Serratia* phages

ABSTRACT

AIMS

This study aimed to isolate virulent bacteriophages (phages) with the capacity to lyse clinical strains belonging to the genus *Serratia*. Furthermore, we aimed to characterize these phages both genomically and in terms of their potential to treat experimental *Serratia* infections.

METHODS AND RESULTS

Virulent phages were isolated from water samples collected close to sewage outlets around Nottinghamshire, UK, using the clinical bloodstream isolate *Serratia marcescens* UMH9 as the host. Two phages, vB_SmaP_10b_1 (Sm10b_1) and vB_SmaS_12 (Sm12) were selected for whole genome sequencing and further characterization. Comparative genomics and proteome-based analyses identified Sm10b_1 represents a novel genus within the order *Autographivirales* whereas Sm12 represents a novel species belonging to a novel genus within the subfamily *Queuovirinae.* Host range analysis against a collection of clinical and environmental *Serratia* spp. (n=19) confirmed both phages produced plaques on at least 40 % of the strains tested. *In vivo* efficacy of Sm10b_1 was determined by infecting *Galleria mellonella* larvae with *S. marcescens* UMH9 or *S. odorifera* Me113 (catheter-associated urinary tract infection isolate) followed by injection of phage Sm10b_1 ($1x10^7$ pfu). Phage treatment significantly improved survival of larvae infected with *S. marcescens* (0 % survival untreated vs 67 % survival treated, $P < 0.0001$) or *S. odorifera* (7 % survival untreated vs 70 % survival treated, $P < 0.0001$).

CONCLUSIONS

Sm10b_1 and Sm12 represent novel lytic phages with broad host ranges against clinical isolates of the genus *Serratia.* Sm10b_1 was found to be highly effective at treating experimental infections caused by *S. marcescens* and *S. odorifera*.

IMPACT STATEMENT

Serratia spp. are an important cause of healthcare-associated infections worldwide. Increased incidence of antibiotic resistance within the genus is a serious concern and novel treatment options are needed. Here we report the isolation of two novel phages with future potential for the treatment of infections caused by *Serratia* spp.

INTRODUCTION

Serratia spp. are Gram-negative, facultatively anaerobic bacilli belonging to the family *Yersiniaceae*. Members of the genus have been isolated from a diverse range of environments and are responsible for causing a range of infections in humans(Mahlen, 2011), animals (Armstrong, 1984; Guardo et al., 1997), insects (Flyg et al., 1980) and plants (Zhang et al., 2005). The most prominent member of the genus is the opportunistic pathogen *Serratia marcescens*. *S. marcescens* is an important cause of healthcare-associated infections, most notably catheter-associated bacteraemia, wound infections, and urinary tract infections (A. Hejazi & F. R. Falkiner, 1997). However, epidemiological studies have identified that *S. marcescens* infections are not limited to hospital settings, with reports of up 47 % of infections developing within the community (Engel et al., 2009). Antibiotic resistance in *Serratia* spp. is a growing concern. *S. marcescens* is intrinsically resistant to multiple frontline antibiotics including ampicillin, and first- and second-generation cephalosporins and macrolides (Tavares-Carreon et al., 2023). Of particular concern are carbapenemase-producing strains, such as those possessing the chromosomally encoded *Serratia marcescens* enzyme (SME) class A carbapenemase (Hopkins et al., 2017), or the plasmid-borne bla_{KPC-2} and bla_{KPC-3} enzymes (Chen et al., 2022; Wang et al., 2022). The potential expansion of multidrug-resistant (MDR) *S. marcescens* strains (Moradigaravand et al., 2016) may limit the therapeutic utility of traditional antibiotic chemotherapy; therefore, alternative treatment strategies for this emerging pathogen are required.

Bacteriophages (phages) are viruses which infect bacterial hosts. Virulent (lytic) phages are characterized by destruction of the host cell on completion of replication, allowing for the release of new virions. Temperate (lysogenic) phages incorporate

their genome into the host chromosome and are often referred to as prophages, with their DNA being replicated upon each round of host chromosomal replication. Temperate phages can also excise their DNA from the host chromosome and enter the lytic cycle. This process is often induced by various stressors, particularly in response to DNA damage which evokes the SOS response (Salsabil Makky et al., 2021). Due to the antimicrobial action of phages, there has been renewed interest in characterizing and developing these viruses as potential therapeutics, especially against MDR strains of bacteria. Although there are previous reports of lytic phages isolated against clinical strains of *S. marcescens* (Matsushita et al., 2009; Tian et al., 2019; Weber et al., 2020), limited data exist regarding the efficacy of phage therapy in experimental models of *Serratia* infection. Moreover, only a limited number of complete genomes exist for phages infecting members of the genus *Serratia*. A recent (April 2025 release) search of the INfrastructure for a PHAge REference Database (INPHARED) (Cook et al., 2021) of complete phage genomes from GenBank identified only 81 complete phage genomes which list the genus *Serratia* as the isolation host. Therefore, this study aimed to characterize the host ranges, genomes and therapeutic potential of two virulent phages isolated against *S. marcescens* UMH9, a bloodstream infection isolate (Anderson et al., 2017) which produces the sialylated KL1 capsule associated with clinical infections (Anderson et al., 2022, 2025).

MATERIALS AND METHODS

Strain and cultivation information

Details of all strains used in this study are provided in **Table 1**. *Serratia* spp. were grown on tryptone soy agar (TSA; Fisher Scientific) unless stated otherwise. Tryptone soy broth (TSB; Fisher Scientific) was used for overnight cultures which were incubated aerobically at 37 °C with shaking (160 rpm) for 16 h.

Isolation of phage

All media used in the isolation, propagation and characterization of phage were supplemented with $CaCl_2$ and $MgCl_2$ (both at 0.5 mM). Water samples (n = 27) were collected from rivers close to sewage outlets around Nottinghamshire, UK from May to June 2022 (**Supplementary Table 1**) and filtered with 0.45 µm cellulose acetate filters (Millipore). For the enrichment of phage, 5 ml aliquots of filtered river water were mixed with an equal volume of 2 x TSB and inoculated with 100 µl of an overnight culture of the indicator strain, *S. marcescens* UMH9. Samples were incubated for 48 h with shaking (150 rpm) at 37 °C. Whole cells and debris were pelleted by centrifugation (6,000 x *g*, 15 min) and the supernatant containing propagated phage was filtered (0.45 µm cellulose acetate filter; Millipore). The filtrate was serially diluted in SM buffer (0.2 M $NaCl_2$, 0.01 M $MgSO_4$, 0.05 M Tris-HCl, pH 7.5) and individual plaques were obtained using the double-layer agar technique with *S. marcescens* UMH9 as the indicator strain. Phages were purified by picking individual plaques into 100 µl sterile SM buffer, followed by vigorous vortexing and ten-fold serial dilution in SM buffer. Single plaques were again obtained using the

double-layer agar technique with *S. marcescens* UMH9 as the indicator strain. To ensure purity, individual plaques were picked and serially diluted a minimum of three times.

Visualization of phage morphology

For transmission electron microscopy (TEM), formvar/carbon grids (200 Mesh Copper, Agar Scientific) were prepared by glow discharge (10 mA, 10 s) using a Q150R ES sputter coater (Quorum Technologies). Bacteriophage suspension (15 µl, approximately 5 x 10^9 pfu ml^{-1}) was pipetted onto the surface of the grids for 30 s before blotting with Whatman filter paper. Samples were then stained by pipetting 15 µl of 2 % phosphotungstic acid onto the grids. Excess stain was removed with Whatman filter paper and grids air dried for a minimum of 5 min. Samples were visualised using a JOEL JEM-2100Plus TEM at an accelerating voltage of 120 kV. Mean capsid diameter and mean tail length were calculated from the measurements of three separate phage images.

Host-range analysis

Phage host range was determined by spot test. Overnight cultures (16 h growth) of prospective host strains (100 µl) were combined with 5 ml of molten 0.6 % TSA and the mixture was gently swirled before being poured onto TSA plates. Once solidified, 5 µl of phage lysate, ~1 x 10^8 plaque forming units (pfu) ml^{-1}, was spotted onto the plates, allowed to dry and plates were incubated at 37 °C for 16 h. The following day, plates were inspected for lysis, with results recorded according to a modification of (Haines et al., 2021).

Efficiency of plating

Efficiency of plating (EOP) was determined by serial dilution of phages and titration to determine to pfu/ml produced against each *Serratia* strain used in the study. Briefly, overnight cultures of *Serratia* spp. (100 µl) were combined with 100 µl of phage dilution and 5 ml of molten 0.6 % TSA and the mixture was gently swirled before being poured onto TSA plates. Plates were incubated for 16 h and number of plaques produced was used to calculate the pfu/ml produced for each prospective host. EOP was determined for each phage by dividing the pfu ml^{-1}formed on the routine propagation host (UMH9) relative to the pfu ml^{-1} observed on a test strain.

Detection of prophages

The presence of potential prophages within the genome of indicator strain *S. marcescens* UMH9 (NCBI accession CP018923) was determined using the prophage prediction tool PHASTEST (Wishart et al., 2023).

Phage DNA sequencing, genome assembly and annotation

Phage lysates (~1 x 10^8 pfu ml^{-1}) were concentrated using centrifugal concentrators and phage DNA was extracted as described previously (Smith-Zaitlik et al., 2022). Sequence data were generated on our in-house Illumina MiSeq platform, with the Nextera XT DNA library preparation kit (Illumina) to produce fragments of approximately 500 bp. Fragmented and indexed samples were run on the sequencer using a Nano flow cell with the MiSeq Reagent Kit v2 (Illumina; 250-bp paired-end reads) following Illumina's recommended denaturation and loading procedures. The

quality of the reads was determined using FastQC v0.11.9. Reads were trimmed with Trimmomatic (Bolger et al., 2014) to an average phred score of 20 and assembled using SPAdes v3.15.4 (default settings) (Bankevich et al., 2012). Assembled genome sequences were annotated using Pharokka v1.6.1 (v1.4.0 databases), with dnaapler used to reorientate the circular genomes so that they began at the terminase large subunit gene, *terL* (Bouras et al., 2023). Completeness and contamination of the two genomes were assessed using CheckV v1.0.1 (checkv-db-v1.5) (Nayfach et al., 2021). Genome sequences of Phage vB_SmaP_10b_1 (Sm10b_1) and Phage vB_SmaS_12 (Sm12) have been deposited with NCBI under accessions PX000100 and PX000101 respectively. Annotated files are available for download via figshare.

Phage comparative genomics

To generate a gene-sharing network, proteomes of 21,625 phages from the INPHARED database (1 February 2024 dataset; https://millardlab-inphared.s3.climb.ac.uk/1Feb2024_vConTACT2_proteins.faa.gz) and the two query phages were clustered using vConTACT2 (v0.9.19; default settings) and data visualized in Cytoscape v3.10.1 using the yFiles organic layout (Bolduc et al., 2017; Cook et al., 2021). The viral clusters (VCs) containing the two query phages were determined based on detection of the first neighbours of both novel phages within the complete network, and phylogenetic trees (bionj) were generated from all phages within the same VCs using VipTree v1.1.2 (default settings) (Nishimura et al., 2017). Genomes belonging to both VCs were annotated using Pharokka (--skip_extra_annotations) but were not reoriented using dnaapler; the large terminase

protein TerL sequences from these annotations were used in subsequent analyses with the TerL sequences of phage Sm12 and phage Sm10b_1. Annotated files are available for download via figshare.

The TerL sequences were aligned using Clustal Omega (v1.2.2), with the multiple sequence alignments used to generate maximum likelihood phylogenetic trees with PHYML 3.3.20180621 (Blosum62; 100 bootstraps) in Geneious Prime 2023.0.1. The intergenomic similarity between phages within the same VCs was determined using VIRIDIC web (v1.0) (Moraru et al. 2020).

Pairwise alignments of phage host recognition proteins were performed in Geneious Prime 2023.0.1. using Clustal Omega.

Purification of phage lysates for use *in vivo*

To remove potential contamination (e.g. lipopolysaccharide) from phage lysates prior to use *in vivo*, phage stocks were further purified by polyethylene glycol (PEG) precipitation, chloroform extraction and ultrafiltration (Hietala et al., 2019). Phage lysates (500 ml) were combined with PEG 8000 (10 % w/v, final concentration) and NaCl (0.5 M, final concentration) and phage were allowed to precipitate overnight at 4 °C. Precipitated phage were pelleted by centrifugation (15,000 x *g*, 15 min, 4 °C) and the pellet resuspended in 50 ml of SM buffer. An equal volume (50 ml) of chloroform was added and the suspension was incubated with gentle agitation at room temperature for 20 min. The phases were separated by centrifugation (5,000 x *g*, 10 min, 21 °C) and the aqueous phase containing the purified phage was carefully recovered. To remove any remnants of chloroform, recovered phage were transferred to a vivaspin ultrafiltration device with a molecular weight cut-off of 30

kDa (Sartorius) and buffer exchanged against SM buffer. Recovered phage were passed through a 0.45 µm cellulose acetate filter (Millipore) and stored at 4 °C until required.

Efficacy of phage therapy in a *Galleria mellonella* model of *Serratia* infection

G. mellonella larvae were sourced from the UK based supplier Livefoods Direct. Any larvae showing signs of melanisation were discarded and the remaining larvae were weighed. Only larvae weighing between 200 and 300 mg were selected for use in experiments. Larvae were used in experiments on their day of arrival. *In vivo* experimental infections were performed with strains *S. marcescens* UMH9 and *Serratia odorifera* Me113, recovered from a catheter-associated urinary tract infection (Eladawy et al., 2025). The infectious dose required to cause at least 80 % mortality within 48 h of injection was first determined by serial dilution of each strain in sterile phosphate-buffered saline followed by injection (10 µl of dilution) into the right hind proleg of the larvae (*n* = 10 per group). The number of colony-forming units (cfu) injected was determined by serial dilution of the injection and plating to TSA. Injected larvae were incubated at 37 °C over a period of six days and survival was recorded every 24 h.

To determine the efficacy of phage treatment in improving survival, larvae *n* = 10 per group, per condition were used. Experiments were performed three times (three biological repeats) giving a total of 30 larvae used for each condition. Larvae were then subjected to the following conditions: Group 1, injected with 10 cfu *Serratia*, not-treated; Group 2, injected with 10 cfu *Serratia*, treated with $1x10^7$ pfu phage; Group 3, injected with $1x10^7$ pfu phage only; Group 4, injected with SM buffer only; Group

5, un-injected control. Treated larvae were injected with PEG-purified phage in SM buffer. Treatment injections were given 30 min post-infection and phage were administered via injection to the opposite hind proleg to infection. Injected larvae were incubated at 37 °C over a period of 144 h and survival was recorded every 24 h. The number of cfu administered was confirmed via serial dilution and plating of injections. Statistically significant differences in survival rates between groups were determined by plotting Kaplan-Meier curves and performing log-rank tests.

In-silico identification of *Serratia* proteases

Potential insecticidal proteases encoded by either *S. marcescens* UMH9 or *S. odorifera* Me113 were identified by performing a megablast of nucleotide query sequences against the genomes of the two *Serratia* spp. Query sequences were serralysin (*prtS*) accession MG020512.1 and the serralysin-like proteases (slp) *slpB*, *slpC*, *slpD* and *slpE*, NCBI accessions MG020513.1, WP_038629638.1, WP_025302762 and KT901292.1, respectively.

RESULTS

Isolation and characterization of *Serratia* spp. phages

For the isolation of phages against *Serratia* spp. we collected water samples from rivers close to sewage outlets around Nottinghamshire, UK, using *S. marcescens* UMH9 as an indicator strain. Firstly, we determined if the genome of *S. marcescens* UMH9 might include complete prophages which could be induced as part of the isolation process and possibly confound downstream analysis. Analysis of the UMH9

genome using the prophage prediction tool PHASTEST did not detect any prophage-like sequences within the genome.

Following initial enrichment of phages in the water samples with strain UMH9, we found that 63 % (17/27) of the samples produced a clear zone of lysis when spotted on the indicator strain suggesting the presence of lytic phage (**Supplementary Table 1**). To maximize the likelihood of further characterizing only genetically distinct phages, we selected plaques with distinct morphologies from two samples (**Figure 1**) for further analysis. Hereafter, these are referred to as Phage vB_SmaP_10b_1 (Sm10b_1) and Phage vB_SmaS_12 (Sm12). Visualization of the phages by TEM revealed phage Sm10b_1 to be a podophage (mean capsid diameter 61.72 nm) and phage Sm12 to be a siphophage (mean capsid diameter 66.33 nm, mean tail length 183.58 nm).

The host range for each phage was determined against a panel of 19 *Serratia* spp. comprising 12 *S. marcescens* isolates, including 8 clinical isolates, 5 clinical isolates of *S. liquefaciens* one environmental isolate of *Serratia plymuthica* and one clinical isolate of *S. odorifera* (**Table 2**). Both phages produced zones of clearing on at least 40 % of the strains included in the panel suggesting relatively broad host ranges. Phage Sm10b_1 exhibited the broadest host range, infecting 52.6 % of strains tested (10/19 strains lysed). Plaques produced by phage Sm12 were seen to be surrounded by large translucent haloes (**Figure 1**), often indicative of a phage-associated depolymerase.

To better characterize the phages, genome sequence data were generated and gene annotations were predicted using Pharokka, a tool developed specifically for the annotation of phage genome (Bouras et al., 2023). Based on Pharokka annotations,

the genome of phage Sm12 was predicted to encode 101 genes, while that of phage Sm10b_1 was predicted to encode 65 genes (**Figure 2**). Both genomes were of high quality according to CheckV (100 % complete, no contamination; DTR high-confidence). Neither phage genome was predicted to encode genes associated with integrases, transcription regulation, CRISPRs, tmRNAs, virulence factors (Virulence Factor Database) or antimicrobial resistance genes (Comprehensive Antibiotic Resistance Database). Sm12 was predicted to encode a Ser tRNA (ID Sm12_tRNA_0001; tRNA1-SerGCT). Sm10b_1 was predicted to encode an RNA polymerase (Sm10b_1_CDS_0050).

A genome-based BLASTN analysis (17 October 2025) identified that phage Sm12 was genetically similar to Klebsiella phage vB_Ko_K4PH164 (91 % coverage, 97 % identity) (accession OY979482; NCBI taxonomy – *Duplodnaviria* › *Heunggongvirae* › *Uroviricota* › *Caudoviricetes* › unclassified *Queuovirinae*). However, it shares little sequence identity with any other entry in NCBI with the next closest nucleotide match being Klebsiella phage vB_KppS-Pokey (8 % coverage, 87 % identity). NCBI BLASTN analysis (17 October 2025) of the *terL* gene sequence of phage Sm12 revealed it shared 95.09 % identity (100 % coverage) with a region of Klebsiella phage vB_Ko_K4PH164.

A genome-based NCBI BLASTN analysis (17 October 2025) showed phage Sm10b_1 was not closely related to any sequence in the public database (e.g. 73.26 % identity and 17 % coverage shared with Cronobacter phage RZ4's genome) (accession LC778449; NCBI taxonomy – *Duplodnaviria › Heunggongvirae › Uroviricota › Caudoviricetes › Autographivirales › Autoscriptoviridae › Stentvirinae › Bonnellvirus*). NCBI BLASTN-based analysis (17

October 2025) of the *terL* gene from phage Sm10b_1 found it shared low identity (73.35 %, 79 % coverage) with a region of Cronobacter phage RZ4's genome.

Proteome-based analyses of the two novel phages' genetic content were undertaken with vConTACT2 and the INPHARED database of 21,625 phage proteomes (**Supplementary Figure 1**). The VCs the two phages belonged to were identified using first neighbour analysis (undirected) in Cytoscape: Sm12 had 247 first neighbours (**Figure 3A**), while Sm10b_1 had 25 first neighbours (**Figure 4A**). Phage Sm12 was found to be closely associated with Klebsiella phage vB_Ko_K4PH164 and other members of the subfamily *Queuovirinae* (namely, *Nonagvirus*, *Seuratvirus* and *Nipunavirus*) (**Figure 3A**), while phage Sm10b_1 was related to members of the order *Autographivirales* (**Figure 4A**).

The genomes of the two sets of first neighbours were annotated using Pharokka and included in ViPTree analyses with the genomes of phage Sm12 (**Figure 3B**) and phage Sm10b_1 (**Figure 4B**), and included in VIRIDIC-based analyses (**Supplementary Figure 2** and **Supplementary Figure 3**). VIRIDIC uses intergenomic similarity thresholds to define phages that belong to the same species (95 %) and genera (70 %). TerL-based analyses were also undertaken for the two phages and their closest relatives (**Figure 3C**, **Figure 4C**).

VIRIDIC analysis of phage Sm12's genome with 62 members of the subfamily *Queuovirinae* showed it shared 89.5 % intergenomic similarity with the genome of Klebsiella phage vB_Ko_K4PH164 (**Supplementary Figure 2**). The genomes of phages Sm12 and vB_Ko_K4PH164 shared 54.0 to 55.3 % intergenomic similarity with Enterobacter phage EC151 (accession MW464860; NCBI taxonomy – *Duplodnaviria* › *Heunggongvirae* › *Uroviricota* › *Caudoviricetes* › unclassified

Queuovirinae) and <20 % intergenomic similarity with all other phage genomes included in the VIRIDIC analysis (**Supplementary Figure 2**). Enterobacter phage EC151, previously described as representing a novel genus related to the genera *Seuratvirus*, *Nonagvirus*, and *Vidquintavirus* (Morozova et al., 2021) was the closest relative of both phage Sm12 and phage vB_Ko_K4PH164 in our ViPTree analysis (**Figure 3B**). TerL-based analysis confirmed this association (100 % bootstrap support (**Figure 3C**). Taken together, our findings from proteome- and genome-based analyses show phage Sm12 and phage vB_Ko_K4PH164 represent two different species of phage belonging to the same novel genus within the subfamily *Queuovirinae*. The genomic arrangement of Sm12 and vB_Ko_K4PH164 showed a high level of synteny (**Supplementary Figure 4**). Previous analysis of phage host recognition proteins encoded by vB_Ko_K4PH164 identified the central tail fiber J protein (accession CAK6605323) as the primary receptor binding protein and the adjacent tail protein (accession CAK6605343) as a potential depolymerase (Ferriol-González et al., 2024). Alignment of these two proteins with their homologues from Sm12 revealed a high degree of amino acid conservation (**Supplementary Figures 5 and 6**). The central tail fibers shared 98.9 % amino acid identity whilst the predicted depolymerases shared 97.8 % identity across the aligned regions.

Our analyses of members of the subfamily *Queuovirinae* also confirmed Escherichia phage PLYYY01 (accession MZ911962) to be a species of the genus *Seuratvirus* (77.1–84.1 % intergenomic similarity, **Supplementary Figure 2**; clusters with the genus based on ViPTree and TerL analyses, **Figure 3B**, **3C**).

According to its VIRIDIC-based analysis, phage Sm10b_1 did not belong to any existing genus of the order *Autographivirales* , sharing between 4.5 and 37.3 % intergenomic similarity with its closest relatives (**Supplementary Figure 3**). Pantoea

phage LIMEzero

[*Duplodnaviria › Heunggongvirae › Uroviricota › Caudoviricetes › Autographivirales › Autoscriptoviridae › Stentvirinae › Waewaevirus › Waewaevirus limezero*;

(Adriaenssens et al., 2011) showed a similar pattern of intergenomic similarity with its closest relatives but shared only 30.6 % intergenomic similarity with phage Sm10b_1. ViPTree- and TerL-based analyses of phage Sm10b_1 and its closest relatives showed the novel phage clustered with members of the genera *Waewaevirus* and *Bonnellvirus* but was distinct from these taxa, with 100 % bootstrap support for the association in the TerL-based analysis (**Figure 4B**, **4C**). Taken together, our findings from proteome- and genome-based analyses show phage Sm10b_1 represents a novel genus within the order *Autographivirales* .

As expected from our phylogenetic analyses (**Figure 4B, 4C**), members of the genus *Bonnellvirus* clustered together in the VIRIDIC-based analysis (**Supplementary Figure 3**), confirming Cronobacter phage RZ4's affiliation with the genus.

In vivo efficacy of phage Sm10b_1 in *G. mellonella*

Based on the wider host range of phage Sm10b_1 and its capacity to cause clear zones of lysis on a second *Serratia* species (*S. odorifera*, **Table 2**), we sought to determine its therapeutic potential *in vivo* using the *G. mellonella* larvae model. *Galleria* larvae were injected with a lethal dose of either *S. marcescens* UMH9 (clinical bloodstream isolate) or *S. odorifera* Me113 (clinical catheter-associated urinary tract infection isolate) followed by an injection of phage Sm10b_1 ($1x10^7$ pfu) 30 min later. Survival of the treated group was compared to that of an untreated cohort over a six-day period. The results in **Figure 5a** show that untreated larvae

injected with *S. marcescens* UMH9 quickly succumbed to infection, with 100 % mortality seen by 24 h. In comparison, larvae treated with Sm10b_1 had a survival rate of 67 % at the end of the six-day study period with phage treatment significantly improving larvae survival versus non-treated controls ($P < 0.0001$, log-rank test). Similarly, phage treatment was found to be effective at preventing lethal infection with *S. odorifera*, with only 7 % of untreated larvae surviving for six days compared to 70 % survival in the treated group (**Figure 5b**) ($P < 0.0001$, log-rank test). In both experiments, controls of non-injected larvae and larvae injected with SM buffer only showed 100 % survival at the end of the study period.

In-silico identification of *Serratia* proteases

As *Serratia* spp. are known to produce the insecticidal proteins serralysin (PrtS) and the serralysin-like proteases SlpB, SlpC, SlpC and SlpE, we undertook a bioinformatic analysis of the genomes of *S. marcescens* UMH9 and *S. odorifera* Me113 to identify potential homologous of these proteins (**Table 3**). We identified that *S. marcescens* UMH9 contained homologues of serralysin (*prtS*), *slpC*, *slpD* and *slpE*. *S. odorifera* contained a homologue of *prtS.*

DISCUSSION

Phage therapy has been investigated for the treatment of numerous important antibiotic-resistant pathogens, including carbapenem-resistant *Acinetobacter baumannii* (Jeon et al., 2019), vancomycin-resistant *Enterococcus faecium* (Biswas et al., 2002) and multidrug-resistant *Staphylococcus aureus* (Kifelew et al., 2020). Favourable outcomes in animal models have resulted in phage therapy reaching

clinical trials for the treatment of various diseases (Karn et al., 2024; Kim et al., 2024; Tamma et al., 2022; Wright et al., 2009). The rise of multidrug-resistant *Serratia* spp. poses a significant threat to hospitalised and immunocompromised patients, and phage therapy may provide a viable alternative treatment. To this end, we sought to isolate virulent phages from rivers using a clinical isolate of *S. marcescens* as a host. Our results suggest that phages of *S. marcescens* may be prevalent in river water located in the vicinity of sewage outlets. We found that water samples collected from distinct locations produced lytic plaques on UMH9 following phage enrichment. Others have also documented that *Serratia* phages are readily isolated from water and sewage (Casey et al., 2017; Hao et al., 2018; Li et al., 2022; Williams et al., 2019) in addition to a diverse range of other locations including compost (Casey et al., 2017) and pig farms (Price et al., 2019).

Although phage therapy is available in countries including Russia and Georgia (Międzybrodzki et al., 2021), the regulatory framework around the future use of phages in human medicine is likely to differ between geographical regions and is still developing (Yang et al., 2023). It has been suggested that high-quality genome sequences should be available for phages used in a therapeutic setting (Abedon et al., 2017; Philipson et al., 2018; Suh et al., 2022). This allows for the detection of genetic elements which may render the phages unsuitable for use *in vivo*. This includes the presence of integrase genes which suggest a temperate lifestyle and the potential for transfer of toxin genes or antibiotic resistance determinants (Torres-Barceló, 2018). The high-quality complete genomes of Sm10b_1 and Sm12 were found to be free from integrases, virulence factors and antimicrobial resistance genes, making them suitable candidates for future phage therapy from a genetic standpoint. Genome sequencing also assists with taxonomy and it can be possible to

infer shared properties if phages share significant sequence similarity. Guidelines from the International Committee on Taxonomy of Viruses suggest that, for genome-based phage taxonomy, assignment to taxonomic ranks should be based on nucleotide sequence identity across entire genomes (Turner et al., 2021). The starting point for such analyses is often identifying closest nucleotide relatives using the basic local alignment search tool (BLAST) and the NCBI database of nucleotide sequences. Our initial BLAST searches identified that Sm12 shared little sequence identity with any entry in the database whereas Sm10b_1 appeared highly related to Klebsiella phage vB_Ko_K4PH164. According to NCBI taxonomy data, Klebsiella phage vB_Ko_K4PH164 is currently assigned as an unclassified member of the sub-family *Queuovirinae* within the *Caudoviricetes* and does not belong to any recognised genus (currently there are eight recognised genera within the sub-family). The ICTV has established that phages belong to the same genus if they share equal to or greater than 70 % nucleotide identity across the full genome (Turner et al., 2021). Using the protein-sharing network tool vConTACT2 and the INPHARED database of phage genomes we were able to identify other phages which formed VCs with Sm10b_1 and Sm12. We then used VIRIDIC to establish the intergenomic similarity between phages identified within these clusters. Our analyses identified that the VC containing Sm12 contained 6 potentially novel genera within the subfamily *Queuovirinae* whilst the VC containing Sm10b_1 contained a total of three potentially novel genera within the order *Autographivirales* . The affiliation of Sm10b_1 to this family is further strengthened by the presence of an RNA polymerase gene within the genome, recognised as a “signature” gene of the family (Lavigne et al., 2008).

Taxonomic assignment to these novel genera was confirmed by whole proteome phylogenetic analysis using ViPTree and further phylogenetic analysis using the terminase large subunit protein sequence (TerL). TerL, is considered a 'hallmark' protein that is frequently used for phylogenetic reconstruction of phages belonging to the *Caudoviricites* (Benler et al., 2021). Trees generated using either whole proteomes or TerL sequences were highly similar. Both approaches resulted in the same nearest neighbours being identified for both phages. These results further highlight the potential for TerL-based phylogeny of *Caudoviricites*, especially where whole genome sequences may not be available.

We found that Sm12 belonged to a new genus with Klebsiella phage vB_Ko_K4PH164. Interestingly, vB_Ko_K4PH164 was isolated using a panel of 77 *Klebsiella* spp. reference strains (Ferriol-González et al., 2024). It is often cited that closely related phages are expected to infect closely related hosts, with host tropism often restricted at the species level (Beatriz Beamud et al., 2023). However, broad host range phages have been described in the literature, some with inter-genus infectivity (Dhungana et al., 2024; Gambino et al., 2020; Hamdi et al., 2017). With regard to *Serratia* phages, phage ΦOT8 originally isolated on *Serratia* sp. ATCC 39006 has been shown to also infect *Pantoea agglomerans* (Evans et al., 2010). Additionally, *S. marcescens* phage UFV01 has been shown to inhibit biofilm formation by members of two different genera belonging to the family *Enterobacteriaceae*, suggesting that some *Serratia* phages can interact with biological receptors belonging to genetically disparate hosts (Vieira et al., 2021). The high level of amino acid sequence conservation between the host recognition proteins (tail fiber protein and predicted depolymerase) of Sm12 and vB_Ko_K4PH164 suggests that these phages may bind similar receptors and share

host-tropism. However, a slight truncation at the C-terminus of the predicted Sm12 depolymerase was observed (31 amino acids fewer than that of vB_Ko_K4PH164). Thus, future work should explore the potential of phage Sm12 to infect members of the genus *Klebsiella.* The therapeutic potential of phage Sm12 would be broadened if capable of infecting two important opportunistic pathogens.

Currently, there are few studies on the therapeutic potential of *Serratia* phages, with most investigations limited to *in vitro* characterization of traits such as host range or biofilm reduction (Dapuliga et al., 2026; Matsushita et al., 2009; Tian et al., 2019). A 2020 study highlighted the potential for *S. marcescens* phage/antibiotic synergy. Application of phage SALSA in combination with ampicillin/sulbactam was found to eradicate drug-resistant bacterial populations where phage treatment alone could not (Weber et al., 2020). A recent study investigated the clinical application of *S. marcescens* phage Spe5P4 to treat a patient suffering from a refractory drug-resistant *S. marcescens*-induced pulmonary infection (Duan et al., 2025). Phage administration in combination with antibiotics showed good efficacy with amelioration of clinical symptoms and no relapse during the follow-up period (140 days after phage treatment). Our results, highlighting that phage Sm12 can rescue *G. mellonella* larvae from otherwise lethal *S. marcescens* and *S. odorifera* infections, build on evidence that phage therapy is a suitable alternative for the treatment of infections caused by *Serratia* spp. We found that *G. mellonella* larvae were highly susceptible to infections caused by *Serratia* spp. We observed that 10 cfu of *S. marcescens* UMH9 caused 100 % mortality within 24 h and the same dose of *S. odorifera* resulted in 90 % mortality within 72 h. *Serratia* spp. are recognised as insect pathogens. For example, *S. marcescens* is known to be pathogenic to over 70 insect species (Grimont et al., 1979). The LD_{50} for *G. mellonella* infected with certain

strains of *S. marcescens* has been reported to be as low as 1 cfu (Chadwick et al., 1990) and others have reported 100 % mortality of larvae infected with 500 cfu (Duan et al., 2025). Previous reports have identified that *S. marcescens* produces a range of enzymes that are highly toxic to *G. mellonella* larvae (Kaska, 1976; Lysenko, 1976; Patil et al., 2011). The most well studied of these insecticidal toxins is the enzyme serralysin (also known as PrtS), a metalloprotease that causes haemolymph bleeding and supresses cellular immunity in silkworms (Ishii et al., 2014). It has also been implicated in pathogenicity towards *G. mellonella* (Tambong et al., 2014). Additionally, serralysin has been shown to kill mammalian cells (Marty et al., 2002) and degrade components of the immune system (Molla et al., 1988). *S. marcescens* is also known to encode a group of proteins known as the serralysin-like proteases (SlpB, SlpC, SlpD and SlpE). SlpB and SlpE have been shown to possess cytotoxic activity towards airway and ocular cell lines *in vitro* (Shanks et al., 2015; Stella et al., 2017). Our analyses identified homologues of serralysin (*prtS*) in both *S. marcescens* UMH9 and *S. odorifera* Me113, whilst UMH9 also contained genes sharing high sequence similarity with *slpC*, *slpD* and *slpE*. It is highly likely these proteases contribute to the rapid death of the larvae in our experiments and that the presence of additional metalloproteases in the genome of UMH9 may account for the rapid death of larvae infected with this strain.

In summary, we have isolated and characterized two novel virulent phages that lyse clinically relevant members of the genus *Serratia*. Extended comparative genomics utilising the INPHARED curated database of phage genomes identified phage Sm10b_1 represents a novel genus within the order *Autographivirales* whilst phage Sm12 belongs to a novel genus within the subfamily *Queuovirinae*. As part of these analyses we identified a total of three novel genera in the order

Autographivirales and six novel genera in the subfamily *Queuovirinae.* Therapeutic profiling of Sm10b_1 utilising a *G. mellonella* model of infection revealed phage therapy can rescue larvae from lethal infections caused by clinical *Serratia* spp. This study contributes to phage taxonomy and the limited data on the therapeutic efficacy of *Serratia* phages.

Acknowledgements

We thank Professor Harry L. T. Mobley, University of Michigan, USA for providing *S. marcescens* UMH9.

Conflicts of interest

None declared.

Author Contributions

MH and YM; Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. CW; Investigation, Methodology, Writing - review & editing. ME; Investigation, Writing - review & editing. LH; Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing - original draft, Writing - review & editing. DN; Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, , Supervision, Writing - original draft, Writing - review & editing.

Funding

CW was funded by a BBSRC doctoral training programme studentship. ME was supported by the Egyptian Ministry of Higher Education & Scientific Research represented by the Egyptian Bureau for Cultural & Educational Affairs in London.

Ethical Approval

The study of anonymised clinical isolates provided by the Nottingham University Hospitals NHS Trust (NUH) Pathogen Bank was approved by NUH Research and Innovation (19MI001).

Data availability

Genome sequences of the bacteriophages isolated as part of this study are available at NCBI under accessions PX000100 and PX000101. Annotated genome files can be accessed via figshare. Supporting data relating to all experiments are available in the online supplementary material

References

References

A. Hejazi, & F. R. Falkiner. (1997). Serratia marcescens. *Journal of Medical Microbiology, 46*(11), 903–912. 10.1099/00222615-46-11-903

Abedon, S. T., García, P., Mullany, P., & Aminov, R. (2017). Editorial: Phage Therapy: Past, Present and Future. *Frontiers in Microbiology, 8*, 981. 10.3389/fmicb.2017.00981

Adriaenssens, E. M., Ceyssens, P., Dunon, V., Ackermann, H., Van Vaerenbergh, J., Maes, M., De Proft, M., & Lavigne, R. (2011). Bacteriophages LIMElight and LIMEzero of Pantoea agglomerans, Belonging to the “phiKMV-Like Viruses”. *Applied and Environmental Microbiology, 77*(10), 3443–3450. 10.1128/AEM.00128-11

Anderson, M. T., Himpsl, S. D., Kingsley, L. G., Smith, S. N., Bachman, M. A., & Mobley, H. L. T. (2025). Infection characteristics among Serratia marcescens capsule lineages. *mBio,* , e0055925. 10.1128/mbio.00559-25

Anderson, M. T., Himpsl, S. D., Mitchell, L. A., Kingsley, L. G., Snider, E. P., & Mobley, H. L. T. (2022). Identification of distinct capsule types associated with Serratia marcescens infection isolates. *PLoS Pathogens, 18*(3), e1010423. 10.1371/journal.ppat.1010423

Anderson, M. T., Mitchell, L. A., Zhao, L., & Mobley, H. L. T. (2017). Capsule Production and Glucose Metabolism Dictate Fitness during Serratia marcescens Bacteremia. *mBio, 8*(3)10.1128/mBio.00740-17

Armstrong, P. J. (1984). Systemic Serratia marcescens infections in a dog and a cat. *Journal of the American Veterinary Medical Association, 184*(9), 1154–1158. 10.2460/javma.1984.184.09.1154

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology, 19*(5), 455–477. 10.1089/CMB.2012.0021

Beatriz Beamud, Neris García-González, Mar Gómez-Ortega, Fernando González-Candelas, Pilar Domingo-Calap, & Rafael Sanjuan. (2023). Genetic determinants of host tropism in Klebsiella phages Genetic determinants of host tropism in Klebsiella phages. *Cell Reports (Cambridge), 42*(2), 112048. https://doaj.org/article/66e865fdb20847649b53ffc620ae2d4d

Benler, S., Yutin, N., Antipov, D., Rayko, M., Shmakov, S., Gussow, A. B., Pevzner, P., & Koonin, E. V. (2021). Thousands of previously unknown phages discovered in whole-community human gut metagenomes. *Microbiome, 9*(1), 78–17. 10.1186/s40168-021-01017-w

Biswas, B., Adhya, S., Washart, P., Paul, B., Trostel, A. N., Powell, B., Carlton, R., & Merril, C. R. (2002). Bacteriophage Therapy Rescues Mice Bacteremic from a Clinical Isolate of Vancomycin-Resistant Enterococcus faecium. *Infection and Immunity, 70*(1), 204–210. 10.1128/IAI.70.1.204-210.2002

Bolduc, B., Jang, H. B., Doulcier, G., You, Z., Roux, S., & Sullivan, M. B. (2017). vConTACT: an iVirus tool to classify double-stranded DNA viruses that infect Archaea and Bacteria. *PeerJ (San Francisco, CA), 5*, e3243. 10.7717/peerj.3243

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a Flexible Trimmer for Illumina Sequence Data. *Bioinformatics (Oxford, England), 30*(15), 2114–2120. 10.1093/bioinformatics/btu170

Bouras, G., Nepal, R., Houtak, G., Psaltis, A. J., Wormald, P., & Vreugde, S. (2023). Pharokka: a fast scalable bacteriophage annotation tool. *Bioinformatics (Oxford, England), 39*(1)10.1093/bioinformatics/btac776

Casey, E., Fitzgerald, B., Mahony, J., Lugli, G. A., Ventura, M., & van Sinderen, D. (2017). Genome Sequence of Serratia marcescens Phage BF. *Genome Announcements, 5*(23), e00211. 10.1128/genomea.00211-17

Chadwick, J. S., Caldwell, S. S., & Chadwick, P. (1990). Adherence patterns and virulence for Galleria mellonella larvae of isolates of Serratia marcescens. *Journal of Invertebrate Pathology, 55*(1), 133–134. 10.1016/0022-2011(90)90044-7

Chen, L., Ai, W., Zhou, Y., Wu, C., Guo, Y., Wu, X., Wang, B., Rao, L., Xu, Y., Zhang, J., Chen, L., & Yu, F. (2022). Outbreak of IncX8 Plasmid–Mediated KPC-3–Producing Enterobacterales Infection, China. *Emerging Infectious Diseases, 28*(7), 1421–1430. 10.3201/eid2807.212181

Cook, R., Brown, N., Redgwell, T., Rihtman, B., Barnes, M., Clokie, M., Stekel, D. J., Hobman, J., Jones, M. A., & Millard, A. (2021). INfrastructure for a PHAge REference Database: Identification of Large-Scale Biases in the Current

Collection of Cultured Phage Genomes. *PHAGE, 2*(4), 214–223. 10.1089/phage.2021.0007

Dapuliga, C. C., Claussen, M., & Schmidt, S. (2026). A novel Serratia marcescens bacteriophage from South Africa – a potential tool to tackle produce-associated antibiotic-resistant Serratia marcescens. *Food Control, 179*, 111534. 10.1016/j.foodcont.2025.111534

Dhungana, G., Nepal, R., Houtak, G., Bouras, G., Vreugde, S., & Malla, R. (2024). Preclinical characterization and in silico safety assessment of three virulent bacteriophages targeting carbapenem-resistant uropathogenic Escherichia coli. *International Microbiology, 27*(6), 1747–1763. 10.1007/s10123-024-00508-8

Duan, X., Liu, W., Xiao, Y., Rao, M., Ji, L., Wan, X., Han, S., Lin, Z., Liu, H., Chen, P., Qiao, K., Zheng, M., Shen, J., Zhou, Y., Asakawa, T., Xiao, M., & Lu, H. (2025). Exploration of the feasibility of clinical application of phage treatment for multidrug-resistant Serratia marcescens -induced pulmonary infection. *Emerging Microbes & Infections, 14*(1), 2451048. 10.1080/22221751.2025.2451048

Eladawy, M., Heslop, N., Negus, D., Thomas, J. C., & Hoyles, L. (2025). Phenotype–genotype discordance in antimicrobial resistance profiles of Gram-negative uropathogens recovered from catheter-associated urinary tract infections in Egypt. *Journal of Antimicrobial Chemotherapy,* 10.1093/jac/dkaf352

Engel, H. J., Collignon, P. J., Whiting, P. T., & Kennedy, K. J. (2009). Serratia sp. bacteremia in Canberra, Australia: a population-based study over 10 years. *European Journal of Clinical Microbiology & Infectious Diseases, 28*(7), 821–824. 10.1007/s10096-009-0707-7

Evans, T. J., Crow, M. A., Williamson, N. R., Orme, W., Thomson, N. R., Komitopoulou, E., & Salmond, G. P. C. (2010). Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, Serratia and Pantoea. *Microbiology (Society for General Microbiology), 156*(1), 240–247. 10.1099/mic.0.032797-0

Ferriol-González, C., Concha-Eloko, R., Bernabéu-Gimeno, M., Fernández-Cuenca, F., Cañada-García, J. E., García-Cobos, S., Sanjuán, R., & Domingo-Calap, P. (2024). Targeted phage hunting to specific Klebsiella pneumoniae clinical isolates is an efficient antibiotic resistance and infection control strategy. *Microbiology Spectrum, 12*(10), e0025424. 10.1128/spectrum.00254-24

Flyg, C., Kenne, K., & Boman, H. G. (1980). Insect pathogenic properties of Serratia marcescens: phage-resistant mutants with a decreased resistance to Cecropia immunity and a decreased virulence to Drosophila. *Journal of General Microbiology, 120*(pt.1 p.173-181), 173–181. https://www.proquest.com/docview/45697196

Gambino, M., Nørgaard Sørensen, A., Ahern, S., Smyrlis, G., Gencay, Y. E., Hendrix, H., Neve, H., Noben, J., Lavigne, R., & Brøndsted, L. (2020). Phage S144, a New Polyvalent Phage Infecting Salmonella spp. and Cronobacter sakazakii. *International Journal of Molecular Sciences, 21*(15), 5196. 10.3390/ijms21155196

Grimont, P. A. D., Grimont, F., & Lysenko, O. (1979). Species and biotype identification of Serratia strains associated with insects [Reevaluation of the idea of using Serratia to biologically control insects, vectors]. *Current Microbiology, 2*(3), 139–142. 10.1007/BF02605870

Guardo, G. D., Battisti, A., Agrimi, U., Forletta, R., Reitano, M. E., & Calderini, P. (1997). Pathology of Serratia marcescens Mastitis in Cattle. *Journal of Veterinary Medicine. Series B, 44*(1-10), 537–546. 10.1111/j.1439-0450.1997.tb01005.x

Haines, M. E. K., Hodges, F. E., Nale, J. Y., Mahony, J., van Sinderen, D., Kaczorowska, J., Alrashid, B., Akter, M., Brown, N., Sauvageau, D., Sicheritz-Pontén, T., Thanki, A. M., Millard, A. D., Galyov, E. E., & Clokie, M. R. J. (2021). Analysis of Selection Methods to Develop Novel Phage Therapy Cocktails Against Antimicrobial Resistant Clinical Isolates of Bacteria. *Frontiers in Microbiology, 12*, 613529. 10.3389/fmicb.2021.613529

Hamdi, S., Rousseau, G. M., Labrie, S. J., Tremblay, D. M., Kourda, R. S., Ben Slama, K., & Moineau, S. (2017). Characterization of two polyvalent phages infecting Enterobacteriaceae. *Scientific Reports, 7*(1), 40349–40349. 10.1038/srep40349

Hao, Y., Shi, H., Li, J., Zhao, C., Huang, H., & Sun, Y. (2018). Complete Genome Sequence of Bacteriophage SM9-3Y Infecting Serratia marcescens. *Genome Announcements (Washington, DC), 6*(1)10.1128/genomeA.01270-17

Hietala, V., Horsma-Heikkinen, J., Carron, A., Skurnik, M., & Kiljunen, S. (2019). The Removal of Endo- and Enterotoxins From Bacteriophage Preparations. *Frontiers in Microbiology, 10*, 1674. 10.3389/fmicb.2019.01674

Hopkins, K. L., Findlay, J., Meunier, D., Cummins, M., Curtis, S., Kustos, I., Mustafa, N., Perry, C., Pike, R., & Woodford, N. (2017). Serratia marcescens producing SME carbapenemases: an emerging resistance problem in the UK? *Journal of Antimicrobial Chemotherapy, 72*(5), dkw567–1537. 10.1093/jac/dkw567

Ishii, K., Adachi, T., Hamamoto, H., & Sekimizu, K. (2014). Serratia marcescens Suppresses Host Cellular Immunity via the Production of an Adhesion-inhibitory Factor against Immunosurveillance Cells. *Journal of Biological Chemistry, 289*(9), 5876–5888. 10.1074/jbc.M113.544536

Jeon, J., Park, J., & Yong, D. (2019). Efficacy of bacteriophage treatment against carbapenem-resistant Acinetobacter baumannii in Galleria mellonella larvae and a mouse model of acute pneumonia. *BMC Microbiology, 19*(1), 70. 10.1186/s12866-019-1443-5

Karn, S. L., Bhartiya, S. K., Pratap, A., Saroj, S. K., Kumar, R., Sahu, M., Gangwar, M., & Nath, G. (2024). A Randomized, Placebo-controlled, Double-blind Clinical Trial of Bacteriophage Cocktails in Chronic Wound Infections. *International Journal of Lower Extremity Wounds,* , 1534734623122634 2. 10.1177/15347346231226342

Kaska, M. (1976). The toxicity of extracellular proteases of the bacterium Serratia marcescens for larvae of greater wax moth, Galleria mellonella. *Journal of Invertebrate Pathology, 27*(2), 271. 10.1016/0022-2011(76)90158-0

Kifelew, L. G., Warner, M. S., Morales, S., Vaughan, L., Woodman, R., Fitridge, R., Mitchell, J. G., & Speck, P. (2020). Efficacy of phage cocktail AB-SA01 therapy in diabetic mouse wound infections caused by multidrug-resistant Staphylococcus aureus. *BMC Microbiology, 20*(1), 204. 10.1186/s12866-020-01891-8

Kim, P., Sanchez, A. M., Penke, T. J. R., Tuson, H. H., Kime, J. C., McKee, R. W., Slone, W. L., Conley, N. R., McMillan, L. J., Prybol, C. J., & Garofolo, P. M. (2024). Safety, pharmacokinetics, and pharmacodynamics of LBP-EC01, a CRISPR-Cas3-enhanced bacteriophage cocktail, in uncomplicated urinary tract

infections due to Escherichia coli (ELIMINATE): the randomised, open-label, first part of a two-part phase 2 trial. *The Lancet Infectious Diseases, 24*(12), 1319–1332. 10.1016/S1473-3099(24)00424-9

Lavigne, R., Seto, D., Mahadevan, P., Ackermann, H., & Kropinski, A. M. (2008). Unifying classical and molecular taxonomic classification: analysis of the Podoviridae using BLASTP-based tools. *Research in Microbiology, 159*(5), 406–414. 10.1016/j.resmic.2008.03.005

Li, Y., Han, P., Pu, M., Li, F., Li, M., An, X., Song, L., Fan, H., Tong, Y., & Chen, Z. (2022). Genomic Analysis of the Serratia marcescens Bacteriophage BUCT660. *Microbiology Resource Announcements, 11*(8), e0040622. 10.1128/mra.00406-22

Lysenko, O. (1976). Chitinase of Serratia marcescens and its toxicity to insects. *Journal of Invertebrate Pathology, 27*(3), 385–386. 10.1016/0022-2011(76)90101-4

Mahlen, S. D. (2011). Serratia Infections: from Military Experiments to Current Practice. *Clinical Microbiology Reviews, 24*(4), 755–791. 10.1128/CMR.00017-11

Marty, K. B., Williams, C. L., Guynn, L. J., Benedik, M. J., & Blanke, S. R. (2002). Characterization of a Cytotoxic Factor in Culture Filtrates of Serratia marcescens. *Infection and Immunity, 70*(3), 1121–1128. 10.1128/IAI.70.3.1121-1128.2002

Matsushita, K., Uchiyama, J., Kato, S., Ujihara, T., Hoshiba, H., Sugihara, S., Muraoka, A., Wakiguchi, H., & Matsuzaki, S. (2009). Morphological and genetic analysis of three bacteriophages of Serratia marcescens isolated from

environmental water. *FEMS Microbiology Letters, 291*(2), 201–208. 10.1111/j.1574-6968.2008.01455.x

Międzybrodzki, R., Hoyle, N., Zhvaniya, F., Łusiak-Szelachowska, M., Weber-Dąbrowska, B., Łobocka, M., Borysowski, J., Alavidze, Z., Kutter, E., Górski, A., & Gogokhia, L. (2021). Current Updates from the Long-Standing Phage Research Centers in Georgia, Poland, and Russia. *Bacteriophages* (pp. 921–951). Springer International Publishing. 10.1007/978-3-319-41986-2_31

Molla, A., Kagimoto, T., & Maeda, H. (1988). Cleavage of immunoglobulin G (IgG) and IgA around the hinge region by proteases from Serratia marcescens. *Infection and Immunity, 56*(4), 916–920. 10.1128/iai.56.4.916-920.1988

Moradigaravand, D., Boinett, C. J., Martin, V., Peacock, S. J., & Parkhill, J. (2016). Recent independent emergence of multiple multidrug-resistant Serratia marcescens clones within the United Kingdom and Ireland. *Genome Research, 26*(8), 1101–1109. 10.1101/gr.205245.116

Morozova, V., Jdeed, G., Kozlova, Y., Babkin, I., Tikunov, A., & Tikunova, N. (2021). A New Enterobacter cloacae Bacteriophage EC151 Encodes the Deazaguanine DNA Modification Pathway and Represents a New Genus within the Siphoviridae Family. *Viruses, 13*(7), 1372. 10.3390/v13071372

Nayfach, S., Camargo, A. P., Schulz, F., Eloe-Fadrosh, E., Roux, S., & Kyrpides, N. C. (2021). CheckV assesses the quality and completeness of metagenome-assembled viral genomes. *Nature Biotechnology, 39*(5), 578–585. 10.1038/s41587-020-00774-7

Nishimura, Y., Yoshida, T., Kuronishi, M., Uehara, H., Ogata, H., & Goto, S. (2017). ViPTree: the viral proteomic tree server. *Bioinformatics, 33*(15), 2379–2380. http://hdl.handle.net/2433/226604

Patil, C. D., Patil, S. V., Salunke, B. K., & Salunkhe, R. B. (2011). Prodigiosin produced by Serratia marcescens NMCC46 as a mosquito larvicidal agent against Aedes aegypti and Anopheles stephensi. *Parasitology Research, 109*(4), 1179–1187. 10.1007/s00436-011-2365-9

Philipson, C., Voegtly, L., Lueder, M., Long, K., Rice, G., Frey, K., Biswas, B., Cer, R., Hamilton, T., & Bishop-Lilly, K. (2018). Characterizing Phage Genomes for Therapeutic Applications. *Viruses, 10*(4), 188. 10.3390/v10040188

Price, L., Rohren, M., Newkirk, H., Liu, M., & Ramsey, J. (2019). Complete Genome Sequence of Serratia marcescens Myophage Moabite. *Microbiology Resource Announcements, 8*(29)10.1128/MRA.00741-19

Salsabil Makky, Alyaa Dawoud, Anan Safwat, Abdallah S. Abdelsattar, Nouran Rezk, & Ayman El-Shibiny. (2021). The bacteriophage decides own tracks: When they are with or against the bacteria The bacteriophage decides own tracks: When they are with or against the bacteria. *Current Research in Microbial Sciences, 2*, 100050. https://doaj.org/article/4f4b9ee1147149ffb70f0dd0347c5a44

Shanks, R. M. Q., Stella, N. A., Hunt, K. M., Brothers, K. M., Zhang, L., & Thibodeau, P. H. (2015). Identification of SlpB, a Cytotoxic Protease from Serratia marcescens. *Infection and Immunity, 83*(7), 2907–2916. 10.1128/IAI.03096-14

Smith-Zaitlik, T., Shibu, P., McCartney, A. L., Foster, G., Hoyles, L., & Negus, D. (2022). Extended genomic analyses of the broad-host-range phages vB_KmiM-

2Di and vB_KmiM-4Dii reveal slopekviruses have highly conserved genomes. *Microbiology (Society for General Microbiology), 168*(9)10.1099/mic.0.001247

Stella, N. A., Callaghan, J. D., Zhang, L., Brothers, K. M., Kowalski, R. P., Huang, J. J., Thibodeau, P. H., & Shanks, R. M. Q. (2017). SlpE is a calcium-dependent cytotoxic metalloprotease associated with clinical isolates of Serratia marcescens. *Research in Microbiology, 168*(6), 567–574. 10.1016/j.resmic.2017.03.006

Suh, G. A., Lodise, T. P., Tamma, P. D., Knisely, J. M., Alexander, J., Aslam, S., Barton, K. D., Bizzell, E., Totten, K. M. C., Campbell, J. L., Chan, B. K., Cunningham, S. A., Goodman, K. E., Greenwood-Quaintance, K. E., Harris, A. D., Hesse, S., Maresso, A., Nussenblatt, V., Pride, D., . . . Patel, R. (2022). Considerations for the Use of Phage Therapy in Clinical Practice. *Antimicrobial Agents and Chemotherapy, 66*(3), e0207121. 10.1128/aac.02071-21

Tambong, J. T., Xu, R., Sadiku, A., Chen, Q., Badiss, A., & Yu, Q. (2014). Molecular detection and analysis of a novel metalloprotease gene of entomopathogenic Serratia marcescens strains in infected Galleria mellonella. *Canadian Journal of Microbiology, 60*(4), 203–209. 10.1139/cjm-2013-0864

Tamma, P. D., Souli, M., Billard, M., Campbell, J., Conrad, D., Ellison, D. W., Evans, B., Evans, S. R., Greenwood-Quaintance, K. E., Filippov, A. A., Geres, H. S., Hamasaki, T., Komarow, L., Nikolich, M. P., Lodise, T. P., Nayak, S. U., Norice-Tra, C., Patel, R., Pride, D., . . . Schooley, R. T. (2022). Safety and microbiological activity of phage therapy in persons with cystic fibrosis colonized with Pseudomonas aeruginosa: study protocol for a phase 1b/2, multicenter,

randomized, double-blind, placebo-controlled trial. *Current Controlled Trials in Cardiovascular Medicine, 23*(1), 1057. 10.1186/s13063-022-07047-5

Tavares-Carreon, F., De Anda-Mora, K., Rojas-Barrera, I. C., & Andrade, A. (2023). Serratia marcescens antibiotic resistance mechanisms of an opportunistic pathogen: a literature review. *PeerJ (San Francisco, CA), 11*, e14399. 10.7717/peerj.14399

Tian, C., Zhao, J., Zhang, Z., Chen, X., Wei, X., Li, H., Lin, W., Ke, Y., Hu, L., Jiang, A., Feng, R., Yang, W., Jing, Y., Yuan, J., Luo, Y., & Zhao, X. (2019). Identification and molecular characterization of Serratia marcescens phages vB_SmaA_2050H1 and vB_SmaM_2050HW. *Archives of Virology, 164*(4), 1085–1094. 10.1007/s00705-019-04169-1

Torres-Barceló, C. (2018). The disparate effects of bacteriophages on antibiotic-resistant bacteria. *Emerging Microbes & Infections, 7*(1), 1–12. 10.1038/s41426-018-0169-z

Turner, D., Kropinski, A. M., & Adriaenssens, E. M. (2021). A Roadmap for Genome-Based Phage Taxonomy. *Viruses, 13*(3), 506. 10.3390/v13030506

Vieira, M. S., Duarte da Silva, J., Ferro, C. G., Cunha, P. C., Vidigal, P. M. P., Canêdo da Silva, C., Oliveira de Paula, S., & Dias, R. S. (2021). A highly specific Serratia-infecting T7-like phage inhibits biofilm formation in two different genera of the Enterobacteriaceae family. *Research in Microbiology, 172*(6), 103869. 10.1016/j.resmic.2021.103869

Wang, X., Xiao, W., Li, L., Jing, M., Sun, M., Chang, Y., Qu, Y., Jiang, Y., & Xu, Q. (2022). Analysis of the molecular characteristics of a blaKPC-2-harbouring

untypeable plasmid in Serratia marcescens. *International Microbiology, 25*(2), 237–244. 10.1007/s10123-021-00172-2

Weber, L., Jansen, M., Krüttgen, A., Buhl, E. M., & Horz, H. (2020). Tackling Intrinsic Antibiotic Resistance in Serratia marcescens with a Combination of Ampicillin/Sulbactam and Phage SALSA. *Antibiotics (Basel), 9*(7), 371. 10.3390/antibiotics9070371

Williams, E. A., Hopson, H., Rodriguez, A., Kongari, R., Bonasera, R., Hernandez-Morales, A. C., & Liu, M. (2019). Complete Genome Sequence of Serratia marcescens Siphophage Serbin. *Microbiology Resource Announcements, 8*(19)10.1128/MRA.00422-19

Wishart, D. S., Han, S., Saha, S., Oler, E., Peters, H., Grant, J., Stothard, P., & Gautam, V. (2023). PHASTEST: faster than PHASTER, better than PHAST. *Nucleic Acids Research, 51*(W1), 443. 10.1093/nar/gkad382

Wright, A., Hawkins, C. H., Änggård, E. E., & Harper, D. R. (2009). A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant Pseudomonas aeruginosa; a preliminary report of efficacy. *Clinical Otolaryngology, 34*(4), 349–357. 10.1111/j.1749-4486.2009.01973.x

Yang, Q., Le, S., Zhu, T., & Wu, N. (2023). Regulations of phage therapy across the world. *Frontiers in Microbiology, 14*, 1250848. 10.3389/fmicb.2023.1250848

Zhang, Q., Melcher, U., Zhou, L., Najar, F. Z., Roe, B. A., & Fletcher, J. (2005). Genomic Comparison of Plant Pathogenic and Nonpathogenic Serratia marcescens Strains by Suppressive Subtractive Hybridization. *Applied and Environmental Microbiology, 71*(12), 7716–7723. 10.1128/AEM.71.12.7716-7723.2005

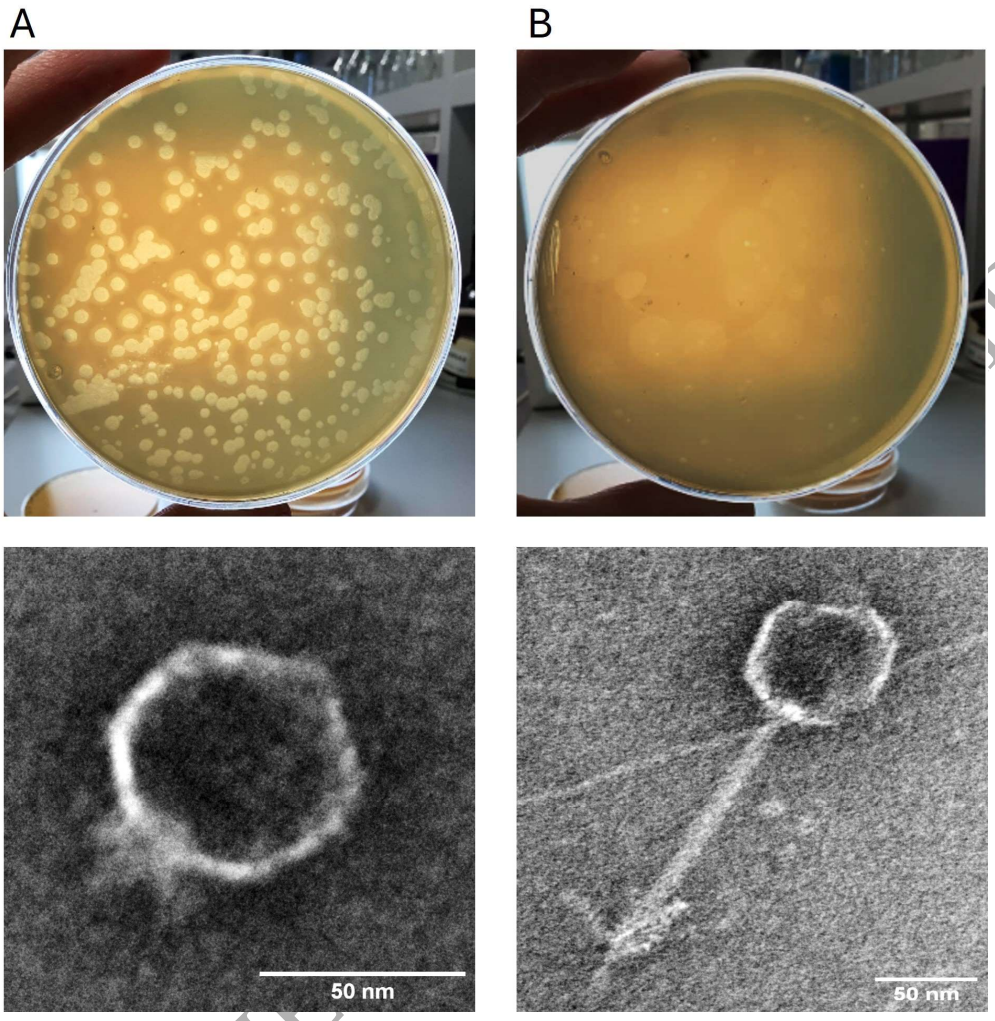

Figure 1. Morphology of the novel *Serratia*-infecting phages. (**A**) phage vB_SmaP_10b_1. (**B**) phage vB_SmaS_12. Top images of each panel show plaque morphologies, while the bottom images show representative TEM micrographs of both phages.

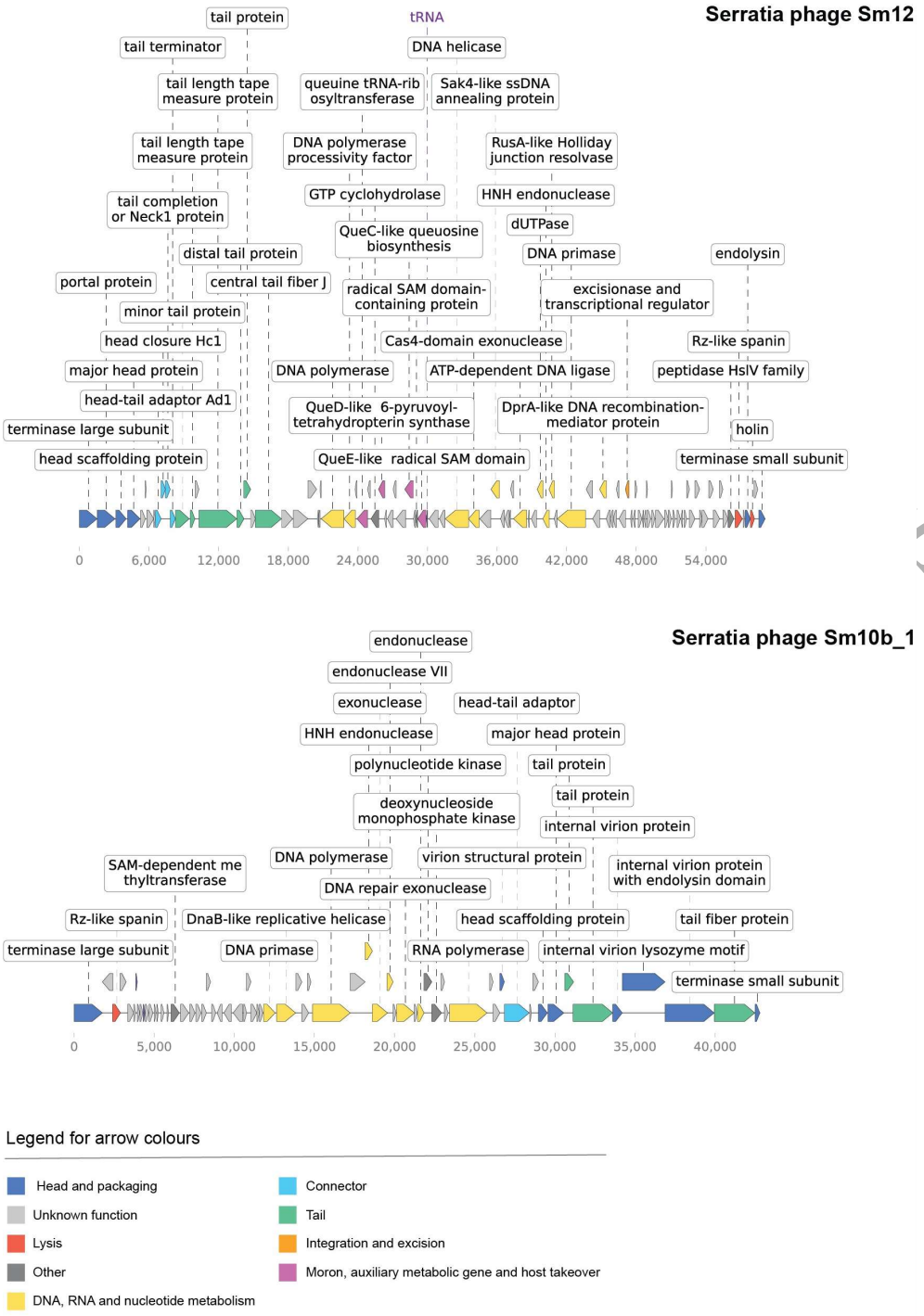

Figure 2. Visualization of gene arrangements for the two novel phages. The images were created using Linear Genome Plot with .gbk annotation files produced by

Pharokka v1.6.1 (v1.4.0 databases). Gene functional annotations were assigned by Pharokka.

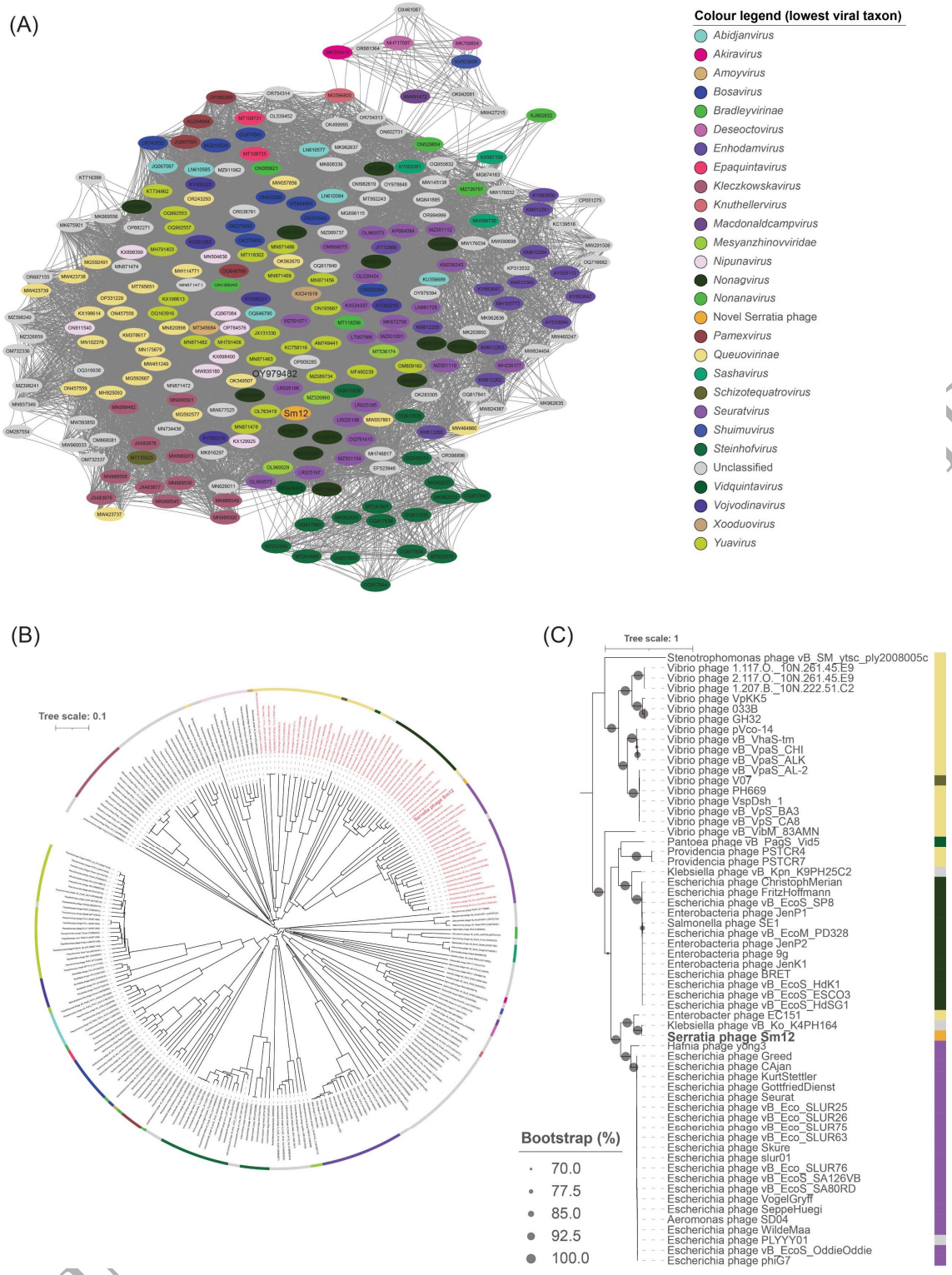

Figure 3. Comparative genomic analyses of phage Sm12. (**A**) First neighbours of Sm12, identified from the vConTACT2 proteome-based network analysis shown in **Supplementary Figure 1**. The subnetwork of neighbours was extracted from the

main network and rearranged using yFiles organic layout. (**B**) ViPTree-based analysis of the genomic data for phage Sm12 and its first neighbours. The tree is rooted at the mid-point, and was constructed based on the distance (1-similarity) matrix (tBLASTx results; bionj option of ViPTree). Phages shown in red were used to produce (**C**), a maximum-likelihood tree (rooted at the mid-point), with bootstrap values shown as a percentage of 100 replications, of TerL sequences of 59 phages most closely related to phage Sm12. No TerL protein was predicted for Vibrio phage PH669 (accession MW423738) or Vibrio phage PH669 (accession MW423739); the TerL sequence for Vibrio phage vB_VnaS-AQKL99 (accession MT795651) was excluded from the analysis shown in (**C**) as it was truncated (258 aa) compared with all other sequences (465–600 aa). Scale bar, average number of amino acid substitutions per position. The colour legend shown for the figure applies to (**A**–**C**).

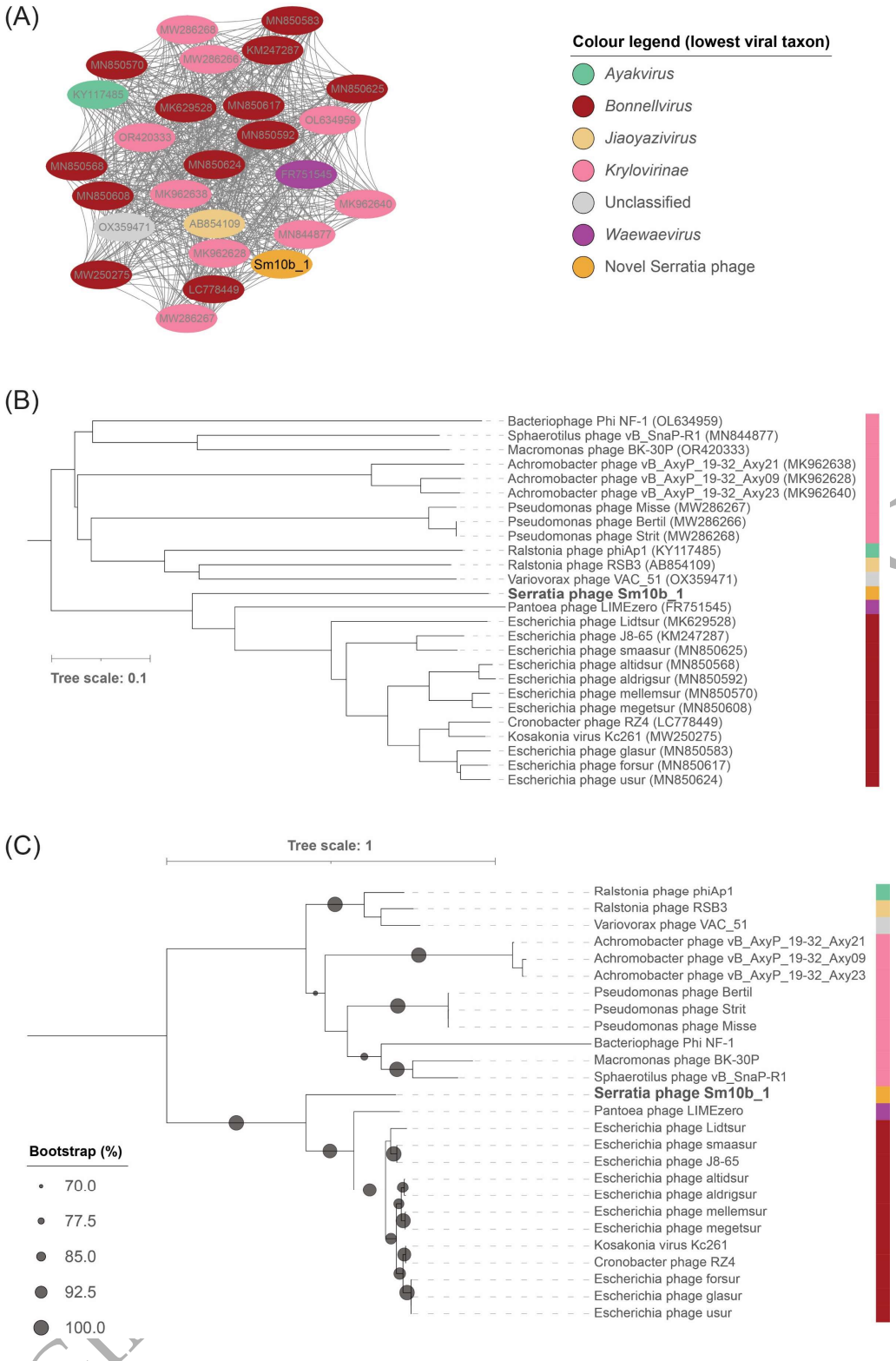

Figure 4. Comparative genomic analyses of phage Sm10b_1. (**A**) First neighbours of Sm10b_1, identified from the vConTACT2 proteome-based network analysis

shown in **Supplementary Figure 1**. The subnetwork of neighbours was extracted from the main network and rearranged using yFiles organic layout. (**B**) ViPTree-based analysis of the genomic data for phage Sm10b_1 and its first neighbours. The tree is rooted at the mid-point, and was constructed based on the distance (1-similarity) matrix (tBLASTx results; bionj option of ViPTree). (**C**) Maximum-likelihood tree (rooted at the mid-point), with bootstrap values shown as a percentage of 100 replications, of the TerL sequences of the phages most closely related to phage Sm10b_1. Scale bar, average number of amino acid substitutions per position. The colour legend shown for the figure applies to (**A**–**C**).

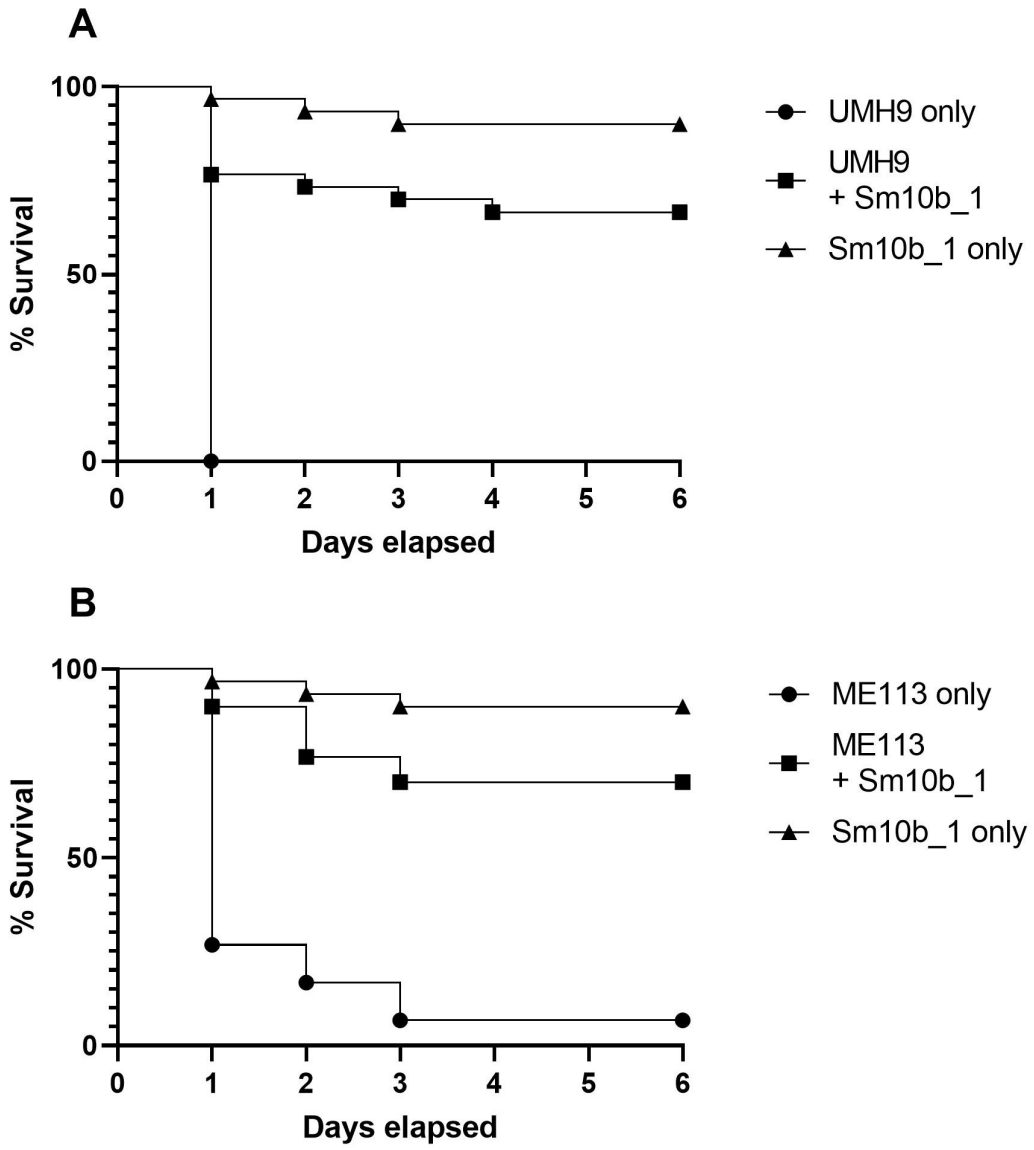

Figure 5. Survival of *G. mellonella* larvae (*n* = 30 per group) infected with 10 cfu of *S. marcescens* UMH9 (**A**) or *S. oderifera* Me113 (**B**) and treated with phage Sm10b_1 (1 x 10^7 pfu). Survival was recorded every 24 h over a period of 6 days. Controls of non-injected larvae and larvae injected with SM buffer only showed 100 % survival at the end of the study period.

Table 1: Details of bacterial strains used in this study

Strain ID	Species	Isolation Source	Provided by	Reference
UMH9	*S. marcescens*	Blood stream infection	Provided by Professor H. Mobley, University of Michigan, USA	Anderson et al. 2017
42	*S. marcescens*	Clinical isolate	Provided by Dr M. Diggle, EMPATH Nottingham University Hospitals UK	NA
JM51	*S. plymuthica*	River water, environmental	Dr Jody Winter, Nottingham Trent University, UK	NA
ME6	*S. marcescens*	Catheter associated infection	Mohamed Eladawy, Mansoura University, Egypt	Eladawy et al. 2025
ME113	*S. odorifera*	Catheter associated infection	Mohamed Eladawy, Mansoura University, Egypt	Eladawy et al. 2025
NTUCC294	*S. marcescens*	Unknown	Nottingham Trent University, Teaching laboratory strain repository, UK.	NA
NTUCC834	*S. marcescens*	Unknown	Nottingham Trent University, Teaching laboratory strain repository, UK.	NA
NTUCC476	*S. marcescens*	Unknown	Nottingham Trent University, Teaching laboratory strain repository, UK.	NA
NTUCC763	*S. marcescens*	Unknown	Nottingham Trent University, Teaching laboratory strain repository, UK.	NA
G2	*S. marcescens*	Sputum, ventilator associated infection	Pathogen bank, Nottingham University Hospitals, UK	NA
G3	*S. marcescens*	Broncho-Alveolar Lavage	Pathogen bank, Nottingham University Hospitals, UK	NA
G4	*S. marcescens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA
G5	*S. marcescens*	Ascitic Fluid	Pathogen bank, Nottingham University Hospitals, UK	NA
G6	*S. marcescens*	Corneal Scrapings	Pathogen bank, Nottingham University Hospitals, UK	NA

C6	*S. liquefaciens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA
C7	*S. liquefaciens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA
C8	*S. liquefaciens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA
C9	*S. liquefaciens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA
C10	*S. liquefaciens*	Blood Culture	Pathogen bank, Nottingham University Hospitals, UK	NA

Table 2: Host range analysis and efficiency of plating (EOP): ++ strong lysis, + weak lysis, and – no lysis. EOP is relative to the routine propagation host, UMH9. Clinical strains indicated by †.

Strain ID	Species	Sm10b_1	EOP	Sm12	EOP
UMH9†	*S. marcescens*	++	1	++	1
42†	*S. marcescens*	-	-	+	0.003
JM51	*S. plymuthica*	-	-	-	-
ME6†	*S. marcescens*	-	-	-	-
ME113†	*S. odorifera*	++	1.485	+	6.583
NTUCC294	*S. marcescens*	++	0.103	++	0.375
NTUCC834	*S. marcescens*	++	0.004	-	-
NTUCC476	*S. marcescens*	+	0.027	-	-
NTUCC763	*S. marcescens*	+	0.009	-	-
G2†	*S. marcescens*	++	0.055	+	2.500
G3†	*S. marcescens*	-	-	-	-
G4†	*S. marcescens*	++	0.001	++	0.005
G5†	*S. marcescens*	++	0.001	++	0.333
G6†	*S. marcescens*	++	0.033	++	0.0003
C6†	*S. liquefaciens*	-	-	-	-
C7†	*S. liquefaciens*	-	-	-	-
C8†	*S. liquefaciens*	-	-	-	-
C9†	*S. liquefaciens*	-	-	-	-
C10†	*S. liquefaciens*	-	-	-	-
% of strains lysed		52.6		42.1	

Table 3: Homologues of proteases identified in the genomes of *S. marcescens* UMH9 and *S. odoifera* ME113 by nucleotide megablast.

Reference sequence	Homologue in UMH9	Percent coverage	Percent identity	Homologue in ME113	Percent coverage	Percent identity
Serralysin *prtS* (accession MG020512.1)	locus tag BVG96_21450, annotated as Serine 3-dehydrogenase	100	96.3	locus tag ACLPHD_15840, annotated as serralysin family protease	99.74	81
slpB (accession MG020513.1)	absent			absent		
slpC (accession WP_038629638.1)	locus tag BVG96_12480, annotated as peptidase serralysin	100	95.6	absent		
slpD (accession WP_025302762)	locus tag BVG96_08260, annotated as protease C	100	95.6	absent		
slpE (accession KT901292.1)	locus tag BVG96_08490, annotated as Serine 3-dehydrogenase	100	92.4	absent		