Two phages, phiIPLA-RODI and phiIPLA-C1C, lyse mono- and dual-staphylococcal biofilms

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Running Head: New lytic phages against staphylococcal biofilms

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Abstract

Phage therapy is a promising alternative to fight against staphylococcal infections. Two lytic phages, vB_SauM_phiIPLA-RODI and vB_SepM_phiIPLA-C1C, belonging to the *Myoviridae* family and exhibiting a wide host range were characterized. The complete genome sequences comprised 142,348 bp and 140,961 bp, and contained 213 and 203 open reading frames, respectively. Gene organization was typical of *Spounavirinae* members, with long direct terminal repeats (LTRs), genes grouped in modules not clearly separated from each other, and several group I introns. In addition, four genes encoding tRNAs were identified in phiIPLA-RODI. Comparative DNA sequences analysis showed its high similarity with two phages GH15 and 676Z, belonging to the *Twort-like virus* genus, (nucleotide identity >84%); for phiIPLA-C1C a high similarity with phage phiIBB-SEP1 was observed (identity of 80%). Challenge assays of phages phiIPLA-RODI and phiIPLA-C1C against staphylococcal planktonic cells confirmed their lytic ability to remove 5 log-units in 8 h. Exposure of biofilms to phages phiIPLA-RODI and phiIPLA-C1C reduced adhered bacteria to about 2 log-units in both mono-species and dual-species biofilms, but phiIPLA-RODI turned out to be as effective as the mixture of both phages. Moreover, the frequency of bacteriophage insensitive mutants (BIMs) of *S. aureus* and *S. epidermidis* to phiIPLA-RODI and phiIPLA-C1C was low, $4.05\text{x}10^{-7} \pm 2.34\text{x}10^{-9}$ and $1.1\text{x}10^{-7} \pm 2.08\text{x}10^{-9}$, respectively. Overall, a general reduced fitness in absence of phages was observed for BIMs, which restored the phage sensitive phenotype in a few generations. These results confirm that lytic bacteriophages can be efficient biofilm disrupting agents, supporting their potential as antimicrobials against staphylococcal infections.

INTRODUCTION

Two staphylococcal species, *Staphylococcus aureus* and *Staphylococcus epidermidis*, are the main cause of nosocomial infections due to their ability to adhere, colonize and develop biofilms in medical devices and human organs (1). Staphylococcal biofilms are complex structures where bacterial cells are surrounded by an extracellular material (polysaccharides, teichoic acids, proteins and eDNA) which confers protection against antibacterial drugs and host immune system. In addition, bacteria growing as a biofilm facilitate the development of antibiotic-resistant organisms (2). *S. epidermidis* is one of the most abundant species in human skin microbiota from where it easily reaches catheters, heart valves and contact lenses. Despite of being regarded as an innocuous bacterium, it is now accepted as an opportunistic pathogen and one of the most common causes of bacteremia in immunocompromised patients (3), preterm infants (4) and biofilm-related infections (5). In addition, resistance to methicillin due to the presence of the *mecA* gene is widely spread in hospital isolates (6). Similarly, virulence in *S. aureus* is mainly due to its ability to adhere, proliferate and attach on biotic and abiotic surfaces (7). In hospital settings *S. aureus* infections affecting internal organs and implanted medical devices have become difficult to eradicate. Moreover, methicillin-resistant strains (MRSA) are often prevalent in hospitals and have been recently spread in no-related environments affecting people without exposure to health care environment (8). MRSA strains have also been isolated from food of animal origin (9) and livestock (10).

Phage therapy exploits the ability of phages to infect and kill bacteria in the treatment of infectious diseases. This represents a potential alternative to antibiotics to fight against multi-resistant pathogenic bacteria or superbugs (11). Indeed, human trials with phages against a number of infections confirmed their safety and showed that phage therapy can provide good results in untreatable chronic infections (12, 13). Specifically, recent results showed the efficacy of phages in animal models such as *S. aureus* septicemia in mice (14) and silkworm (15). Safety and efficacy of phage products to remove this bacterium in a sinusitis sheep model has also been proven (16). Other applications of phages against *S. aureus* encompass the improvement in wound healing developed on diabetic patients (17, 18) and the treatment of chronic wounds (19). Previous results also showed the ability of phages to remove biofilms formed by staphylococcal species (20-22).

In this study, we report a complete morphological and genetic characterization of two new phages infecting staphylococcal species, named vB_SauM_phiIPLA-RODI and vB_SepM_phiIPLA-C1C (in short, phiIPLA-RODI and phiIPLA-C1C), following the nomenclature proposed by Kropinski, et al. (23). The lytic abilities of these phages including host range and biofilm removal were analyzed. Furthermore, the frequency of bacteriophage insensitive mutants (BIMs) was calculated for both phages against planktonic cells and a preliminary characterization of these resistant bacteria is also presented.

MATERIAL AND METHODS

Bacterial strains, bacteriophages and growth conditions. Forty four different staphylococcal species and one *Macrococcus caseolyticus* strain were used in this study (Table 1). All the bacteria were isolated in Baid-Parker Agar (BP) and were routinely cultured in TSB broth (Tryptic Soy Broth, Scharlau, Barcelona, Spain) at 37°C with shaking or on TSB plates containing 2% (w/v) bacteriological agar (TSA).

To select *S. aureus* IPLA16 colonies resistant to rifampicin (*S. aureus* IPLA16-rif^R), 100 µl of overnight cultures were plated onto TSA plates supplemented with 100 µg/ml of rifampin. Plates were incubated for 16 h at 37ºC. Single colonies were picked up and grown in fresh TSB at 37ºC with shaking for further studies.

Bacteriophages phiIPLA-RODI and phiIPLA-C1C were propagated on *S. aureus* IPLA1 and *S. epidermidis* F12, respectively, as previously described (24).

Bacteriophage isolation and propagation. Bacteriophages were isolated from a sewage treatment plant in Colunga, Asturias (Spain). For isolation of staphylococcal phages, 1 liter of sewage was centrifuged twice at 13,600 ×*g* for 30 min and the supernatant filtered using sequentially 0.45 μm and 0.22 μm cellulose acetate membrane filters (VWR, Spain). Enrichment cultures were performed by mixing 20 ml of TSB concentrated five times (TSB 5x), 80 ml of filtrated sewage and 100 μl of overnight cultures from four mixtures of *S. aureus* strains (mixture 1: *S. aureus* IPLA3, IPLA4, IPLA6, IPLA15, IPLA16, IPLA17, IPLA18; mixture 2: *S. aureus* IPLA5, IPLA8, IPLA14; mixture 3: *S. aureus* IPLA1, IPLA2, IPLA9, IPLA10; mixture 4: *S. aureus* IPLA7, IPLA13). After incubation for 16 h at 37ºC, the samples were centrifuged and filtered. A total of three enrichments were carried out to obtain a higher phage titration. To assess the presence of phages, 5 μl of the supernatants from the different combinations were spotted onto a bacterial lawn of each of the *S. aureus* and *S. epidermidis* strains following the double layer technique (24). The presence of an inhibition halo is representative of phage sensitivity. Each inhibition halo was further purified to isolate different phages. Two phages were re-isolated, propagated and purified by a CsCl continuous density gradient as described by (24). As host bacteria, *S. aureus* IPLA1 and *S. epidermidis* F12 were used for the propagation and purification of phages phiIPLA-RODI and phiIPLA-C1C, respectively.

Bacteriophage one-step growth curve, EOP, and stability to pH and temperature. One-step growth curves were made for phages phiIPLA-RODI and phiIPLA-C1C, on the sensitive strains *S. aureus* IPLA16 and *S. epidermidis* LO5081, respectively, as previously described (24). Bacteriophage host range was performed using phiIPLA-RODI (10^9 PFU/ml) and phiIPLA-C1C (10^9 PFU/ml) by drop test, and a titration of the phages was further carried out in all sensitive strains to differentiate between infection and lysis due to bacteriocins. Efficiency of plaque formation (EOP) was determined by dividing the phage titer on the test strain by the phage titer on the reference strain (*S. aureus* IPLA1 for phage phiIPLA-RODI and *S. epidermidis* F12 for phage phiIPLA-C1C).

The pH stability of the phage particles was tested by incubation in the Britton-Robinson pH universal buffer (150 mM KCl, 10 mM KH_2PO_4, 10 mM sodium citrate, 10 mM H_3BO_3; adjusting the pH in a range from 3 to 11) for 3 h at room temperature. Similarly, the temperature stability was examined by incubating the phages in SM buffer at different temperatures (ranging from 40ºC to 90ºC), for 30 min. Phage suspensions in SM buffer at 4ºC were used as controls.

Bacteria-phage challenge test against staphylococcal planktonic cultures. Ten ml of TSB broth was inoculated with an overnight culture until an optical density (OD_{600}) of 0.05 and incubated at 37ºC with shaking until reaching an OD_{600nm} of 0.1 (10^7 CFU/ml). A 100-fold dilution of the culture was infected with a multiplicity of infection (MOI) of 100 (10^7 PFU/ml). Infected cultures were incubated for 8 h at 37ºC, and samples were taken at 2 h intervals. Phage and cell counts were plated in triplicate.

Biofilm formation and biofilm-phage challenge test. Overnight cultures of *S. aureus* IPLA16-rif^R and *S. epidermidis* LO5081 were diluted to 10^6 CFU/ml into fresh TSB supplemented with 0.25% glucose. Aliquots of 200 µl of each single culture or mixture of both strains (100 µl of each strain) were poured into a 96 microwell plate (Thermo Scientific, Madrid, Spain). Biofilms were grown during 24 h at 37ºC. Wells were then washed twice with PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4; pH 7.4). To test biofilm degradation by each phage, 100 µl of SM and 100 µl of phiIPLA-RODI or phiIPLA-C1C were added to each well (10^9 PFU/well). To test the combined effect of both phages, 200 µl of the mixture of both phages was added to the well (10^9 PFU/well of each phage). SM buffer was added for control purposes. The plates were incubated for 4 h at 37ºC, the supernatants were removed and serial dilutions plated on TSB. The cells that were still bound after phage treatment were collected by scratching twice with a sterile swab, suspended in 9 ml of SM buffer and vigorously vortexed for 1 min. Serial dilutions were plated for bacterial counting. For mixed biofilms, *S. epidermidis* counts were calculated as the difference between total staphylococcal counts in TSA and the *S. aureus* IPLA16-rif^R counts in TSA supplemented with 100 µg/ml of rifampicin.

Alternatively, the biomass adhered to the well was observed by staining with crystal violet (0.1% w/v) as described previously (25).

Isolation and characterization of Bacteriophage-Insensitive Mutants (BIMs). Bacteriophage-Insensitive Mutants to phages phiIPLA-RODI and phiIPLA-C1C were obtained from the strains *S. aureus* IPLA16 and *S. epidermidis* LO5081, respectively. Aliquots of 100 µl of overnight cultures of each strain (10^8 CFU) were incubated with 100 µl of phage (10^9 PFU) for 10 min at 37 ºC. Then, the mixture was poured onto a 2% TSA plate and covered with 3 ml of 0.7% TSA. Plates were incubated for 16 h at 37 ºC. Surviving colonies were picked up and grown in fresh TSB medium for 16 h at 37ºC. Bacteriophage susceptibility was tested by the drop assay (24). BIM frequency was calculated as the ratio between the number of surviving colonies and the initial number of bacteria incubated in the presence of phage.

Surface hydrophobicity was determined according to the microbial-adhesion-to-solvents (MATS) assay using hexadecane (Sigma–Aldrich, Madrid, Spain) and stationary-phase cells washed with 0.15 M NaCl adjusted to a final OD_{600} of 0.8 (26). Each measurement was performed in triplicate, and the assay was carried out twice with independent cultures.

The susceptibility of *S. aureus* IPLA16, *S. epidermidis* LO5081 and their respective BIMs to several NaCl concentrations was evaluated by the LD50 values, defined as the concentration of NaCl that inhibits the growth of the strain by 50% compared with the control culture of the same strain growing in standard TSB (0.5% NaCl). Overnight cultures were diluted to OD_{600} of 0.05 in TSB containing different NaCl concentrations (0.5-20%). Aliquots of 0.2 ml were put into 96 microwell plates (NunclonDsurface, Nunc, Roskilde, Denmark). Plates were incubated at 37°C and growth was monitored in a Benchmark Plus Microplate Spectrophotometer (Bio-Rad Laboratories) until the control samples reached an OD_{600} of 0.9±0.1. Growth rates were estimated from linear regression after plotting $Ln(OD_{600})$ as a function of time during the exponential growth phase as described previously (27).The adsorption kinetics and the adsorption rate constant (*k*) of the phages were calculated as previously described (28).

Electron microscopy of phage particles. Electron microscope examination was performed after negative staining of the phage particles with 2% uranyl acetate; electron micrographs were taken using a JEOL 12.000 EXII transmission electron microscope (JEDL USA Inc, Peabody, MA, USA).

DNA extraction and protein analysis. To prepare bacterial DNA-free samples for sequence analysis, the purified phages were treated as described previously (20). Analysis of virion proteins was carried out by SDS-PAGE analysis and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/TOF), as previously described (29).

Bacteriophage genome analysis and characterization. The genome sequence of phages phiIPLA-RODI and phiIPLA-C1C was determined by GenProbio SRL (Parma, Italy) using the Ion Torrent Personal Genome Machine (PGM; Life Technologies, USA). The MIRA program (version 3.4.0) was used for assembly of genome sequences, resulting in coverage of 138-fold for phiIPLA-C1C and 432-fold for phiIPLA-RODI. Additional sequencing using specific primers was done to elucidate regions with ambiguities. Phage genomes were auto-annotated using RAST (30) and manually curated. BLASTX and BLASTP were used to search for similar proteins (31). Structural predictions and motif searches were performed with InterProScan (32). Putative promoters and Shine-Dalgarno sites were predicted using the software MEME (33) followed by visual inspection. ARNold (34) and TransTerm (35) were used to detect potential rho-independent terminators. Putative tRNA were predicted using tRNAscan-SE (36) and ARAGORN (37). Genomic comparison at the nucleotide level was made with EMBOSS Stretcher (38) and with MAUVE (39), using the genome sequences available in public databases (July, 2014) from phages of the *Myoviridae* family infecting *Staphylococcus*. Before the global alignments could be performed, the genomes were manually colinearized, placing the arbitrary starting point at the end of the *orf* preceding the large terminase subunit. The genome organization of the phages and a comparative BLASTN figure was generated using CGView server (40). Annotation was done on the basis of their homology with previously described phages.

The sequences of phiIPLA-RODI and phiIPLA-C1C have been deposited in the GenBank under accession numbers KP027446 and KP027447, respectively.

Statistical analysis. Statistical analyses were performed to establish any significant difference between the control and the tested strains. The differences were expressed as the mean ± standard deviation of three biological replicates in all the assays, and were determined by one-way analysis of variance (ANOVA) followed by the Bonferroni multi-comparison test. Statistical significance was considered at $p<0.05$.

RESULTS

Bacteriophages phiIPLA-RODI and phiIPLA-C1C, two new members of the *Myoviridae* family infecting staphylococcal species. Two phages were isolated from sewage after enrichment with four different mixtures of *S. aureus* strains. From the mixtures 1 and 4 two phages were further isolated, propagated and purified using *S. aureus* IPLA1 as host for phage phiIPLA-RODI and *S. epidermidis* F12 for phage phiIPLA-C1C. The host range of the isolated bacteriophages was tested against a collection of 47 bacterial strains (Table 1). Both phages showed a wide host range infecting the 81% (phiIPLA-RODI) and 40% (phiIPLA-C1C) of *Staphylococcus* strains. For *S. aureus*, all strains were infected by phiIPLA-RODI, whereas phiIPLA-C1C only infected three strains. All *S. epidermidis* strains were infected by phiIPLA-C1C, indicating that phage phiIPLA-C1C is more specific for *S. epidermidis*. In addition, ten different species belonging to *Staphylococcus* genus were also sensitive to phiIPLA-RODI and six of them were sensitive to phiIPLA-C1C. Both phages were able to infect the *Macrococcus caseolyticus* IPLA101 strain (Table 1).

Virions of both phages were observed under transmission electron microscopy, and showed isometric capsids and long contractile tails typical for the *Myoviridae* family (Fig. 1A). PhiIPLA-RODI has a capsid of 73±8 nm of diameter and a tail of 95±8 nm long. PhiIPLA-C1C has a capsid of 88±10 nm of diameter and a tail of 110±13 nm long.

A double baseplate upon tail contraction, which is typical from SPO1-related phages was clearly observed in both phages (41).

One-step growth curves under standardized conditions were determined for both phages (Fig. 1B). The eclipse and latent periods of phiIPLA-RODI on *S. aureus* IPLA16 and for phiIPLA-C1C in *S. epidermidis* LO5081 were 15 and 20 min, respectively. The burst sizes were estimated as 25 and 15 phage particles per infected cell for phiIPLA-RODI and phiIPLA-C1C, respectively (Fig. 1B).

Both phages appeared quite stable at temperatures below 60°C but a total inactivation over 70°C was observed (Fig. S1). Concerning pH stability, a notable reduction of 3.6 log-units in the phage titer was observed in phage phiIPLA-C1C at pH 11 while phiIPLA-RODI was found to be quite stable at this pH. No viable phages were recovered after incubation at pH 3 (Fig. S1).

Genomic organization of phages phiIPLA-RODI and phiIPLA-C1C is typical of the *Spounaviridae* subfamily. The genome of phiIPLA-RODI and phiIPLA-C1C is a double-stranded DNA linear molecule of 142,348 bp (encoding 213 putative ORFs) and 140,961 bp (encoding 203 putative ORFs), respectively, with ORFs preceded by potential Shine-Dalgarno sequences (Tables S1 and S2). Up to 40 and 27 putative promoters (Table S3) were identified, respectively, most of them showed AT-rich sequences upstream of the -35 region and would correspond to middle or early promoters. In addition, 52 and 33 putative rho-independent terminators in phiIPLA-RODI and phiIPLA-C1C were predicted, respectively (Table S4). Two main transcriptional units were identified in both phages (Fig. 2 and 3).

Based on BLAST analysis and conserved domains screening, putative functions have been assigned to 93 of the predicted ORFs (44%) from phiIPLA-RODI and 80 of the predicted ORFs (39%) from phiIPLA-C1C (Tables S1 and S2, respectively). ORFs were annotated based on the similarity of phiIPLA-RODI and phiIPLA-C1C to phage K (Accession number NC_005880) and *S. epidermidis* phage phiIBB-SEP1 (Accession number KF021268.1), respectively.

Overall, genes of both phages are organized into four functional modules including long terminal repeats, morphogenesis, cell lysis and replication/transcription. Furthermore, the phiIPLA-RODI genome encodes a tRNA gene encoding $tRNA_{Met}$ (located between *orf18* and *orf19*) and three tRNA genes encoding $tRNA_{Asp}$, $tRNA_{Phe}$ and $tRNA_{Trp}$, (between *orf59* and *orf60*). The presence of a conserved sequence (TGTCAAGTTAATTT) was detected near these tRNAs, at positions 8852-8865, 31999-32012 and 32179-32192, which may be binding sites for a transcriptional regulatory factor (42). No tRNA genes were identified in the phiIPLA-C1C genome.

The ends of the genome of both phages are flanked by long terminal repeats (LTRs), putatively involved in the recombination of phage genome inside the cell. These regions encode small proteins implicated in host takeover, redirecting cell metabolism to phage production (43). The exact boundaries between these LTRs and the rest of the genome have not been determined but comparison of the terminal repeat proteins with other phages suggests that they could span from TreA (*orf192*) until BofL (*orf6*) encoding genes in phiIPLA-RODI. In this fragment, 28 putative terminal repeat proteins were detected. Homologous to the previously described TreA, TreB, TreC, TreE, TreF, TreJ, TreK, TreN, TreP, TreU and TreT were recognized in a region of 10,330 bp. In addition, a putative group I homing HNH endonuclease (*orf206*), typical from this region, was also identified. In phiIPLA-C1C, LTRs could be expanded from the pentapeptide repeat protein (*orf143*) until BofL (*orf165*) with a total of 11,844 bp and 23 putative proteins. In this region, three putative terminal repeat proteins with homology to TreK (*orf150*), TreO (*orf152*) and TreN (*orf155*) were identified.

The morphogenesis module was split in two regions in both genomes. In phiIPLA-RODI these regions (*orf67-orf108* and *orf135-orf139*) were separated by the replication/transcription module, whereas the LTRs region and the replication/transcription module were located in between of the two morphogenetic regions (*orf1* to *orf42* and *orf169* to *orf171*) in phiIPLA-C1C. Genes encoding large terminase subunit, portal protein, prohead protease, major capsid, major tail sheath and tape measure protein (TMP) were identified. The large terminase subunit of phiIPLA-RODI (*orf67*) presented a group I intron protein interspaced in the gene; while in phiIPLA-C1C (*orf2*) this intron was not present. The remainder of the proteins encoded in these regions failed to show similarity to the terminase small subunits. The TMP encoding gene is followed by two putative genes in phiIPLA-RODI: *orf*95 which encodes a tail-associated protein with muralytic activity (as deduced from the presence of a CHAP domain), and *orf96*, which contains a predicted endopeptidase domain. Moreover, the product of *orf97* showed homology with glycerol-phosphodiester hydrolytic activities. In phiIPLA-C1C, the TMP encoding gene is followed by four putative genes: *orf28* that encodes a glucosaminidase, *orf29* encoding a lytic transglycosylase, *orf30* that encodes an amidase with a CHAP domain, and *orf31* which encodes a peptidase. The other genes in these modules are likely to encode baseplate, structural and assembly proteins. Protein analysis of viral particles allowed the identification of the adsorption-associated tail protein (*orf104*), major tail sheath protein (*orf85*), capsid protein (*orf78*) and major tail protein (*orf136*) in phage phiIPLA-RODI. In phage phiIPLA-C1C, a tail protein (*orf40*), major tail sheath (*orf18*), major capsid protein (*orf11*) and a hypothetical protein (*orf85*) were also identified (Fig. 4).

The lysis modules containing genes involved in bacterial lysis (holin and endolysin) were located upstream of the morphogenetic module. In addition, putative transglycosylase encoding genes (*orf53* in phiIPLA-RODI and *orf174* in phiIPLA-C1C) which could be involved in cell wall hydrolysis were also identified. Moreover, in phiIPLA-C1C a second holin gene (*orf78*) was located downstream of the replication/transcription module.

In the replication and transcription module several genes related with DNA replication (DNA helicase, DNA primase, resolvase, DNA polymerase and DNA repair protein), synthesis of DNA precursors (ribonucleotide reductase) and gene regulation (sigma factor and integration host factor) were identified. Additionally, two direct repeats of 41 nucleotides were found in phiIPLA-RODI genome between *orf60* and *orf61* (AAAAAGTACGTATTTAGAAAATAAGGAACTCTCCTATTATA). These sequences share the 27 first nucleotides with that sequence of 28 nucleotides conserved in the *Myoviridae* family of phages infecting *Staphylococcus* (except in phages Romulus, Remus, SA11, phiIBB-SEP1 and Twort). These regions are supposed to be potential binding sites for the replication initiator protein.

A group I intron associated to a VRS endonuclease was detected in the middle of the terminase large subunit (*orf68*) in phiIPLA-RODI, while in phiIPLA-C1C two introns and one intein were identified. The group I intron GIY-YIG homing endonucleases were located interrupting the ribonucleotide reductase large subunit (*orf61*), and the DNA polymerase (*orf69*). The intein DOD homing endonuclease is located after the recombination protein (*orf73).* Additionally, in phiIPLA-C1C two intronless GIY-YIG and HNH homing endonucleases were located in intergenic regions downstream from *orf171* and *orf185*, respectively.

Comparative genomics. To perform comparative genomics, genomes of *Myoviridae* phages infecting *Staphylococcus* were colinearized to start at the terminase large subunit. At the nucleotide level, phage phiIPLA-C1C shares a similarity of 80.2%

with the only *S. epidermidis* specific myophage phiIBB-SEP1 (Table 2), whereas similarity with the rest of the *S. aureus* phages is lower than 55%, suggesting that phiIPLA-C1C might be specific for *S. epidermidis*. Phage phiIPLA-RODI is more closely related to the other phages in the database (identity over 80%), excluding phage phiIBB-SEP1 and the representative phage Twort to which the similarity is lower than 56% (Table 2).

A MAUVE comparison allowed us to determine that all of these phages possess the same general module structure including the morphogenesis, replication/transcription, long terminal repeats and lysis. The morphogenesis and the replication/ transcription modules are conserved between the *Myoviridae* phages infecting *Staphylococcus* (Fig. S2, Fig. 2 and Fig. 3). The internal organization in these regions is highly dependent on the presence of homing endonucleases and transposases. Regarding the LTR region, phage phiIPLA-RODI shared homology with all the phages except Twort, phiIPLA-C1C and phiIBB-SEP1. The phages phiIPLA-C1C and phiIBB-SEP1 possess a specific organization in this LTR region, not shared with the other phages. The most variable regions at the nucleotide level are located upstream the LTR region, in which differences regarding the length and gene arrangement are found (Fig. S2).

Killing of staphylococcal planktonic cultures by phiIPLA-RODI and phi IPLA-C1C. Phages phiIPLA-RODI and phiIPLA-C1C had the same lytic activity on *S. aureus* IPLA16, since no viable bacteria were detected after 8 h of incubation and a considerable decrease of the bacterial population was already achieved after 6 h of treatment (7.9 log-units compared to the control). Noteworthy, both phages halted growth during the first 4 h keeping bacterial counts at 10^5 CFU/ml (Fig. 5A). When *S. epidermidis* LO5081 was infected by either phage, no viable counts were detected after 8 h of incubation (Fig. 5B). However, phiIPLA-C1C killed host cells more quickly than phiIPLA-RODI and after 4 h of incubation only 10 CFU/ml viable cells remained. As expected, the number of phages increased in all infected cultures to about 10^9 PFU/ml (data not shown).

PhiIPLA-RODI proved to be more effective than phiIPLA-C1C for removal of mono- and dual-species staphylococcal biofilms. To perform challenge assays against mono and dual-species biofilms, a *S. aureus* IPLA16-derived strain, resistant to rifampin (*S. aureus* IPLA16-rif^R), was isolated which kept the same phage sensitivity and biofilm formation compared to its parent (data not shown). *S. aureus* IPLA16-rif^R and *S. epidermidis* LO5081 were grown in both mono and dual-species biofilms and treated with the phages individually and as a mixture. Surface-adhered bacteria were successfully reduced after phage treatment (Figure 6A). In the presence of phiIPLA-RODI a reduction of 2.43 log-units was achieved for *S. aureus* IPLA16-rif^R and 1.89 log-units for *S. epidermidis* LO5081. Phage phiIPLA-C1C showed a reduced lytic ability against both staphylococcal biofilms with reductions of 1.84 and 1.16 log-units in viable counts of *S. aureus* IPLA16-rif^R and *S. epidermidis* LO5081, respectively. No significant reduction beyond that recorded for individual phages was observed on biofilms treated with a mixture of phages (Fig. 6A).

Planktonic cells of *S. aureus* IPLA16-rif^R were more sensitive to lysis by phage phiIPLA-RODI (reduction of 4.27 log-units) compared to phage phiIPLA-C1C (reduction of 0.76 log-units) (Fig. 6A). However, neither individual phage nor the phage mixture were able to kill planktonic *S. epidermidis* LO5081 (ANOVA, $p>0.05$) (Fig. 6A).

In dual species biofilms, treatment with phage phiIPLA-RODI showed a reduction of 4.27 log-units in adhered cells for *S. aureus* IPLA16-rif^R and 2.66 log-units for *S.*

epidermidis LO5081 strains (Fig. 6B). Treatment of biofilms with phiIPLA-C1C was found to be more effective than that observed in individual biofilms, with a decrease in the adhered cells of 3.23 log-units for *S. aureus* IPLA16-rif^R and 2.64 log-units for *S. epidermidis* LO5081. Similar to mono-species biofilm challenge, the mixture of phages did not clearly improve results obtained by individual phages.

Regarding the planktonic cells, the efficacy of phiIPLA-RODI was higher than that shown by phiIPLA-C1C against both strains forming the dual-biofilm (Fig. 6B). A reduction of 5.69 log-units in *S. aureus* IPLA16-rif^R, and 0.64 log-units in *S. epidermidis* LO5081 was obtained. For phiIPLA-C1C only a weak reduction in viable counts was detected for *S. aureus* IPLA16-rif^R (Fig. 6B). Treatment with a mixture of phages gave similar results to those obtained using phiIPLA-RODI.

Crystal violet staining was used to confirm the reduction in the total biomass of phage treated biofilms (Fig. 6C). Overall, removal of biomass by phage treatment in mono and dual species biofilms was in accordance with the viable counts results (see above). However, in terms of total biomass, the phage treatment turned out to be more effective when a mixture of both phages was applied in both *S. epidermidis* LO5081 single biofilm and the dual species biofilm (Fig. 6C). Similarly, the treatment with phiIPLA-RODI was more effective than with phiIPLA-C1C. However, in biofilms only formed by *S. aureus* IPLA16-rif^R, all the treatments were found to be similar in detaching ability.

Phage-resistance phenotype has an important fitness cost and is highly unstable. BIMs of *S. aureus* IPLA16 after the treatment with phiIPLA-RODI (MOI 100) and of *S. epidermidis* LO5081 treated with phiIPLA-C1C (MOI 1000) emerge at frequencies of $4.05 \times 10^{-7} \pm 2.34 \times 10^{-9}$ and $4.05 \times 10^{-7} \pm 2.34 \times 10^{-9}$, respectively. To test whether resistance implies fitness costs, three phage-resistant colonies from *S. aureus*

IPLA16 (*S. aureus* IPLA16-R40, *S. aureus* IPLA16-R53 and *S. aureus* IPLA16-R71) and two phage-resistant colonies from *S. epidermidis* LO5081 (*S. epidermidis* LO5081-R49 and *S. epidermidis* LO5081-R32) were randomly selected to further microbiological characterization (Table 3).

In phage-free liquid cultures all the BIMs formed aggregates, as observed under optical microscopy (Fig. S3). Moreover, the growth rate of BIMs was clearly reduced compared to wild-type strains (Table 3). Other parameters indicative of cellular fitness, such as the ability of BIMs to grow in high NaCl concentrations and to form biofilms on polystyrene surfaces, were determined. *S. aureus* IPLA16-derived BIMs were sensitive to 8% NaCl (Table 3) whereas *S. epidermidis* LO5081-derived BIMs showed a similar resistance to NaCl than control cultures. Phage resistant bacteria of both species had a reduced capacity to form biofilms on polystyrene surfaces (to up 4-fold reduction in *S. aureus* IPLA16 BIMs and 25-fold reduction in *S. epidermidis* LO5081-R33), except for *S. epidermidis* LO5081-R49 which showed similar values to the wild-type strain (Table 3).

Regardless of the phage against which BIMs had been arisen, they were also resistant to either phiIPLA-RODI or phiIPLA-CIC as well as to phages vB_SauS_phiIPLA88 (44) and vB_SepS_phiIPLA5 (24), two *Siphoviridae* staphylococcal phages (data not shown). Cross-resistance suggested that impaired phage infection could be due to a lower or inexistent adsorption of phages. The kinetics of phage binding indicated that adsorption of phiIPLA-RODI and phiIPLA-C1C proceeded up to 87-90% in 15 min for wild type strains while the adsorption rates to BIMs were reduced up to 38-62% in *S. aureus* and 23% in *S. epidermidis* BIMs (Table 3) indicating that phage infection was prevented by low adsorption of phages to bacterial surface.

Changes in cell surface properties were further confirmed by the significantly less hydrophobic character of the BIMs than the wild type strains (Table 3).

To check whether the phage-resistance phenotype of BIMs is stable without the selective pressure of phages, BIMs were subcultured for 57 generations and the sensitivity to phages phiIPLA-RODI and phiIPLA-C1C tested. *S. aureus* IPLA16-R40, IPLA16-R53 and IPLA16-R71 strains showed a highly unstable phenotype and sensitive cultures (a defined transparent halo was observed) were obtained after 27 generations. More variability was observed for *S. epidermidis* LO5081 BIMs as *S. epidermidis* LO5081-R49 reverted to the sensitive phenotype after 17 generations, while *S. epidermidis* LO5081-R33 lost phage-resistance only after 57 generations (Table 3). Recovery of phage sensitivity was linked to the reestablishment of sensitivity to either phiIPLA-RODI or phiIPLA-C1C and the two *Siphoviridae* phages. Moreover, the original growth rate was also restored (data not shown).

DISCUSSION

Within the bacteriophage therapy context, we have characterized two new lytic phages, phiIPLA-RODI and phiIPLA-C1C, which show the typical wide host range of polyvalent phages such phage K and phi812 (45, 46), in contrast to other monovalent myophages like Stau2, Romulus and Remus, (28, 47), which host range is limited to *S. aureus* strains, and phage phiIBB-SEP1 only infecting *S. epidermidis* strains (48).

With on the perspective of a feasible therapeutic application, we have determined that the stability of phages phiIPLA-RODI and phiIPLA-C1C to pH and temperature was very similar to those described for related phages as MSA6 (49) and Romulus and Remus (28) and therefore, suitable for a feasible design of different pharmaceutical formulations by lyophilization (50), spray drying (51) and aerosolization (52). Bioinformatic analysis of phiIPLA-RODI and phiIPLA-C1C genomes showed the typical characteristics of the “Twort-like viruses” (*Spounavirinae* subfamily): strictly virulent, with large genomes (127-140 kb) containing long direct terminal repeats (LTRs), genes grouped in modules not clearly separated, a few genes encoding tRNAs, and the presence of group I introns (42). The genome of phage phiIPLA-C1C differs from these phages in the lack of genes encoding tRNAs, a peculiarity already observed in *S. epidermidis* phage phiIBB-SEP1 (48). In addition, nucleotide genome sequence of phiIPLA-RODI showed the lack of restriction sites for endonucleases *Sau3*AI, *BamH*I and *Bgl*II, while they are present in the phiIPLA-C1C genome. This appears to be a general strategy among *S. aureus* bacteriophages such as Twort, K, G1, Sb-1 and MSA6 to avoid restriction by the host bacteria (49, 53).

Both phages encode homing endonucleases, found within group I introns, intergenic regions or inteins, which is in accordance with previous results reported for other myophages such as T4 where 11% of the *orf*s correspond to homing endonucleases (54). In T4-related phages, most of the homing endonucleases belong to the GIY-YIG and HNH families, with multiple functions like recombination, binding to and repair of DNA (54). Homing endonucleases encoded by phiIPLA-C1C were not identified in other phages genomes, which is consistent with the idea that these proteins are a recent evolutionary acquisition that could have an influence on gene arrangement, function and, in addition, they could promote their own spread between phages (54).

Once the phages were morphological and genetically characterized, we proceeded to study their lytic activity against staphylococcal bacteria in both planktonic cultures and pre-formed biofilms. Results of biofilm removing assays confirmed that phage infection in planktonic cell cultures is more efficient that in biofilms. Overall, *S. aureus* IPLA16 biofilms were well infected by both phages while, *S. epidermidis* LO5081 biofilms were found to be more resistant to phage predation. A likely explanation is the higher content of extracellular matrix formed by *S. epidermidis* LO5081 compared to *S. aureus* IPLA16 which may hinder the access of phages to bacteria. Some phages encode polysaccharide depolymerase proteins which degrade the extracellular matrix of biofilms facilitating the access of phages to target bacteria (20). However, no genes encoding proteins with these catalytic domains were detected either in phiIPLA-RODI or in phiIPLA-C1C genomes. After biofilm treatment, it was also quite surprising that detached cells were not killed by phages, except those released from *S. aureus* IPLA16 biofilms treated with phiIPLA-RODI. These data suggest that cells released from inside the biofilm would not be susceptible to phage infection due to their unique physiological state (22). The biomass reduction of *S. aureus* IPLA16 biofilms by these phages was similar (67% for phage phiIPLA-RODI and 69% for phage phiIPLA-C1C) to those obtained with phages ISP and Romulus and Remus (28). The complete eradication of biofilms by phages has not been described in the literature to date. However, prevention of biofilm formation has been achieved for *S. aureus* Xen29 biofilms using a combination of phage K and modified derivatives (21). In addition, *S. aureus* biofilms could be efficiently eradicated with a combination of phage SAP-26 and rifampicin (55).

Phage control of dual species biofilm was approached using *S. aureus* and *S. epidermidis* as a proof of concept to evaluate whether the mixture of both phages could be more effective than each individual phage. Our results provide evidence that phages can reduce the cell numbers of both species but the application of a phage mixture against each of the hosts was not always more effective than the use of only one phage. Indeed, the addition of the single phage phiIPLA-RODI resulted in a reduction similar to that achieved by the mixture of phages. The low efficacy of phiIPLA-C1C may be due to the lower burst size of this phage comparing with phiIPLA-RODI. Overall, these results are in agreement with those obtained by other authors (56, 57), who reported a reduction of about 3-4 units-log in viable cells from mixed biofilms treated with phages Mixed species biofilms are complex communities in which the physiological state of cells and the availability of phage receptors will play an important role in the behavior of phages (58). These features could be drastically altered by competition with other bacterial species. In mixed cultures of *E. coli* and *Salmonella*, phages against *E. coli* were more effective in removing this species than in monocultures of *E. coli*. It seems that for some bacteria the competition with other bacterial species may enhance the effectiveness of phages because phage bacterial resistance can decrease the competitive ability of bacteria (59). Similar data were observed in our staphylococcal biofilms in which treatment with phages turned to be more effective in dual biofilms than in mono-species biofilms.

Phage resistance is often a major concern in the therapeutic application of phages as it could compromise the efficacy of the treatment (60). In this regard, the frequency of bacteria acquiring resistance to phages phiIPLA-RODI and phiIPLA-C1C was determined to be low, and most important, with the global fitness of staphylococcal BIMs clearly affected. This reduced fitness of phage-resistant bacteria was also observed for different species such as *Vibrio cholerae* (61) and *Pseudomonas aeruginosa* (62). We have not determined the molecular basis of the phage resistance mechanisms in the BIMs. However, it is known that wall teichoic acid (WTA) serves as a receptor for several staphylococcal siphoviruses and myoviruses (63). Therefore, lack of WTA in *S. aureus* and *S. epidermidis* BIMs could explain the resistance of these strains to phages belonging to *Siphoviridae* and *Myoviridae* families. In addition, phiIPLA-RODI and phiIPLA-C1C BIMs were shown to be more sensitive to high temperatures, showed a higher degree of cell aggregation, and a reduced capacity to form biofilms, as previously observed in *S. aureus* WTA-deficient mutants (64). However, we cannot disregard the presence of a capsular polysaccharide in staphylococcal BIMs, which could modify the bacterial surface properties and prevent phage adsorption to cell (65).

The data presented in this study support the potential of the lytic bacteriophages phiIPLA-RODI and phiIPLA-C1C to be used in phage therapy. Their characterization indicated a wide host range, an adequate stability to environmental conditions, lack of virulence factors, capability to remove biofilms and low frequency of BIMs.

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FIGURE LEGENDS

Figure 1. A) Transmission electron microphotographs of phages phiIPLA-RODI and phiIPLA-C1C; scale bars correspond to 100 nm. B) One-step growth curves of phage phiIPLA-RODI in *S. aureus* IPLA16 and phiIPLA-C1C in *S. epidermidis* LO5081. Values correspond to the number of PFU per infected cell in chloroform-treated cultures (■) and in untreated cultures (●). Each data point is the mean ± standard deviation of three independent experiments.

Figure 2. Genome organization of phage phiIPLA-RODI and BLASTN comparison. The outer ring with the arrows represents the *orf*s of the circularized phage. The predicted gene functions are also indicated. The different functional modules in the genome are shown as a coloured shadow. BLASTn, that is represented by each inner ring, was performed with the representative phage Twort (pink), the phage K (green) and the most similar GH15 (light blue).

Figure 3. Genome organization of phage phiIPLA-C1C and BLASTN comparison. The outer ring with the arrows represents the *orf*s of the circularized phage. The predicted gene functions are indicated. The different functional modules in the genome are shown as a coloured shadow. BLASTn, that is represented by each inner ring, was performed with the representative phages Twort (pink), and K (green) and the most similar phiIBB-SEP1 (light blue).

Figure 4. Analysis by SDS-PAGE electrophoresis and silver staining of the structural proteins of phages phiIPLA-RODI and phiIPLA-C1C. Protein molecular size markers (kDa) are shown on the left (Lane L). Bands marked with a white arrow were identified by mass-spectrophotometry: In phage phiIPLA-RODI (1) adsorption-associated tail protein (*orf104*), (2) major tail sheath protein (*orf85*), (3) capsid protein (*orf78*) and (4) major tail protein (*orf136*). In phage phiIPLA-C1C, (5) tail protein (*orf40*), (6) major tail sheath (*orf18*) (7) major capsid protein (*orf11*) and (8) hypothetical protein (*orf85*).

Figure 5. Susceptibility of the strains (A) *S. aureus* IPLA16 and (B) *S. epidermidis* LO5081 to phages phiIPLA-RODI and phiIPLA-C1C. Cell counts of control cultures (■) and treated cultures with phiIPLA-RODI (▲) and phiIPLA-C1C (●) are represented as log (CFU/ml). Each value corresponds to the mean ± standard deviation of three independent experiments.

Figure 6. Bacteriophage mediated removal of 24 h-old *S. aureus* and *S. epidermidis* biofilms. (A) Mono- or (B) dual-species biofilms of *S. aureus* IPLA16-rif^R and *S. epidermidis* LO5081 were treated with phage phiIPLA-RODI (dark grey), phage phiIPLA-C1C (light grey) or with a mixture of both phages (white), for 4 h. Control biofilms are presented in black. Adhered cell counts and supernatant cell counts were expressed as Log (CFU/well). Bacteria detection threshold [<10 Log (CFU/ml)].

Alternatively, biomass was calculated by crystal violet staining of adhered cells after phage treatment (C). Absorbance was measured at a wavelength of 595 nm. Means and standard deviations were calculated from three biological replicates. Bars having an asterisk are significantly different from the control (ANOVA; $P<0.05$) and bars with a lower case 'a' indicates a significantly different decrease in biomass between the treatment with the mixture of phages and the individual treatment either with phiIPLA-RODI or phiIPLA-C1C (ANOVA; $P<0.05$).

TABLE LEGENDS

Table 1. Strains used in this study, origin and phage sensitivity expressed as efficiency of plaque formation (EOP). Means ± standard deviations were calculated using three independent experiments. (-) Resistance to the phage.

Table 2. Comparative genome analysis using Emboss Strecher of the *Myoviridae* phages infecting *Staphylococcus.*

Table 3. Fitness of *S. aureus* IPLA16 and *S. epidermidis* LO5081 and their BIMs for phages phiIPLA-RODI and phiIPLA-C1C, respectively. Values represent the mean ± standard deviation of three biological replicates. The presence of an asterisk indicates those values that are significantly different from the wild type strain (ANOVA; $P<0.05$).

SUPPLEMENTARY MATERIAL

Figure S1. Phage sensitivity to environmental conditions. (A) Temperature and (B) pH, of phages phiIPLA-RODI (black) and phiIPLA-C1C (white). Values represent the mean ± standard deviation of three independent experiments. Bars having an asterisk are significantly different from the control (ANOVA; $P<0.05$).

Figure S2: Progressive MAUVE comparison at the nucleotide level of phages belonging to the *Myoviridae* family infecting Staphylococcal species. Coloured blocks surround a region of the genome sequence that aligns and is homologous to part of another genome. Regions with lack of homology are outside these blocks or white inside the blocks. The height of the similarity profile corresponds to the average of conservation in that region of the genome sequence.

Figure S3. Optical microphotographs of (A) *S. aureus* IPLA16; (B) *S. aureus* IPLA16-R71 resistant to phage phiIPLA-RODI; (C) *S. epidermidis* LO5081 and (D) *S. epidermidis* LO5081-R49 resistant to phage phiIPLA-C1C. Cultures were grown in TSB at 37ºC with shacking during 16 h.

Table S1: Features of bacteriophage phiIPLA-RODI *orfs*, gene products (gp) and functional assignments.

Table S2: Features of bacteriophage phiIPLA-C1C *orfs*, gene products (gp) and functional assignments.

Table S3: Putative promoters of phages phiIPLA-RODI and phiIPLA-C1C. -10 and -35 boxes are underlined. Nucleotide positions and presence of the TG dinucleotide were also indicated. * Promoters without AT-rich upstream sequences.

Table S4: Putative terminators of phages phiIPLA-RODI and phiIPLA-C1C. The underline sequence corresponds to the terminator stem. Lowercase letter in RNA motifs indicates the spacer element between the stem-loop and T-rich region.

	Strain	Origin	Reference	EOP	
				phiIPLA-RODI	phiIPLA-C1C
S. aureus	IPLA-1	Dairy industries surfaces	66	1	-
	IPLA-2			0.98±0.03	-
	IPLA-3			0.87±0.06	-
	IPLA-4			1.03±0.04	-
	IPLA-5			0.91±0.01	-
	IPLA-6	Meat industries surfaces		1.01±0.03	-
	IPLA-7			0.99±0.03	-
	IPLA-8			0.85±0.11	-
	IPLA-9			0.92±0.23	-
	IPLA-10			0.87±0.07	-
	IPLA-11			0.89±0.06	0.09±0.01
	IPLA-12			0.79±0.05	-
	IPLA-13			1.02±0.03	-
	IPLA-14			0.96±0.02	-
	IPLA-15			1.02±0.03	-
	IPLA-16			1.23±0.04	1.09±0.07
	IPLA-17			1.06±0.01	-
	IPLA-18			0.96±0.02	0.39±0.05
	IPLA-19	Milk sample	Unpublished	1.12±0.03	-
	15981	Clinical isolate	67	0.99±0.02	-
	V329	Bovine subclinical mastitis	68	1.01±0.01	-
S. epidermidis	F12	Women breast milk	69	-	1
	B			0.19±0.01	0.78±0.01
	DH3LIK			-	0.56±0.07
	YLIC13			-	0.77±0.06
	Z2LDC14			-	0.62±0.03
	DG2ñ			-	0.88±0.06
	ASLD1			-	0.62±0.04
	LO5081			0.87±0.04	1.23±0.04
	LX5RB4			-	0.89±0.02
	LO5RB1			-	0.97±0.03
Staphylococcus haemoliticus	ZL89-3	Women breast milk	70	0.91±0.03	-
	ZL114-1			0.87±0.02	-
Staphylococcus hominis	ZL31-13			0.77±0.05	-
	ZL5-5			0.68±0.01	-
Staphylococcus arlettae	ZL114-5			0.45±0.03	-
	ZL98-5			0.55±0.08	0.23±0.04
Staphylococcus	ZL5-11			0.69±0.04	0.56±0.05

lugdunensis					
Staphylococcus gallinarum	ZL90-5			0.71±0.06	0.68±0.01
Staphylococcus kloosii	ZL74-2			0.46±0.03	0.65±0.01
Staphylococcus pasteuri	ZL16-6			0.45±0.06	-
Staphylococcus xylosus	ZL61-2			0.65±0.02	0.56±0.01
Staphylococcus saprophyticus	ZL112-15			0.89±0.08	0.37±0.04
Staphylococcus sciuri	IPLA301			0.98±0.05	-
Macrococcus caseolyticus	IPLA101	Dairy industry surface	Unpublished	0.65±0.03	0.06±0.03

Table 1. Strains used in this study, origin and phage sensitivity expressed as efficiency of plaque formation (EOP). Means ± standard deviations were calculated using three independent experiments. (-) Resistance to the phage.

	phiIPLA-C1C	phiIPLA-RODI
phiIPLA-C1C	100	54.8
phiIPLA-RODI	54.8	100
G1	54.6	83.5
GH15	54.2	84.3
JD007	54.1	83.5
K	53.6	81.1
Twort	53.4	54.5
vB_SauM_Remus	53.6	54.6
vB_SauM_Romulus	52.9	54.2
SA11	54.7	56.2
SA1	44.1	44.7
ISP	54.7	83.8
A5W	54.7	83.2
Sb-1	54.1	79
SA5	54.3	83.1
S25-3	55.1	81.2
S25-4	55.3	80.1
SA012	54.8	81.6
phiIBB-SEP1	80.2	55.8
P4W	55.1	83.6
MSA6	54.9	81.7
Fi200w	55	84.2
676Z	55	84.3
A3R	55.1	82.6
Staph1N	54.7	83.1

Table 2. Comparative genome analysis using Emboss Strecher of the *Myoviridae* phages infecting *Staphylococcus.*

	Growth rate (μ) (h^{-1})	LD50 of NaCl (%)	Biofilm formation (Absorbance OD_{595} nm)	% of phage adsorption in 15 min	Adsorption rate constant (*k*) (ml/min)	Hydrophobicity (% adhesion to hexadecan)	Reversion after generation
S. aureus IPLA16	0.76±0.02	8.14±0.42	1.13±0.06	87.67±3.74	$8.9 x10^{-11}$±$7.5 x10^{-12}$	85.62±5.47	-
S. aureus IPLA16-R40	0.56±0.02 (*)	6.12±0.33 (*)	0.31±0.01 (*)	62.66±2.09 (*)	$3.1 x10^{-10}$±$7.1 x10^{-11}$(*)	44.47±7.67 (*)	27
S. aureus IPLA16-R53	0.59± 0.04 (*)	4.80±0.23 (*)	0.28±0.02 (*)	38.33±6.21 (*)	$6.5 x10^{-10}$±$1.7 x10^{-11}$ (*)	32.13±8.37 (*)	27
S. aureus IPLA16-R71	0.49± 0.03(*)	2.82±0.34 (*)	0.41±0.02 (*)	39.66±3.25 (*)	$6.2 x10^{-10}$±$1.2 x10^{-11}$(*)	34.77±8.17 (*)	27
S. epidermidis LO5081	0.74±0.03	8.23±0.74	9.81±0.38	90.60±8.19	$6.5 x10^{-11}$±$2.3 x10^{-12}$	95.43±2.06	-
S. epidermidis LO5081-R32	0.58±0.02 (*)	8.15±0.25	0.39±0.06 (*)	17.60±7.23 (*)	$6.9 x10^{-10}$±$1.3 x10^{-10}$(*)	33.78±6.65 (*)	57
S. epidermidis LO5081-R49	0.52±0.06 (*)	8.13±0.08	8.99±0.47	23.00±5.69 (*)	$9.9 x10^{-10}$±$1.7 x10^{-10}$ (*)	77.05±11.31 (*)	17

Table 3. Fitness of *S. aureus* IPLA16 and *S. epidermidis* LO5081 and their BIMs for phages phiIPLA-RODI and phiIPLA-C1C, respectively. Values represent the mean ± standard deviation of three biological replicates. The presence of an asterisk indicates those values that are significantly different from the wild type strain (ANOVA; *P*<0.05).

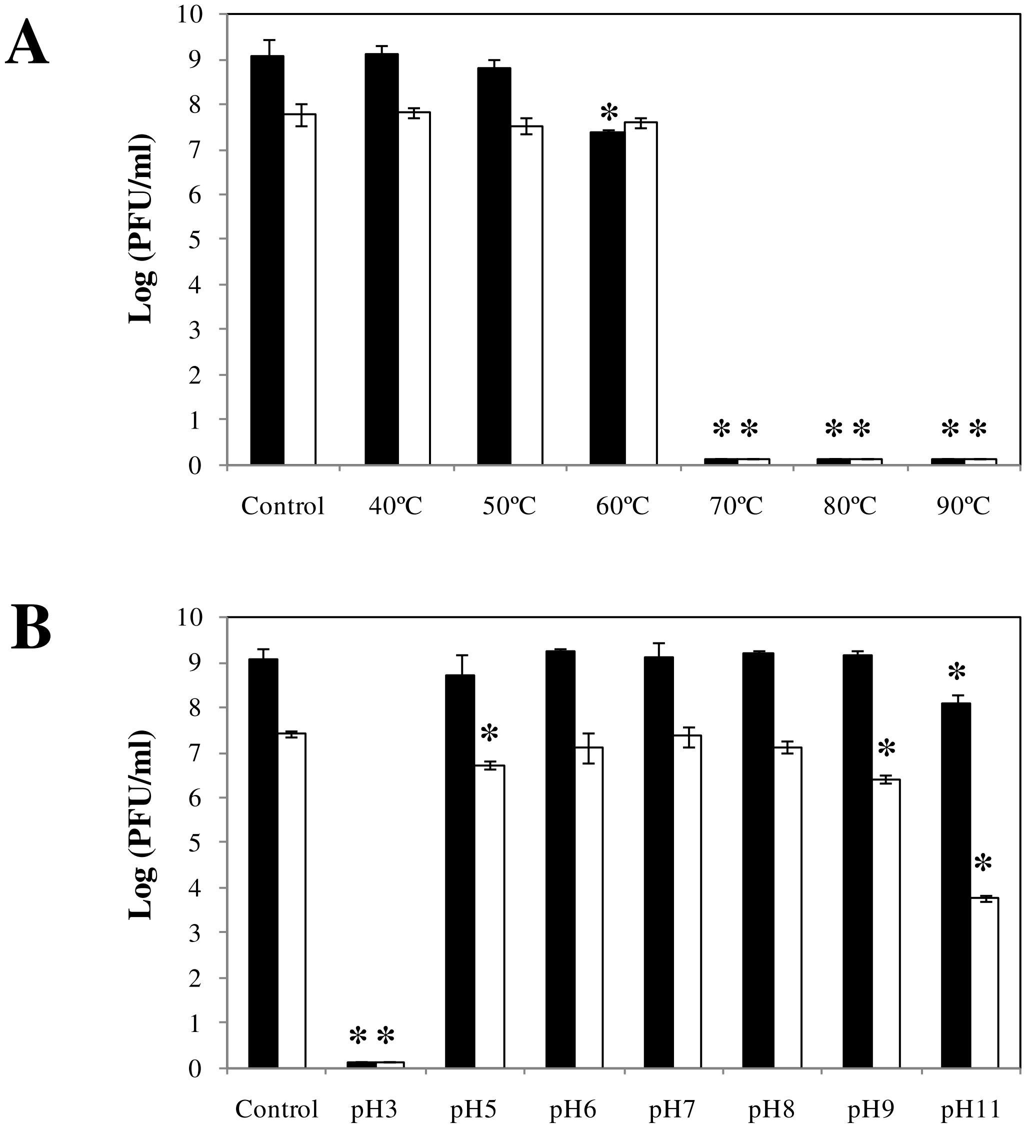
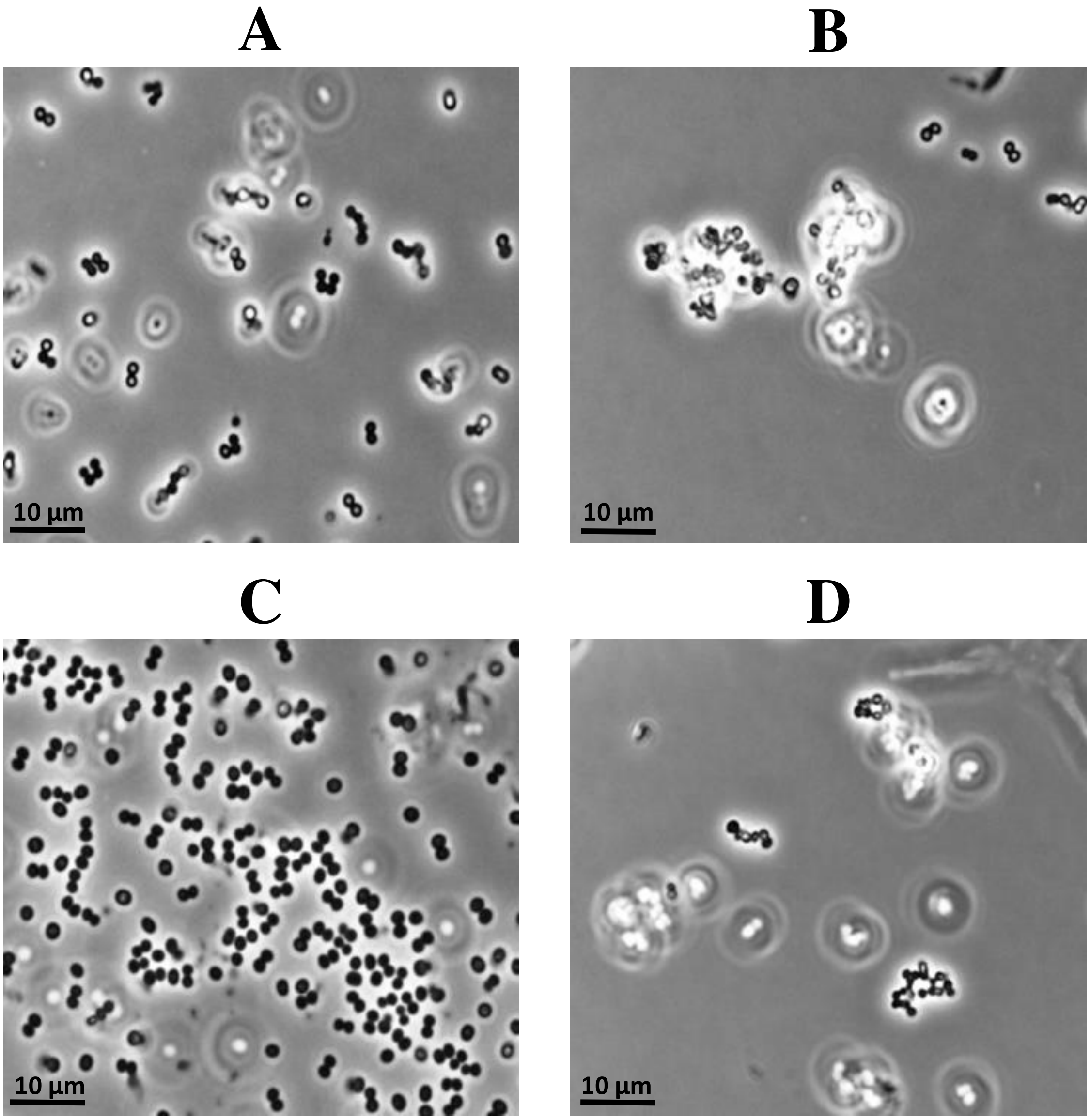


Figure S1. Phage sensitivity to environmental conditions. (A) Temperature and (B) pH, of phages phiIPLA-RODI (black) and phiIPLA-C1C (white). Values represent the mean ± standard deviation of three independent experiments. Bars having an asterisk are significantly different from the control (ANOVA; $P<0.05$).


Morphogenesis
Replication/
Transcription
Long terminal
repeats
Lysis
10000
20000
30000
40000
50000
60000
70000
80000
90000
100000
110000
120000
130000
140000
phiIPLA-RODI
676Z
GH15
P4W
Fi200w
Staph1N
ISP
JD007
K
MSA6
G1
A5W
SA5
S25-3
A3R
Sb-1
SA012
S25-4
SA11
Remus
Romulus
Twort
phiIPLA-C1C
IBB-SEP1

Figure S2: Progressive MAUVE comparison at the nucleotide level of phages belonging to the *Myoviridae* family infecting Staphylococcal species. Coloured blocks surround a region of the genome sequence that aligns and is homologous to part of another genome. Regions with lack of homology are outside these blocks or white inside the blocks. The height of the similarity profile corresponds to the average of conservation in that region of the genome sequence.



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orf	**From**	**To**	**Length**	**aa**	**kDa (pI)**	**Predictive Function**	**Closes hit (E value)**	**% aa identity / % similarity**	**Accesion no.**	**Predicted domain (E value)**
1	14	496	483	160	18.25 (3.60)	Terminal repeat-encoded protein	Terminal repeat-encoded protein [*Staphylococcus* phage phiSA012] (1e-66)	71% (83%)	BAO47048.1	
2	575	739	165	54	6.3 (4.96)	Terminal repeat-encoded protein	Hypothetical protein [*Staphylococcus* phage S25-4] (7e-28)	98% (98%)	YP_008853972.1	
3	739	1008	270	89	10.1 (5.12)	TreT	Hypothetical protein GH15_015 [*Staphylococcus* phage GH15] (1e-50)	93% (96%)	YP_007002138.1	
4	1093	1314	222	73	8.65 (4.51)	TreU	Hypothetical protein [*Staphylococcus* phage S25-4] (3e-37)	90% (93%)	YP_008853974.1	
5	1932	1642	291	96	11.59 (4.27)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (3e-62)	99% (98%)	YP_007112845.1	
6	2272	2036	237	78	9.55 (4.59)	BofL	gp ORF004 [*Staphylococcus* phage A5W] (4e-49)	100% (100%)	ACB88995.1	
7	2759	2274	486	161	19.16 (7.44)	Hypothetical protein	ORF088 [*Staphylococcus* phage G1] (8e-105)	96% (97%)	YP_241045.1	
8	3179	2772	408	135	16.46 (5.06)	Hypothetical protein	ORF109 [*Staphylococcus* phage G1] (4e-93)	100% (100%)	YP_241046.1	
9	3610	3179	432	143	17.24 (4.33)	UboA	UboA [*Staphylococcus* phage Fi200W] (8e-95)	100% (100%)	AFN38459.1	
10	3804	3613	192	63	7.88 (10.21)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (4e-36)	100% (100%)	YP_007112842.1	
11	4286	3801	486	161	18.41 (9.6)	Membrane protein	Membrane protein [*Staphylococcus* phage phiSA012] (5e-105)	98% (99%)	BAO47055.1	
12	4710	4279	432	143	167.44 (3.95)	Hypothetical protein	Hypothetical protein KgORF2 [*Staphylococcus* phage K] (5e-97)	99% (100%)	YP_024433.1	
13	5266	4724	543	180	21.55 (9.68)	Nucleotidyl transferase	Hypothetical protein KgORF3 [*Staphylococcus* phage K] (5e-123)	99% (99%)	YP_024434.1	COG1665 (9.88e-03)
14	5766	5278	489	162	19.5 (9.89)	ribA/ribD-fused hypothetical protein	Hypothetical protein KgORF4 [*Staphylococcus* phage K] (4e-115)	98% (100%)	YP_024435.1	ribofla_fusion[TIGR02464], (4.99e-75)
15	6177	5779	399	132	16.75 (9.4)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-3] (5e-87)	99% (99%)	YP_008854162.1	
16	6881	6174	708	235	27.62 (4.88)	Phosphatase	Putative protein phosphatase [*Staphylococcus* phage K] (9e-169)	98% (100%)	YP_024437.1	MPP_PPP_family[cd00144] (9.50e-27)
17	7535	6981	555	184	21.22 (4.33)	Hypothetical protein	Hypothetical protein KgORF7 [*Staphylococcus* phage K] (1e128)	99% (99%)	YP_024438.1	
18	7868	7551	318	105	11.80 (7.19)	Hypothetical protein	ORF138 [*Staphylococcus* phage G1] (4e-66)	99% (100%)	YP_241056.1	
19	9402	8854	549	182	21.95 (4.25)	Hypothetical protein	Hypothetical protein KgORF8 [*Staphylococcus* phage K] (6e-123)	99% (100%)	YP_024439.1	

20	9624	9406	219	72	8.41 (4.18)	Hypothetical protein	ORF201 [*Staphylococcus* phage G1] (3e.43)	100% (100%)	YP_241058.1	
21	9819	9625	195	64	7.64 (4.39)	Hypothetical protein	ORF218 [*Staphylococcus* phage G1] (1e-37)	100% (100%)	YP_241059.1	
22	10546	9809	738	245	28.67 (5.98)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (1e-171)	99% (99%)	YP_007112830.1	
23	10713	10609	105	34	4.13 (4.37)	Hypothetical protein	ORF437 [*Staphylococcus* phage G1] (7e-12)	85% (97%)	YP_241061.1	
24	10962	10735	228	75	89.2 (4.31)	Aspartate aminotransferase	Hypothetical protein [*Staphylococcus* phage S25-4] (3e-45)	97% (97%)	YP_008853991.1	PRK05937[PRK05937], (3.65e-03)
25	11350	10964	387	128	14.62 (4.57)	Hypothetical protein	Hypothetical protein GH15_039 [*Staphylococcus* phage GH15] (6e-89)	100% (100%)	YP_007002162.1	
26	11620	11447	174	57	6.81 (4.94)	Hypothetical protein	Hypothetical protein GH15_040 [*Staphylococcus* phage GH15] (8e-34)	100% (100%)	YP_007002163.1	
27	12143	11661	483	160	18.98 (4.52)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (8e-108)	98% (100%)	YP_008853994.1	
28	12735	12193	543	180	20.55 (4.60)	Hypothetical protein	Hypothetical protein GH15_042 [*Staphylococcus* phage GH15] (1e-114)	94% (96%)	YP_007002165.1	
29	13265	12735	531	176	20.51 (3.92)	Hypothetical protein	Hypothetical protein GH15_043 [*Staphylococcus* phage GH15] (7e-122)	99% (100%)	YP_007002166.1	
30	13432	13268	165	54	6.15 (9.9)	Membrane protein	ORF256 [*Staphylococcus* phage G1] (1e-26)	94% (98%)	YP_241068.1	
31	13722	13435	288	95	11.29 (9.45)	Membrane protein	Membrane protein [*Staphylococcus* phage JD007] (2e-06)	52% (69%)	YP_007112822.1	
32	14567	13722	846	281	31.77 (4.41)	Hypothetical protein	Hypothetical protein GH15_046 [*Staphylococcus* phage GH15] (0.0)	98% (99%)	YP_007002169.1	
33	15698	14580	1119	372	42.24 (4.48)	AAA family ATPase	Hypothetical protein [*Staphylococcus* phage S25-4] (0.0)	99% (99%)	YP_008854000.1	PHA02244 ATPase-like protein (0.0)
34	16177	15851	327	108	12.81 (4.52)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (9e-71)	97% (98%)	YP_008854001.1	
35	16586	16170	417	138	16.01 (5.21)	Hypothetical protein	Hypothetical protein GH15_049 [*Staphylococcus* phage GH15] (8e-94)	99% (99%)	YP_007002172.1	
36	17020	16718	303	100	11.30 (4.58)	NTP-PPase	Hypothetical protein KgORF17 [*Staphylococcus* phage K] 87e-64)	100% (100%)	YP_024448.1	NTP-PPase_u3[cd11540], (1.92e-24)
37	17208	17020	189	62	7.321 (4.04)	Hypothetical protein	Hypothetical protein GH15_051 [*Staphylococcus* phage GH15] (2e-34)	100% (100%)	YP_007002174.1	
38	17413	17252	162	53	6.37 (4.39)	Hypothetical protein	ORF259 [*Staphylococcus* phage G1] (3e-29)	100% (100%)	YP_241076.1	
39	19464	17413	2052	683	79.79 (6.52)	Hypothetical protein	Hypothetical protein GH15_053 [*Staphylococcus* phage GH15] (0.0)	99% (99%)	YP_007002176.1	
40	19806	19543	264	87	10.26 (4,97)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (2e-52)	98% (97%)	YP_008854007.1	
41	19996	19823	174	57	6.67 (7.15)	Peptidoglycan binding	Hypothetical protein [*Staphylococcus* phage	98% (100%)	YP_007112812.1	LysM[cd00118], (1.06e-07)

						protein	JD007] (4e-31)			
42	20581	20003	579	192	21.46 (8.93)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (4e-130)	100% (100%)	YP_008854009.1	
43	21173	20574	600	199	22.58 (4.65)	Nucleoside 2-deoxyribosyltransferase	Hypothetical protein [*Staphylococcus* phage S25-4] (2e-114)	82% (89%)	YP_008854010.1	Nuc_deoxyrib_tr[pfam05014], (3.71e-03)
44	22059	21166	894	297	34.55 (5)	RNA ligase	Putative DNA ligase [*Staphylococcus* phage GH15] (0.0)	95% (99%)	YP_007002182.1	RNA_ligase[pfam09414], (1.06e-11)
45	22287	22063	225	74	8.15 (9.42)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (6e-40)	100% (100%)	YP_008854012.1	
46	23096	22356	741	246	28.53 (4.96)	PhoH-related protein	Putative PhoH-related protein [*Staphylococcus* phage K] (0.0)	99% (100%)	YP_024453.1	PhoH[pfam02562], (5.49e-25)
47	23762	23148	615	204	23.08 (3.97)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (1e-142)	99% (99%)	YP_008854014.1	
48	24203	23778	426	141	15.79 (7.15)	Ribonuclease	Putative ribonuclease [*Staphylococcus* phage GH15] (2e-194)	100% (100%)	YP_007002186.1	
49	24384	24193	192	63	7.47 (5.61)	Hypothetical protein	ORF222 [*Staphylococcus* phage G1] (3e-37)	100% (100%)	YP_241086.1	
50	25048	24407	642	213	24.58 (3.82)	Hypothetical protein	Hypothetical protein KgORF25 [*Staphylococcus* phage K] (4e-143)	99% (100%)	YP_024456.1	
51	25268	25038	231	76	8.83 (9.12)	DNA binding protein	ORF187 [*Staphylococcus* phage G1] (1e-46)	100% (100%)	YP_241088.1	HTH_XRE[cd00093], (3.67e-10)
52	25498	25271	228	75	9.26 (10.21)	Hypothetical protein	ORF190 [*Staphylococcus* phage G1] (2e-42)	96% (98%)	YP_241089.1	
53	26299	25607	693	230	24.90 (4.75)	Transglycosylase	Putative transglycosylase IsaA [*Staphylococcus* phage GH15] (4e-167)	100% (100%)	YP_007002191.1	LT_GEWL[cd00254], (3.26e-5)
54	27291	26497	795	264	29.29 (9.28)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (0.0)	100% (100%)	YP_007002192.1	
55	27599	27291	309	102	12.13 (9.31)	Hypothetical protein	Hypothetical protein GH15_070 [*Staphylococcus* phage GH15] (6e-65)	100% (100%)	YP_007002193.1	
56	28332	27712	621	206	24.55 (9.41)	Hypothetical protein	Hypothetical protein KgORF27 [*Staphylococcus* phage K] (9e-42)	42% (58%)	YP_024458.1	
57	29885	28395	1491	496	54.81 (9.8)	Endolysin	Putative lysin [*Staphylococcus* phage GH15] (0.0)	99% (100%)	YP_007002194.1	CHAP[pfam05257], (1.42e-11) Amidase_2[pfam01510],_(4.58e-10) SH3b[smart00287], (2.66e-03)
58	30388	29885	504	167	18.11 (3.88)	Holin	Putative holin [*Staphylococcus* phage GH15] (2e-114)	98% (99%)	YP_007002195.1	Phage_holin_1[pfam04531], (1.28e-31)
59	30658	30473	186	61	7.06 (4.81)	Hypothetical protein	ORF233 [*Staphylococcus* phage G1] (1e-33)	100% (100%)	YP_241098.1	
60	32429	32211	219	72	8.67 (9.36)	Hypothetical protein	ORF200 [*Staphylococcus* phage G1] (4e-45)	100% (100%)	YP_241099.1	
61	33118	32909	210	69	7.76 (5.57)	Hypothetical protein	Hypothetical protein GH15_075 [*Staphylococcus* phage GH15] (1e-40)	100% (100%)	YP_007002198.1	

62	33463	33131	333	110	12.50 (5.08)	Hypothetical protein	Hypothetical protein GH15_076 [*Staphylococcus* phage GH15] (8e-69)	99% (100%)	YP_007002199.1	
63	33802	33476	327	108	13.01 (5.14)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (1e-69)	98% (99%)	YP_007002200.1	
64	34243	34629	387	128	14.84 (9.31)	Membrane protein	Hypothetical protein [*Staphylococcus* phage S25-4] (8e-81)	98% (100%)	YP_008854032.1	
65	34607	34885	279	92	10.57 (10.03)	Hypothetical protein	ORF161 [*Staphylococcus* phage G1] (2e-60)	100% (100%)	YP_241104.1	
66	34882	35292	411	136	15.62 (4.38)	Hypothetical protein	ORF133 [*Staphylococcus* phage G1] (6e-91)	99% (100%)	YP_241105.1	
67	35307	35657	351	116	13.56 (9.9)	Terminase large subunit	Putative terminase large subunit [*Staphylococcus* phage GH15] (2e-76)	100% (100%)	YP_007002205.1	
68	35897	36667	771	256	30.01 (9.2)	Group I intron VSR homing endonuclease	Group I intron protein [*Staphylococcus* phage vB_SauM_Romulus] (7e-134)	89% (94%)	YP_007677505.1	Very-short-patch-repair endonuclease [Replication, recombination, and repair] (3.42e-03)
69	36734	38194	1461	486	56.37 (5.74)	Terminase large subunit	Ter [*Staphylococcus* phage MSA6] (0.0)	100% (100%)	AFN38730.1	Terminase_GpA[pfam05876] (1.62e-17)
70	38187	39008	822	273	30.6 (4.92)	Hypothetical protein	Hypothetical protein GH15_083 [*Staphylococcus* phage GH15] (0.0)	96% (99%)	YP_007002206.1	
71	39165	39644	480	159	18.53 (4.62)	Hypothetical protein	Hypothetical protein GH15_085 [*Staphylococcus* phage GH15] (5e-109)	100% (100%)	YP_007002208.1	
72	39686	40936	1251	416	45.82 (3.89)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (0.0)	94% (95%)	YP_007002209.1	
73	41021	41362	342	113	12.82 (9.61)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (1e-71)	100% (100%)	YP_007002210.1	
74	41372	41752	381	126	14.84 (6.14)	Hypothetical protein	Hypothetical protein KgORF40 [*Staphylococcus* phage K] (2e-82)	99% (100%)	YP_024470.1	
75	41756	43447	1692	563	64.04 (6.19)	Portal protein	Putative portal protein [*Staphylococcus* phage GH15] (0.0)	99% (100%)	YP_007002212.1	Phage_portal[pfam04860], (1.99e-14)
76	43641	44414	774	257	28.62 (4.71)	Prohead protease	Hypothetical protein KgORF42 [*Staphylococcus* phage K] (0.0)	99% (100%)	YP_024472.1	Peptidase_U35[pfam04586], (2.03e-05)
77	44433	45383	951	316	35.74 (4.17)	Hypothetical protein	Hypothetical protein KgORF43 [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024473.1	
78	45499	46890	1392	463	51.27 (4.9)	Capsid protein	Putative capsid protein [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024474.1	
79	46982	47278	297	98	11.28 (9.88)	Hypothetical protein	ORF151 [*Staphylococcus* phage G1] (1e-59)	100% (100%)	YP_240904.1	
80	47291	48199	909	302	34.16 (4.93)	Hypothetical protein	Hypothetical protein KgORF45 [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024475.1	
81	48213	49091	879	292	33.71 (5.99)	Hypothetical protein	hypothetical protein GH15_095 [*Staphylococcus* phage GH15] (0.0)	99% (100%)	YP_007002218.1	
82	49091	49711	621	206	23.73 (10.85)	Hypothetical protein	Hypothetical protein GH15_096	99% (100%)	YP_007002219.1	

							[*Staphylococcus* phage GH15] (9e-148)			
83	49730	50566	837	278	31.76 (4.47)	Hypothetical protein	Hypothetical protein KgORF48 [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024478.1	
84	50568	50783	216	71	8.28 (9.14)	Hypothetical protein	ORF202 [*Staphylococcus* phage G1] (3e-45)	100% (100%)	YP_240909.1	
85	50810	52573	1764	587	64.43 (4.69)	Major tail sheath protein	Major tail sheath protein [*Staphylococcus* phage 812] (0.0)	99% (100%)	ABL87117.1	pfam04984: Phage_sheath_1 (1.73e-4)
86	52646	52984	339	112	12.45 (9.01)	Tail tube protein	TmpA [*Staphylococcus* phage A3R] (4e-73)	98% (99%)	AFN38130.1	
87	53779	54783	1005	334	39.42 (9.17)	Ioh	Ioh [*Staphylococcus* phage A3R] (3e-39)	35% (51%)	AFN38131.1	
88	54840	54980	141	46	5.39 (11.19)	Hypothetical protein	Hypothetical protein phi_A3R_ORF076 [*Staphylococcus* phage A3R] (4e-16)	76% (89%)	AFN38132.1	
89	55023	55481	459	152	18.12 (10.02)	Hypothetical protein	Hypothetical protein KgORF51 [*Staphylococcus* phage K] (9e-105)	97% (100%)	YP_024481.1	
90	55494	55688	195	64	7.15 (9.87)	Hypothetical protein	ORF215 [*Staphylococcus* phage G1] (3e-34)	100% (100%)	YP_240914.1	
91	55770	56081	312	103	12.25 (5.8)	Hypothetical protein	Hypothetical protein KgORF52 [*Staphylococcus* phage K] (9e-66)	100% (100%)	YP_024482.1	
92	56213	56671	459	152	18.15 (4.50)	Hypothetical protein	Hypothetical protein KgORF53 [*Staphylococcus* phage K] (5e-105)	100% (100%)	YP_024483.1	
93	56715	57251	537	178	20.92 (4.01)	Tail morphogenetic protein	Hypothetical protein KgORF54 [*Staphylococcus* phage K] (1e-125)	100% (100%)	YP_024484.1	
94	57307	61362	4056	1351	143.77 (9.51)	TMP	Tail morphogenetic protein, tape measure protein [*Staphylococcus* phage phiSA012] (0.0)	99% (100%)	BAO47136.1	TACC[Cdd:pfam05010], (7.75e-06)
95	61441	63867	2427	808	91.25 (6.30)	CHAP domain protein	Hypothetical protein KgORF56 [*Staphylococcus* phage K] (0.0)	99% (99%)	YP_024486.1	CHAP[pfam05257], (6.45e-17)
96	63881	64768	888	295	34.58 (4.21)	Protease	Hypothetical protein GH15_109 [*Staphylococcus* phage GH15] (0.0)	99% (99%)	YP_007002232.1	IPR000064 Endopeptidase (0.00054)
97	64768	67314	2547	848	95.99 (4.61)	Glycerophosphoryl diester phosphatase	Glycerophosphoryl diester phosphodiesterase [*Staphylococcus* phage JD007] (0.0)	99% (99%)	YP_007112758.1	GDPD_SaGlpQ_like[cd08601] (6.54e-69)
98	67421	68212	792	263	29.32 (8.75)	Hypothetical protein	Hypothetical protein KgORF59 [*Staphylococcus* phage K] (0.0)	99% (100%)	YP_024489.1	
99	68212	68736	525	174	19.95 (4.24)	Hypothetical protein	ORF078 [*Staphylococcus* phage G1] (3e-121)	100% (100%)	YP_240925.1	
100	68736	69440	705	234	26.58 (4.44)	Baseplate protein	Putative bacteriophage baseplate protein [*Staphylococcus* phage K] (6e-171)	100% (100%)	YP_024491.1	
101	69455	70501	1047	348	39.2 (4.53)	Baseplate protein	Hypothetical protein KgORF62 [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024492.1	LysM[cd00118] (4.35e-03) [COG3628], Phage baseplate assembly protein W (2.23e-04)

102	70522	73587	3066	1021	116.45 (4.76)	Hypothetical protein	Hypothetical protein KgORF63 [*Staphylococcus* phage K] (0.0)	90% (94%)	YP_024493.1	IPR006949 Baseplate assembly protein J-like (4.5e-26)
103	73698	74219	522	173	19.23 (5.12)	Baseplate protein	Hypothetical protein KgORF64 [*Staphylococcus* phage K] (4e-122)	100% (100%)	YP_024494.1	Phage-Gp8[pfam09215], (1.40e-03)
104	74240	77698	3459	1152	129.33 (4.84)	Adsorption-associated tail protein	Adsorption-associated tail protein [*Staphylococcus* phage JD007] (0.0)	99% (99%)	YP_007112751.1	
105	77747	77905	159	52	62.8 (9.20)	Hypothetical protein	ORF262 [*Staphylococcus* phage G1] (9e-27)	100% (100%)	YP_240931.1	
106	77906	79825	1920	639	72.4 (6.22)	Carbohydrate binding domain protein	Hypothetical protein [*Staphylococcus* phage JD007] (0.0)	97% (98%)	YP_007112749.1	IPR003305Carbohydrate-binding, (5.4e-07)
107	79847	80218	372	123	14.49 (4.58)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (7e-83)	100% (100%)	YP_007112748.1	
108	80225	81601	1377	458	50.49 (5.64)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (0.0)	98% (98%)	YP_007112747.1	
109	81691	83439	1749	582	67.23 (5.46)	DNA helicase	Putative helicase [*Staphylococcus* phage K] (0.0)	99% (100%)	YP_024499.1	Helicase, C-terminal (IPR001650) (7.6e-15)
110	83451	85064	1614	537	63.14 (8.28)	Rep protein	Putative Rep protein [*Staphylococcus* phage GH15] (0.0)	99% (99%)	YP_007002246.1	HTH_ARSR[cd00090] (1.49e-04)
111	85057	86499	1443	480	54.58 (5.38)	ATPase	Putative ATPase [*Staphylococcus* phage K] (0.0)	99% (99%)	YP_024501.1	IPR003593 AAA+ ATPase domain (1.4e-05)
112	86578	86997	420	139	16.18 (5.39)	Hypothetical protein				
113	86997	88022	1026	341	39.34 (4.76)	Exonuclease	Hypothetical protein [*Staphylococcus* phage S25-4] (0.0)	96% (98%)	YP_008854079.1	MPP_Mre11_N[cd00840], (9.51e-25)
114	88022	88399	378	125	15.17 (4.81)	Hypothetical protein	Hypothetical protein KgORF73 [*Staphylococcus* phage K] (9e-72)	84% (92%)	YP_024503.1	
115	88399	90318	1920	639	73.34 (4.96)	ATPase	Hypothetical protein [*Staphylococcus* phage S25-4] (0.0)	98% (99%)	YP_008854081.1	ABC_ATPase[cd00267], (1.18e-04)
116	90318	90914	597	198	23.20 (5.96)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (5e-140)	98% (99%)	YP_007112958.1	
117	90929	91996	1068	355	41.04 (8.50)	DNA Primase	Putative primase [*Staphylococcus* phage K] (0.0)	99% (99%)	YP_024506.1	TOPRIM_DnaG_primases[cd03364], (2.75e-07)
118	92062	92400	339	112	12.88 (3.98)	Hypothetical protein	ORF127 [*Staphylococcus* phage G1] (1e-69)	98% (99%)	YP_240943.1	
119	92400	92852	453	150	17.10 (4.69)	Hypothetical protein	Hypothetical protein phi_676Z_ORF107 [*Staphylococcus* phage 676Z] (2e-96)	95% (99%)	AFN38356.1	
120	92839	93447	609	202	23.64 (5.36)	Resolvase	Resolvase [*Staphylococcus* phage JD007] (4e-146)	99% (100%)	YP_007112954.1	tRNA endonuclease-like domain (IPR011856)(4.6e-06)
121	93437	93856	420	139	15.75 (10.18)	Ribonucleotide reductase flavodoxin	gp ORF109 [*Staphylococcus* phage A5W] (9e-92)	98% (98%)	ACB89102.1	nrdI[PRK03600],(2.13e-25)
122	93871	95985	2115	704	80.30 (5.39)	Ribonucleotide reductase	Putative ribonucleotide reductase large	99% (99%)	YP_007002258.1	Ribonuc_red_lgC[pfam02867], (6.96e-176)

						large subunit	subunit [*Staphylococcus* phage GH15] (0.0)			
123	95999	97048	1050	349	40.45 (4.5)	Ribonucleotide reductase small subunit	Ribonucleoside-diphosphate beta subunit [*Staphylococcus* phage JD007] (0.0)	98% (99%)	YP_007112951.1	RNRR2[cd01049], (1.58e-59)
124	97066	97395	330	109	12.48 (4.39)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (2e-69)	96% (97%)	YP_007112950.1	
125	97379	97699	321	106	12.04 (4.57)	Thioredoxin-like protein	Thioredoxin-like protein [*Staphylococcus* phage K] (4e-68)	99% (100%)	YP_024513.1	TRX_family[cd02947], (7.08e-08)
126	97906	98502	597	198	23.59 (6.25)	Hypothetical protein	Hypothetical protein GH15_139 [*Staphylococcus* phage GH15] (2e-140)	100% (100%)	YP_007002262.1	
127	98512	98817	306	101	11.92 (5.62)	Integration host factor	Putative integration host factor [*Staphylococcus* phage K] (5e-66)	100% (100%)	YP_024515.1	HU_IHF[cd00591], (7.28e-08)
128	98893	102111	3219	1072	124.57 (5.21)	DNA polymerase	DNA polymerase I [*Staphylococcus* phage phiSA012] (0.0)	99% (99%)	BAO47171.1	DNA_pol_A_pol_I_C[cd08637] (5.79e-80)
129	102181	102423	243	80	9.26 (3.83)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (2e-48)	98% (100%)	YP_008854095.1	
130	102440	102922	483	160	18.94 (5.12)	Hypothetical protein	Hypothetical protein GH15_143 [*Staphylococcus* phage GH15] (2e-113)	99% (100%)	YP_007002266.1	
131	103009	104280	1272	423	47.01 (4.44)	Hypothetical protein	Hypothetical protein GH15_144 [*Staphylococcus* phage GH15] (0.0)	98% (99%)	YP_007002267.1	
132	104340	105596	1257	418	46.76 (4.95)	DNA repair protein	Putative DNA repair protein [*Staphylococcus* phage GH15] (0.0)	99% (100%)	YP_007002268.1	recA[cd00983] (1.49e-36)
133	105600	105953	354	117	13.38 (4.89)	Hypothetical protein	ORF121 [*Staphylococcus* phage G1] (6e-79)	100% (100%)	YP_240963.1	
134	105940	106602	663	220	26.60 (5.07)	Sigma factor	Putative sigma factor [*Staphylococcus* phage K] (1e-155)	100% (100%)	YP_024522.1	
135	106730	107362	633	210	23.21 (4.46)	Putative Ig-like protein	Hypothetical protein KgORF95 [*Staphylococcus* phage K] (3e-149)	99% (100%)	YP_024523.1	
136	107385	107897	513	170	17.83 (4.13)	Major tail protein	Putative major tail protein [*Staphylococcus* phage K] (4e-113)	99% (100%)	YP_024524.1	Big_2[pfam02368], (1.65e-05)
137	107912	108139	228	75	7.81 (4.17)	Tail protein	ORF189 [*Staphylococcus* phage G1] (9e-45)	100% (100%)	YP_240967.1	
138	108235	108495	261	86	10.27 (5.52)	Hypothetical protein	Hypothetical protein GH15_151 [*Staphylococcus* phage GH15] (8e-55)	100% (100%)	YP_007002274.1	
139	108499	109254	756	251	29.15 (4.21)	Hypothetical protein	Hypothetical protein KgORF97 [*Staphylococcus* phage K] (8e-180)	100% (100%)	YP_024525.1	
140	109247	110497	1251	416	47.56 (5.64)	DNA polymerase	DNA polymerase [*Staphylococcus* phage JD007] (0.0)	99% (100%)	YP_007112932.1	
141	110511	110879	369	122	13.99 (5.56)	Hypothetical protein	Hypothetical protein KgORF99 [*Staphylococcus* phage K] (2e-80)	100% (100%)	YP_024527.1	
142	110866	111177	312	103	12.01 (4.43)	Hypothetical protein	Hypothetical protein KgORF100	100% (100%)	YP_024528.1	

							[*Staphylococcus* phage K] (7e-68)			
143	111241	111777	537	178	20.78 (6.39)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage phiSA012] (3e-126)	99% (100%)	BAO47187.1	
144	111770	112537	768	255	30.04 (9.83)	Hypothetical protein	Hypothetical protein KgORF101 [*Staphylococcus* phage K] (0.0)	100% (100%)	YP_024529.1	
145	112515	112961	447	148	17.33 (10.63)	Hypothetical protein	Hypothetical protein KgORF102 [*Staphylococcus* phage K] (4e-103)	100% (100%)	YP_024530.1	
146	112961	113824	864	287	32.35 (5.40)	Hypothetical protein	ORF036 [*Staphylococcus* phage G1] (0.0)	100% (100%)	YP_240976.1	
147	114196	114927	732	243	28.35 (5.05)	Hypothetical protein	Hypothetical protein KgORF103 [*Staphylococcus* phage K] (1e-172)	100% (100%)	YP_024531.1	
148	114945	115403	459	152	17.84 (4.62)	Hypothetical protein	Hypothetical protein GH15_161 [*Staphylococcus* phage GH15] (2e-105)	100% (100%)	YP_007002284.1	
149	115468	115911	444	147	17.50 (5.96)	Hypothetical protein	Hypothetical protein KgORF105 [*Staphylococcus* phage K] (2e-98)	100% (100%)	YP_024533.1	
150	115928	116632	705	234	27.36 (4.37)	Hypothetical protein	Hypothetical protein KgORF106 [*Staphylococcus* phage K] (4e-167)	99% (100%)	YP_024534.1	
151	116694	117092	399	132	15.42 (9.30)	Hypothetical protein	Hypothetical protein KgORF107 [*Staphylococcus* phage K] (6e-90)	100% (100%)	YP_024535.1	
152	117239	117481	243	80	9.39 (9.79)	Hypothetical protein	ORF182 [*Staphylococcus* phage G1] (3e-48)	100% (100%)	YP_240982.1	
153	117486	118043	558	185	21.67 (9.88)	Membrane protein	Hypothetical protein PhageK_168 [*Staphylococcus* phage K] (2e-130)	99% (100%)	AHB80083.1	
154	118079	118255	177	58	6.98 (4.37)	Hypothetical protein	ORF240 [*Staphylococcus* phage G1] (2e-32)	100% (100%)	YP_240984.1	
155	118248	118496	249	82	9.04 (9.72)	Membrane protein	Hypothetical protein [*Staphylococcus* phage JD007] (1e-46)	94% (98%)	YP_007112917.1	
156	118489	118722	234	77	88.93 (7.61)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-3] (5e-42)	90% (96%)	YP_008854298.1	
157	118804	119448	645	214	25.20 (5.49)	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit	Putative ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Staphylococcus* phage GH15] (1e-145)	98% (99%)	YP_007002293.1	
158	119464	119712	249	82	9.04 (9.72)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (1e-35)	94% (100%)	YP_008854124.1	
159	119724	119900	177	58	6.9 (10.19)	Hypothetical protein	Hypothetical protein phi_676Z_ORF149 [*Staphylococcus* phage 676Z] (1e-30)	98% (98%)	AFN38398.1	
160	119893	120189	297	98	11.47 (8.99)	Hypothetical protein	Hypothetical protein GH15_172 [*Staphylococcus* phage GH15] (3e-62)	98% (99%)	YP_007002295.1	
161	120237	120419	183	60	7.13 (9.35)	Membrane protein	ORF219 [*Staphylococcus* phage G1] (2e-32)	98% (100%)	YP_240988.1	
162	120432	120803	372	123	14.34 (3.99)	Hypothetical protein	Hypothetical protein GH15_174 [*Staphylococcus* phage GH15] (3e-75)	93% (97%)	YP_007002297.1	

163	120816	121163	348	115	12.97 (4.37)	Hypothetical protein	Hypothetical protein GH15_175 [*Staphylococcus* phage GH15] (4e-76)	99% (100%)	YP_007002298.1	
164	121169	121441	273	90	9.99 (4.03)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (4e-53)	98% (99%)	YP_007002299.1	
165	121511	121816	306	101	12.14 (9.61)	Hypothetical protein	ORF140 [*Staphylococcus* phage G1] (8e-67)	100% (100%)	YP_240992.1	
166	121831	122181	351	116	136.67 (10.24)	Hypothetical protein	Hypothetical protein GH15_178 [*Staphylococcus* phage GH15] (2e-75)	100% (100%)	YP_007002301.1	
167	122215	122394	180	59	7.3 (7.17)	Hypothetical protein	Hypothetical protein GH15_180 [*Staphylococcus* phage GH15] (1e-33)	97% (100%)	YP_007002303.1	
168	122620	123030	411	136	15.32 (4.37)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (9e-86)	94% (99%)	YP_007002304.1	
169	123032	123256	225	74	8.51 (6.54)	Hypothetical protein	Hypothetical protein GH15_198 [*Staphylococcus* phage GH15] (5e-44)	96% (100%)	YP_007002321.1	
170	123269	123469	201	66	7.60 (4.90)	Hypothetical protein	ORF211 [*Staphylococcus* phage G1] (5e-41)	100% (100%)	YP_241008.1	
171	123470	123760	291	96	11.13 (9.48)	Membrane protein	Putative membrane protein [*Staphylococcus* phage GH15] (6e-59)	100% (100%)	YP_007002323.1	
172	123853	124146	294	97	11.41 (6.15)	Hypothetical protein	Hypothetical protein GH15_201 [*Staphylococcus* phage GH15] (1e-63)	100% (100%)	YP_007002324.1	
173	124143	125051	909	302	34.92 (4.98)	Phosphoribosyl pyrophosphate synthetase	Putative ribose-phosphate pyrophosphokinase [*Staphylococcus* phage GH15] (0.0)	96% (98%)	YP_007002325.1	ibP_PPkin[TIGR01251], (7.63e-17)
174	125069	126538	1470	489	56.04 (5.05)	Nicotinamide phosphoribosyltransferase	Nicotinamide phosphoribosyl transferase [*Staphylococcus* phage phiSA012] (0.0)	98% (98%)	BAO47229.1	NAPRTase_PncB[cd01567], (3.65e-95)
175	126629	126940	312	103	11.87 (9.34)	Hypothetical protein				
176	126957	127190	234	77	9.27 (4.56)	Hypothetical protein				
177	127270	127464	195	64	7.41 (4.46)	Hypothetical protein	Hypothetical protein GH15_196 [*Staphylococcus* phage GH15] (5e-20)	65% (80%)	YP_007002319.1	
178	127478	127801	324	107	12.53 (6.37)	Hypothetical protein				
179	127814	128176	363	120	14.21 (4.51)	Hypothetical protein				
180	128176	128415	240	79	9.28 (4.16)	Hypothetical protein	Hypothetical protein GH15_212 [*Staphylococcus* phage GH15] (5e-07)	54% (70%)	YP_007002335.1	
181	128488	128898	411	136	16.29 (4.72)	Hypothetical protein	ORF113 [*Staphylococcus* phage G1] (6e-78)	94% (97%)	YP_241014.1	
182	128903	129157	255	84	9.88 (4.38)	Hypothetical protein	Hypothetical protein PhageK_205 [*Staphylococcus* phage K] (7e-40)	78% (92%)	AHB80120.1	Clr2[Cdd:pfam10383], (9.04e-03)
183	129261	129659	399	132	15.12 (5.15)	Hypothetical protein				
184	129673	130101	429	142	16.52 (4.31)	Hypothetical protein				
185	130103	130378	276	91	10.70 (4.57)	Hypothetical protein	Hypothetical protein GH15_213	72% (88%)	YP_007002336.1	

							[*Staphylococcus* phage GH15] (4e-32)			
186	130392	130781	390	129	14.35 (5.03)	Hypothetical protein				
187	130897	131523	627	208	23.19 (4.24)	Hypothetical protein	Putative uncharacterized protein [*Staphylococcus equorum*] (3e-20)	36% (46%)	WP_002512196.1	
188	131604	131720	117	38	4.51 (10.52)	Hypothetical protein				
189	131734	132135	402	133	16.03 (8.85)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage phiSA012] (8e-45)	59% (77%)	YP_024544.1	
190	132167	132376	210	69	8.33 (4.04)	Hypothetical protein	Hypothetical protein [*Staphylococcus epidermidis*] (2e-07)	47% (65%)	WP_002493393.1	
191	132376	132732	357	118	13.49 (5.03)	Hypothetical protein				
192	133332	133631	300	99	11.56 (4.18)	TreA	gp ORF182 [*Staphylococcus* phage A5W] (1e-62)	96% (100%)	ACB89175.1	
193	133647	133832	186	61	6.67 (7.68)	TreB	ORF231 [*Staphylococcus* phage G1] (3e-25)	87% (93%)	YP_241023.1	
194	133939	134226	288	95	10.82 (4.43)	TreC	TreC [*Staphylococcus* phage A3R] (5e-60)	99% (100%)	AFN38033.1	
195	134226	134552	327	108	12.61 (4.07)	TreE	Hypothetical protein KgORF117 [*Staphylococcus* phage K] (9e-70)	99% (99%)	YP_024545.1	
196	134567	134860	294	97	11.62 (4.43)	TreE	TreE [*Staphylococcus* phage A3R] (3e-61)	97% (98%)	AFN38035.1	
197	134864	135049	186	61	7.44 (8.98)	TreF	ORF175 [*Staphylococcus* phage G1] (8e-35)	100% (100%)	YP_241027.1	
198	135186	135479	294	97	11.62 (4.43)	TreE	TreE [*Staphylococcus* phage A3R] (3e-61)	97% (98%)	AFN38035.1	
199	135483	135740	258	85	10.23 (6.10)	TreF	ORF175 [*Staphylococcus* phage G1] (4e-54)	100% (100%)	YP_241027.1	Peptidase_C26[pfam07722], (5.58e-03)
200	135828	136067	240	79	9.13 (4.63)	Hypothetical protein	gp ORF187 [*Staphylococcus* phage A5W] (2e-45)	94% (96%)	ACB89180.1	
201	136078	136425	348	115	13.59 (4.76)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (1e-71)	91% (99%)	YP_007112864.1	
202	136968	136630	339	112	13.47 (4.33)	Hypothetical protein	ORF128 [*Staphylococcus* phage G1] (3e-70)	98% (100%)	YP_241030.1	
203	137279	137587	309	102	11.79 (4.63)	TreJ	ORF145 [*Staphylococcus* phage G1] (8e-68)	99% (99%)	YP_241031.1	
204	137793	138077	285	94	10.98 (8.90)	TreK	gp ORF190 [*Staphylococcus* phage A5W] (1e-60)	97% (98%)	ACB89183.1	
205	138152	138343	192	63	7.67 (9.90)	Hypothetical protein	Hypothetical protein GH15_004 [*Staphylococcus* phage GH15] (4e-37)	100% (100%)	YP_007002127.1	
206	139325	138843	483	160	19.42 (9.61)	HNH homing endonuclease	ORF085 [*Staphylococcus* phage G1] (4e-93)	85% (89%)	YP_241035.1	HNH_3[pfam13392](1.26e-11)
207	139493	139651	159	52	60.56 (10.90)	TreN	Hypothetical protein [*Staphylococcus* phage S25-4] (2e-26)	100% (100%)	YP_008853962.1	
208	139724	139870	147	48	5.61 (9.82)	Hypothetical protein				

209	140036	140359	324	107	12.35 (4.97)	TreP	Hypothetical protein GH15_006 [*Staphylococcus* phage GH15] (2e-65)	93% (96%)	YP_007002129.1	
210	140445	140840	396	131	15.39 (4.30)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-3] (4e-66)	75% (87%)	YP_008854145.1	
211	141309	141530	222	73	8.46 (3.96)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage S25-4] (5e-45)	100%(100%)	YP_008853966.1|	
212	141789	141953	165	54	6.42 (4.67)	Hypothetical protein	Hypothetical protein [*Staphylococcus* phage JD007] (4e-29)	100% (100%)	YP_007112853.1	
213	142033	142269	237	78	9.00 (4.03)	Hypothetical protein	hypothetical protein [*Staphylococcus* phage S25-4] (9e-39)	85% (97%)	YP_008853969.1	

Table S1: Features of bacteriophage phiIPLA-RODI *orfs*, gene products (gp) and functional assignments.

orf	**From**	**To**	**Length**	**aa**	**kDa (pI)**	**Predictive Function**	**Closes hit (E value)**	**% aaidentity / % similarity**	**Accesion no.**	**Predicted domain (E value)**
1	1	216	216	71	8.43 (10.51)	Hypothetical protein	ORF151 [*Staphylococcus* phage Twort] (2e-39)	87% (98%)	YP_238726.1	
2	621	2168	1548	515	59.78 (5.76)	Terminase large subunit	Terminase large subunit [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48129.1	Terminase_GpA[pfam05876], Phage terminase large subunit (GpA) (1.39e-20)
3	2182	2985	804	267	30.63 (4.98)	Hypothetical protein	Hypothetical protein SEP1_002 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48131.1	
4	2972	3133	162	53	6.56 (9.53)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (5e-26)	100% (100%)	AGR48132.1	
5	3147	3638	492	163	19.21 (4.68)	Hypothetical protein	Hypothetical protein SEP1_004 [*Staphylococcus* phage phiIBB-SEP1] (2e-110)	99% (100%)	AGR48133.1	
6	3715	4101	387	128	14.85 (9.57)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-44)	100% (100%)	AGR48134.1	
7	4082	4453	372	123	14.57 (4.84)	Hypothetical protein	Hypothetical protein SEP1_006 [*Staphylococcus* phage phiIBB-SEP1](1e-82)	99% (100%)	AGR48135.1	
8	4455	6146	1692	563	64.21 (5.85)	Portal protein	Portal protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48136.1	Phage_portal[pfam04860], Phage portal protein (4.87e-15)
9	6288	7058	771	256	28.70 (4.92)	Prohead protease	Hypothetical protein SEP1_008 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48137.1	Peptidase_U35[pfam04586], Caudovirus prohead protease (6.91e-06)
10	7061	8074	1014	337	38.40 (4.27)	Hypothetical protein	Hypothetical protein SEP1_009 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (98%)	AGR48138.1	
11	8201	9592	1392	463	51.28 (4.89)	Major capsid protein	Major capsid protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48139.1	
12	9692	9958	267	88	10.12 (10.00)	Hypothetical protein	Hypothetical protein SEP1_011 [*Staphylococcus* phage phiIBB-SEP1] (4e-51)	99% (100%)	AGR48140.1	
13	9968	10876	909	302	33.99 (4.65)	Hypothetical protein	Hypothetical protein SEP1_012 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48141.1	
14	10890	11765	876	291	33.45 (5.82)	Capsid protein	Hypothetical protein SEP1_013 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	98% (99%)	AGR48142.1	
15	11765	12400	636	211	24.29 (11.22)	Hypothetical protein	Hypothetical protein SEP1_014 [*Staphylococcus* phage phiIBB-SEP1] (4e-41)	95% (96%)	AGR48143.1	
16	12416	13273	858	285	32.27 (4.46)	Hypothetical protein	Hypothetical protein SEP1_015 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (98%)	AGR48144.1	
17	13248	13469	222	73	8.25 (9.31)	Hypothetical protein	Hypothetical protein SEP1_016 [*Staphylococcus* phage phiIBB-SEP1] (2e-45)	100% (100%)	AGR48145.1	
18	13489	15273	1785	594	65.59 (4.72)	Major tail sheath	Tail sheath protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48146.1	Phage_sheath_1[pfam04984], Phage tail sheath protein (1.83e-05)
19	15333	15698	366	121	13.53 (9.04)	Hypothetical protein	Hypothetical protein SEP1_018 [*Staphylococcus* phage phiIBB-SEP1] (6e-	97% (98%)	AGR48147.1	

							148)			
20	16510	17448	939	312	36.68 (9.42)	Hypothetical protein	ORF018 [*Staphylococcus* phage Twort] (3e-92)	50% (66%)	YP_238556.1	
21	17505	17639	135	44	5.45 (11.18)	Hypothetical protein	Hypothetical protein SEP1_022 [*Staphylococcus* phage phiIBB-SEP1] (5e-19)	93% (95%)	AGR48150.1	
22	17639	18100	462	153	18.17 (9.72)	Hypothetical protein	Hypothetical protein SEP1_023 [*Staphylococcus* phage phiIBB-SEP1] (3e-102)	96% (98%)	AGR48151.1	
23	18105	18302	198	65	7.59 (4.55)	Hypothetical protein	Hypothetical protein SEP1_024 [*Staphylococcus* phage phiIBB-SEP1] (7e-32)	97% (98%)	AGR48152.1	
24	18368	18661	294	97	11.69 (5.82)	Hypothetical protein	Hypothetical protein SEP1_025 [*Staphylococcus* phage phiIBB-SEP1] (4e-60)	100% (100%)	AGR48153.1	
25	18787	19197	411	136	16.00 (4.53)	Hypothetical protein	Hypothetical protein SEP1_026 [*Staphylococcus* phage phiIBB-SEP1] (2e-93)	100% (100%)	AGR48154.1	
26	19229	19738	510	169	20.17 (4.03)	Hypothetical tail protein	Hypothetical protein SEP1_027 [*Staphylococcus* phage phiIBB-SEP1] (1e-117)	100% (100%)	AGR48155.1	
27	19793	23110	3318	1105	118.12 (10.11)	Tail lysin	Tail lysin [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48156.1	SMC_prok_A[TIGR02169], chromosome segregation protein SMC (7.96e-03)
28	23855	24436	582	193	21.17 (9.72)	Glucosaminidase	Tail lysin [*Staphylococcus* phage phiIBB-SEP1] (6e-131)	100% (100%)	AGR48156.1	Glucosaminidase[pfam01832], Mannosyl-glycoproteinendo-beta-N-acetylglucosaminidase (2.51e-09)
29	24499	25296	798	265	28.59 (9.34)	Lytic transglycosylase	SLT-domain containing protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48157.1	SLT[pfam01464], Transglycosylase SLT domain (7.70e-06)
30	25353	27959	2607	868	98.03 (5.88)	Amidase	Putative N-acetylmuramoyl-L-alanine amidase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48158.1	CHAP[pfam05257], CHAP domain (1.28e-21)
31	27974	28882	909	302	35.68 (4.24)	Endopeptidase	Hypothetical protein SEP1_032 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48159.1	IPR000064 Endopeptidase, NLPC/P60 domain(4.1e-05)
32	28885	31038	2154	717	81.76 (4.63)	Chromosome segregation protein	Hypothetical protein SEP1_033 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	98% (99%)	AGR48160.1	PRK01156 chromosome segregation protein (9.17e-05)
33	31059	31727	669	222	24.91 (4.94)	Phage-related replication protein	Hypothetical protein SEP1_034 [*Staphylococcus* phage phiIBB-SEP1] (5e-158)	99% (99%)	AGR48161.1	COG4195 Phage-related replication protein (3.31e-47)
34	31841	32638	798	265	26.69 (9.53)	Hypothetical protein	Hypothetical protein SEP1_035 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48162.1	
35	32638	33162	525	174	20.54 (5.08)	Hypothetical protein	Hypothetical protein SEP1_036 [*Staphylococcus* phage phiIBB-SEP1] (2e-120)	100% (100%)	AGR48163.1	
36	33162	33866	705	234	27.13 (4.72)	Baseplate wedge subunit	Baseplate wedge subunit [*Staphylococcus* phage phiIBB-SEP1] (1e-160)	93% (99%)	AGR48164.1	COG3628[COG3628], Phage baseplate assembly protein W (1.91e-07)

37	33880	34926	1047	348	39.65 (4.69)	Baseplate J protein	Baseplate J protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48165.1	Baseplate_J[pfam04865], Baseplate J-like protein (8.10e-05)
38	34943	37600	2658	885	102.93 (4.63)	Hypothetical protein	Hypothetical protein SEP1_039 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48166.1	
39	37724	38245	522	173	19.41 (5.67)	Baseplate protein	Hypothetical protein SEP1_040 [*Staphylococcus* phage phiIBB-SEP1] (5e-123)	99% (99%)	AGR48167.1	Phage-Gp8[pfam09215], Bacteriophage T4, Gp8 (3.48e-03)
40	38266	41721	3456	1151	129.16 (5.29)	Tail protein	Hypothetical protein SEP1_041 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	98% (99%)	AGR48168.1	
41	41781	41933	153	50	5.95 (9.22)	Hypothetical protein	Hypothetical protein SEP1_042 [*Staphylococcus* phage phiIBB-SEP1] (3e-16)	94% (98%)	AGR48169.1	
42	41939	43867	1929	642	73.16 (5.06)	Hypothetical protein	Hypothetical protein SEP1_043 [*Staphylococcus* phage phiIBB-SEP1](0.0)	83% (91%)	AGR48170.1	PHA01818[PHA01818]
43	43880	44272	393	130	15.11 (4.22)	Methyltransferase subunit G	Hypothetical protein SEP1_044 [*Staphylococcus* phage phiIBB-SEP1] (4e-76)	95% (96%)	AGR48171.1	DUF2977[pfam11192], Protein of unknown function (DUF2977) (4.56e-06) PRK01026[PRK01026], tetrahydromethanopterin S-methyltransferase subunit G (8.57e-03)
44	44279	45649	1371	456	50.94 (6.50)	Hypothetical protein	Hypothetical protein SEP1_045 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48172.1	PHA01818[PHA01818], Hypothetical protein (3.54e-116)
45	45741	48743	3003	1000	115.97 (7.86)	DNA helicase	ORF006 [*Staphylococcus*phageTwort] (0.0)	83% (92%)	YP_238583.1	HELICc[cd00079], Helicase superfamily c-terminal domain (7.70e-12) Hint[cd00081], Hedgehog/Intein domain (8.98e-12)
46	48759	50372	1614	537	63.52 (9.26)	Rep protein	Transcriptional regulator [*Staphylococcus* phage phiIBB-SEP1] (0.0)	98% (99%)	AGR48174.1	IPR011991 Winged helix-turn-helix DNA-binding domain (2.2e-05)
47	50386	51492	1107	368	43.67 (10.47)	Transposase	Transposase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	80% (91%)	AGR48183.1	OrfB_IS605[pfam01385], Probable transposase (4.42e-10) HTH_OrfB_IS605[pfam12323], Helix-turn-helix domain (1.38e-07) tspaseT_teng_C[TIGR01766], transposase, IS605 OrfB family, central region (1.42e-05)
48	51717	53117	1401	466	53.76 (5.32)	Helicase	DNA helicase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48175.1	GP4d_helicase[cd01122] (7.91e-06)
49	53189	53494	306	101	11.78 (4.38)	Hypothetical protein	Hypothetical protein SEP1_049 [*Staphylococcus* phage phiIBB-SEP1] (6e-63)	100% (100%)	AGR48176.1	
50	53494	54057	564	187	22.133 (5.15)	Hypothetical protein	Hypothetical protein SEP1_050 [*Staphylococcus* phage phiIBB-SEP1] (2e-127)	100% (100%)	AGR48177.1	PTZ00211[PTZ00211], ribonucleoside-diphosphate reductase small subunit (2.12e-03)
51	54057	55091	1035	344	39.78 (4.86)	Exonuclease	DNA repair exonuclease [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48178.1	MPP_Mre11_N[cd00840], Mre11 nuclease, N-terminal metallophosphatase domain (1.35e-24)
52	55169	55558	390	129	15.42 (4.66)	Hypothetical protein	Hypothetical protein KgORF73	81% (93%)	YP_024503.1	PHA02275[PHA02275], Hypothetical

							[*Staphylococcus* phage K] (2e-70)			protein (1.74e-24)
53	55551	57464	1914	637	73.95 (5.12)	Exonuclease	Putative exonuclease [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	YP_007677555.1	ABC_ATPase[cd00267], ATP-binding cassette transporter nucleotide-binding domain (2.14e-05) ABC_sbcCD[cd03279], ATP-binding cassette domain of sbcCD (1.11e-13)
54	57471	58070	600	199	23.58 (9.51)	Hypothetical protein	Hypothetical protein SEP1_054 [*Staphylococcus* phage phiIBB-SEP1] (3e-140)	100% (100%)	AGR48181.1	
55	58082	59137	1056	351	40.53 (7.63)	DNA primase	Primase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48182.1	TOPRIM_DnaG_primases[cd03364], TOPRIM_DnaG_primases (1.69e-09) ZnF_CHCC[smart00400], zinc finger (1.80e-06)
56	59199	59510	312	103	11.92 (3.83)	Hypothetical protein	Hypothetical protein SEP1_057 [*Staphylococcus* phage phiIBB-SEP1] (6e-64)	99% (100%)	AGR48184.1	
57	59510	59944	435	144	16.71 (4.69)	Hypothetical protein	Hypothetical protein SEP1_058 [*Staphylococcus* phage phiIBB-SEP1] (1e-94)	100% (100%)	AGR48185.1	PHA02277[PHA02277], Hypothetical protein (1.96e-08)
58	59937	60548	612	203	23.51 (4.81)	Resolvase	Hypothetical protein SEP1_059 [*Staphylococcus* phage phiIBB-SEP1] (2e-146)	99% (99%)	YP_240945.1	COG1591[COG1591], Holliday junction resolvase - archaeal type [DNA replication, recombination, and repair] (7.68e-05)
59	60566	60964	399	132	15.02 (9.83)	Flavoprotein	FlavoproteinNrdI [*Staphylococcus* phage phiIBB-SEP1] (2e-90)	100% (100%)	AGR48187.1	Flavodoxin_NdrI[pfam07972], NrdI Flavodoxin like (1.86e-32)
60	60969	62123	1155	384	43.98 (5.13)	Ribonucleotide reductase large subunit	Ribonucleotide reductase large subunit [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48188.1	RNR_I[cd01679], Class I ribonucleotide reductase (2.84e-71) RNR_N[pfam08343], Ribonucleotide reductase N-terminal (6.97e-22)
61	62253	62837	585	194	22.36 (9.95)	Group I intron GIY-YIG homing endonuclease	Group I intron endonuclease [*Staphylococcus* phage vB_SepS_SEP9] (8e-22)	36% (53%)	AHG24002.1	GIY-YIG_ SegABCDEFG [cd10444], N-terminal catalytic GIY-YIG domain of bacteriophage T4 segABCDEFG gene encoding proteins (1.05e-10)
62	63115	64038	924	307	34.73 (5.85)	Ribonucleotide reductase large subunit	Ribonucleotide reductase large subunit [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48188.1	RNR_I[cd01679], Class I ribonucleotide reductase (9.03e-72)
63	64053	65099	1047	348	40.76 (4.62)	Ribonucleotide reductase small subunit	Ribonucleotide reductase beta subunit [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48189.1	RNRR2[cd01049], Ribonucleotide Reductase, R2/beta subunit, ferritin-like diiron-binding domain (2.18e-69)
64	65139	65450	312	103	12.11 (4.29)	Hypothetical protein	Hypothetical protein SEP1_063 [*Staphylococcus* phage phiIBB-SEP1] (3e-66)	100% (100%)	AGR48190.1	
65	65453	65776	324	107	12.11 (4.08)	Thioredoxin-like	Thioredoxin-like protein [*Staphylococcus* phage phiIBB-SEP1] (7e-68)	100% (100%)	AGR48192.1	TRX_family[cd02947], TRX family (3.35e-07)
66	65842	66567	726	241	28.59 (9.06)	Hypothetical protein	Hypothetical protein SEP1_066 [*Staphylococcus* phage phiIBB-SEP1] (1e-172)	100% (100%)	AGR48193.1	

67	66576	66875	300	99	11.85 (5.86)	DNA binding protein	DNA-binding protein [*Staphylococcus* phage phiIBB-SEP1] (5e-63)	100% (100%)	AGR48194.1	Bac_DNA_binding [pfam00216], Bacterial DNA-binding protein (1.40e-12)
68	66956	69187	2232	743	86.85 (6.10)	DNA polymerase	DNA polymerase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48195.1	DNA_polA_I_Ecoli_like_exo [cd06139] (4.46e-15) 35EXOc[smart00474], 3'-5' exonuclease (2.71e-07) UDG_F4_TTUDGA_like [cd10030] (1.17e-03)
69	69353	70162	810	269	31.39 (10.13)	Group I intron HNH homing endonuclease	HNH endonuclease [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48196.1	NUMOD4 [pfam07463], NUMOD4 motif (5.02e-08)
70	70429	71271	843	280	32.30 (5.32)	DNA polymerase	DNA polymerase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48195.1	DNA_pol_A_pol_I_C [cd08637] (4.07e-50)
71	71325	71807	483	160	18.75 (5.92)	Hypothetical protein	Hypothetical protein SEP1_071 [*Staphylococcus* phage phiIBB-SEP1] (1e-112)	99% (100%)	AGR48197.1	
72	71898	73142	1245	414	47.22 (4.50)	Hypothetical protein	Hypothetical protein SEP1_072 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48198.1	
73	73200	73424	225	74	80.01 (8.66)	Recombination protein	Recombination protein [*Staphylococcus* phage phiIBB-SEP1] (9e-40)	100% (100%)	AGR48199.1	SSF52540 P-loop containing nucleoside triphosphate hydrolases (2.7e-07)
74	73769	74737	969	322	38.36 (9.53)	Intein DOD homing endonuclease	I-MsaII [*Staphylococcus* phage MSA6] (0.0)	98% (99%)	AFN38796.1	IPR004042 Intein DOD homing endonuclease (4.4e-07)
75	74885	75805	921	306	34.33 (5.20)	DNA repair protein	Recombination protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48199.1	recA[cd00983] (2.79e-18)
76	75802	76170	369	122	14.32 (6.37)	Hypothetical protein	Hypothetical protein SEP1_074 [*Staphylococcus* phage phiIBB-SEP1] (3e-83)	100% (100%)	AGR48200.1	
77	76151	76807	657	218	26.09 (5.59)	Sigma factor	Putative sigma factor [*Staphylococcus* phage phiIBB-SEP1] (8e-150)	99% (99%)	AGR48201.1	
78	76883	77227	345	114	13.03 (10.19)	Holin	Holin [*Staphylococcus* phage phiIBB-SEP1] (1e-70)	100% (100%)	AGR48202.1	Holin_SPP1[TIGR01592], holin, SPP1 family (1.73e-06)
79	77244	77903	660	219	24.89 (4.40)	Hypothetical protein	Hypothetical protein SEP1_078 [*Staphylococcus* phage phiIBB-SEP1] (5e-158)	100% (100%)	AGR48203.1	PHA02283[PHA02283], Hypothetical protein (2.59e-80)
80	78011	78271	261	86	10.13 (5.71)	Hypothetical protein	Hypothetical protein SEP1_079 [*Staphylococcus* phage phiIBB-SEP1] (2e-54)	98% (100%)	AGR48204.1	
81	78274	79014	741	246	28.97 (5.63)	Hypothetical protein	Hypothetical protein SEP1_080 [*Staphylococcus* phage phiIBB-SEP1] (4e-163)	93% (97%)	AGR48205.1	PHA02284[PHA02284], Hypothetical protein (1.81e-21)
82	79017	80282	1266	421	48.18 (5.71)	Mre11 nuclease	Putative metallophosphatase [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (100%)	AGR48206.1	MPP_Mre11_N[cd00840], Mre11 nuclease, N-terminal metallophosphatase domain (2.77e-05)
83	80295	80633	339	112	13.11 (9.37)	Hypothetical protein	Hypothetical protein SEP1_082 [*Staphylococcus* phage phiIBB-SEP1] (1e-71)	99% (100%)	AGR48207.1	

84	80698	81237	540	179	20.64 (8.74)	Hypothetical protein	Hypothetical protein SEP1_083 [*Staphylococcus* phage phiIBB-SEP1] (8e-125)	100% (100%)	AGR48208.1	
85	81227	81976	750	249	29.45 (9.81)	Hypothetical protein	Hypothetical protein SEP1_084 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48209.1	
86	81969	82382	414	137	16.08 (10.81)	Hypothetical protein	Hypothetical protein SEP1_085 [*Staphylococcus* phage phiIBB-SEP1] (3e-93)	99% (100%)	AGR48210.1	
87	82382	83227	846	281	32.40 (5.49)	Hypothetical protein	Hypothetical protein SEP1_086 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48211.1	
88	83311	83811	501	166	18.97 (4.16)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-109)	98% (98%)	AGR48212.1	
89	84149	84874	726	241	28.15 (5.15)	Hypothetical protein	Hypothetical protein SEP1_088 [*Staphylococcus* phage phiIBB-SEP1] (9e-167)	98% (98%)	AGR48213.1	
90	84899	85387	489	162	19.04 (4.56)	Hypothetical protein	Hypothetical protein SEP1_089 [*Staphylococcus* phage phiIBB-SEP1] (1e-111)	100% (100%)	AGR48214.1	
91	85430	85870	441	146	17.33 (9.28)	Hypothetical protein	Hypothetical protein SEP1_090 [*Staphylococcus* phage phiIBB-SEP1] (7e-98)	100% (100%)	AGR48215.1	IPR009057 Homeodomain-like(1e-05)
92	85903	86604	702	233	26.97 (4.48)	Hypothetical protein	Hypothetical protein SEP1_091 [*Staphylococcus* phage phiIBB-SEP1] (8e-165)	99% (100%)	AGR48216.1	PHA02290[PHA02290], Hypothetical protein (1.56e-17)
93	86669	87052	384	127	14.63 (9.93)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (3e-69)	90% (98%)	AGR48217.1	PHA02291[PHA02291], Hypothetical protein (1.03e-11)
94	87189	87371	183	60	7.32 (10.34)	Hypothetical protein	Hypothetical protein SEP1_093 [*Staphylococcus* phage phiIBB-SEP1] (2e-31)	93%(95%)	AGR48218.1	
95	87364	87645	282	93	10.95 (6.13)	Hypothetical protein	Hypothetical protein SEP1_094 [*Staphylococcus* phage phiIBB-SEP1] (5e-58)	96% (100%)	AGR48219.1	
96	87642	87995	354	117	13.95 (10.08)	Hypothetical protein	Hypothetical protein SEP1_096 [*Staphylococcus* phage phiIBB-SEP1] (9e-28)	96% (100%)	AGR48221.1	
97	87995	88495	501	166	19.42 (5.64)	Hypothetical protein	Hypothetical protein SEP1_097 [*Staphylococcus* phage phiIBB-SEP1] (2e-113)	100% (100%)	AGR48222.1	
98	88499	88822	324	107	12.20 (4.52)	Hypothetical protein	Hypothetical protein SEP1_098 [*Staphylococcus* phage phiIBB-SEP1] (2e-63)	95% (96%)	AGR48223.1	
99	88915	89475	561	186	22.05 (9.82)	Hypothetical protein	Hypothetical protein SEP1_099 [*Staphylococcus* phage phiIBB-SEP1] (4e-123)	96% (97%)	AGR48224.1	
100	89529	89876	348	115	13.20 (10.06)	Membrane protein	Membrane protein [*Staphylococcus* phage	71% (80%)	AGR48225.1	

							phiIBB-SEP1] (4e-48)			
101	89890	90117	228	75	8.69 (4.43)	Hypothetical protein	Hypothetical protein SEP1_101 [*Staphylococcus* phage phiIBB-SEP1] (7e-46)	100% (100%)	AGR48226.1	
102	90135	92471	2337	778	92.03 (6.24)	RNA ligase	Hypothetical protein SEP1_102 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48227.1	RNA_lig_T4_1[pfam09511], RNA ligase (2.26e-38) MPP_PPP_family[cd00144], phosphoprotein phosphatases of the metallophosphatase superfamily (5.91e-15) AAA_33[pfam13671], AAA domain (1.49e-23)
103	92669	92812	144	47	5.49 (4.71)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (9e-10)	96% (95%)	AGR48228.1	
104	92815	93621	807	268	29.79 (9.08)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (0.0)	100% (100%)	AGR48229.1	Band_7[pfam01145], SPFH domain / Band 7 family (1.80e-26)
105	93740	93928	189	62	6.76 (4.65)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-29)	100% (100%)	AGR48230.1	
106	93943	94119	177	58	6.89 (4.83)	Hypothetical protein	Hypothetical protein SEP1_106 [*Staphylococcus* phage phiIBB-SEP1] (7e-30)	98% (98%)	AGR48231.1	
107	94135	94644	510	169	19.96 (9.90)	Hypothetical protein	Hypothetical protein SEP1_107 [*Staphylococcus* phage phiIBB-SEP1] (8e-116)	98% (99%)	AGR48232.1	
108	94698	95807	1110	369	43.41 (7.99)	Hypothetical protein				
109	95883	96077	195	64	7.78 (10.02)	Hypothetical protein	Hypothetical protein PhageK_209 [*Staphylococcus* phage K] (0.38)	50% (78%)	AHB80124.1	
110	96110	96619	510	169	20.24 (5.16)	Hypothetical protein				
111	96649	96816	168	55	6.14 (4.43)	Hypothetical protein				
112	96951	97259	309	102	11.85 (5.51)	Hypothetical protein				
113	97286	97447	162	53	6.32 (5.02)	Hypothetical protein	Hypothetical protein PhageK_209 [*Staphylococcus* phage K] (4e-06)	46% (72%)	AHB80124.1	UPF0182[pfam03699], Uncharacterized protein family (UPF0182) (6.06e-03)
114	97463	97969	507	168	19.72 (4.44)	Hypothetical protein	Hypothetical protein [*Paenibacilluspolymyxa*] (1e-11)	28% (53%)	WP_019687640.1	
115	97996	98232	237	78	9.16 (4.65)	Hypothetical protein	Hypothetical protein IPLA7_0054 [*Staphylococcus*phage vB_SepiS-phiIPLA7] (1e-44)	91% (93%)	YP_006561216.1	
116	98256	98666	411	136	15.76 (8.46)	Membrane protein	Membrane protein [*Staphylococcus* phage vB_SepS_SEP9] (9e-52)	89% (90%)	YP_009007710.1	
117	98672	99208	537	178	21.08 (4.92)	Hypothetical protein	Hypothetical protein SEP1_108 [*Staphylococcus* phage phiIBB-SEP1] (2e-109)	87% (94%)	AGR48234.1	
118	99224	99424	201	66	7.82 (5.14)	Hypothetical protein	Hypothetical protein SEP1_109 [*Staphylococcus* phage phiIBB-SEP1] (1e-39)	100% (100%)	AGR48235.1	
119	99450	99707	258	85	9.89 (5.53)	Hypothetical protein	Hypothetical protein SEP1_110	99% (100%)	AGR48236.1	

							[*Staphylococcus* phage phiIBB-SEP1] (2e-53)			
120	99709	100209	501	166	19.28 (5.92)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-106)	97% (98%)	AGR48237.1	
121	100220	100609	390	129	14.38 (5.51)	Membrane protein	Membrane protein [*Staphylococcus* phage vB_SepS_SEP9] (3e-53)	73% (87%)	YP_009007709.1	
122	100699	101094	396	131	15.57 (4.47)	YopX	Phage conserved Hypothetical protein TIGR01671 [*Staphylococcus epidermidis*] (5e-44)	64% (72%)	WP_002504181.1	YopX [pfam09643], YopXprotein (4.94e-24)
123	101095	101412	318	105	12.29 (5.03)	Hypothetical protein	Hypothetical protein, partial [*Staphylococcus aureus*] (1e-23)	54% (73%)	WP_000193480.1	
124	101502	101795	294	97	11.31 (5.02)	Hypothetical protein	Hypothetical protein SEP1_112 [*Staphylococcus* phage phiIBB-SEP1] (2e-62)	98% (97%)	AGR48238.1	
125	101816	102034	219	72	8.61 (4.28)	Hypothetical protein	Hypothetical protein SEP1_113 [*Staphylococcus* phage phiIBB-SEP1] (8e-40)	96% (97%)	AGR48239.1	
126	102038	102253	216	71	8.35 (4.42)	Hypothetical protein	Hypothetical protein SEP1_114 [*Staphylococcus* phage phiIBB-SEP1] (2e-43)	100% (100%)	AGR48240.1	
127	102297	102650	354	117	13.28 (4.85)	Hypothetical protein	Hypothetical protein SEP1_115 [*Staphylococcus* phage phiIBB-SEP1] (9e-80)	99% (100%)	AGR48241.1	
128	102679	103077	399	132	15.19 (4.88)	Hypothetical protein	Hypothetical protein SEP1_116 [*Staphylococcus* phage phiIBB-SEP1] (5e-91)	99% (99%)	AGR48242.1	
129	103131	103334	204	67	8.10 (9.83)	Hypothetical protein	Hypothetical protein [*Staphylococcus epidermidis*] (2e-39)	99% (100%)	WP_002469455.1	
130	103346	103615	270	89	10.61 (5.73)	Hypothetical protein	Hypothetical protein SEP9_088 [*Staphylococcus* phage vB_SepS_SEP9] (4e-49)	91% (95%)	YP_009007756.1	
131	103627	104040	414	137	16.00 (4.65)	Hypothetical protein	Hypothetical protein SEP1_120 [*Staphylococcus* phage phiIBB-SEP1] (4e-77)	85% (94%)	AGR48246.1	
132	104272	104673	402	133	16.02 (9.87)	Hypothetical protein				
133	104686	104886	201	66	7.63 (9.20)	Hypothetical protein	Hypothetical protein SEP9_038 [*Staphylococcus* phage vB_SepS_SEP9] (2e-34)	92% (98%)	YP_009007708.1	
134	104918	105334	417	138	16.42 (4.50)	Hypothetical protein	Hypothetical protein SEP1_122 [*Staphylococcus* phage phiIBB-SEP1] (3e-90)	97% (98%)	AGR48248.1	
135	105337	105573	237	78	9.31 (4.19)	Hypothetical protein	Hypothetical protein SEP1_123 [*Staphylococcus* phage phiIBB-SEP1] (9e-47)	99% (100%)	AGR48249.1	
136	105589	106032	444	147	17.63 (9.81)	Hypothetical protein	Hypothetical protein SEP1_124	88% (93%)	AGR48250.1	

							[*Staphylococcus* phage phiIBB-SEP1] (3e-87)			
137	106051	106389	339	112	13.04 (4.58)	Hypothetical protein				
138	106410	106853	444	147	17.46 (5.39)	Hypothetical protein	Hypothetical protein SEP1_126 [*Staphylococcus* phage phiIBB-SEP1] (3e-88)	90% (94%)	AGR48252.1	
139	106867	107259	393	130	15.53 (7.32)	Hypothetical protein				
140	108656	108390	267	88	10.26 (4.14)	Hypothetical protein	Hypothetical protein SEP1_131 [*Staphylococcus* phage phiIBB-SEP1 (3e-52)	98%/98%	AGR48257.1	
141	108935	108675	261	86	9.87 (4.09)	Hypothetical protein	Hypothetical protein SEP1_132 [*Staphylococcus* phage phiIBB-SEP1] (4e-52)	100% (100%)	AGR48258.1	
142	109349	109011	339	112	13.50 (4.21)	Hypothetical protein	Hypothetical protein SEP1_133 [*Staphylococcus* phage phiIBB-SEP1] (2e-54)	84% (84%)	AGR48259.1	
143	109491	110165	675	224	25.45 (4.54)	Pentapeptide repeat protein	Pentapeptide repeat protein [*Staphylococcus* phage phiIBB-SEP1] (3e-140)	92% (92%)	AGR48263.1	IPR001646 Pentapeptide repeat (3.3e-08)
144	110252	110596	345	114	13.53 (5.08)	Hypothetical protein	Hypothetical protein SEP1_137 [*Staphylococcus* phage phiIBB-SEP1] (3e-71)	98% (100%)	AGR48264.1	
145	110738	111073	336	111	13.30 (8.88)	Hypothetical protein	Hypothetical protein SEP1_138 [*Staphylococcus* phage phiIBB-SEP1 (1e-69)	100% (100%)	AGR48265.1	
146	111098	111298	201	66	7.84 (4.51)	Hypothetical protein	Hypothetical protein SEP1_139 [*Staphylococcus* phage phiIBB-SEP1 (1e-35)	100% (100%)	AGR48266.1	
147	112349	112149	201	66	7.83 (10.58)	Hypothetical protein	Hypothetical protein SEP1_142 [*Staphylococcus* phage phiIBB-SEP1 (1e-32)	98% (100%)	AGR48269.1	
148	112603	112382	222	73	8.62 (5.04)	Hypothetical protein	Hypothetical protein SEP1_143 [*Staphylococcus* phage phiIBB-SEP1 (9e-47)	100% (100%)	AGR48270.1	
149	113189	113497	309	102	11.71 (5.08)	Hypothetical protein	Hypothetical protein SEP1_144 [*Staphylococcus* phage phiIBB-SEP1 (7e-67)	100% (100%)	AGR48271.1	
150	113700	113981	282	93	10.92 (5.73)	TreK	Hypothetical protein SEP1_145 [*Staphylococcus* phage phiIBB-SEP1] (9e-51)	90% (91%)	AGR48272.1	
151	114031	114303	273	90	10.47 (4.35)	Hypothetical protein				
152	114979	115110	132	43	5.09 (10.36)	TreO	Hypothetical protein SEP1_146 [*Staphylococcus* phage phiIBB-SEP1 (1e-20)	98% (100%)	AGR48273.1	
153	115176	115316	141	46	5.61 (4.53)	Hypothetical protein	Hypothetical protein SEP1_147 [*Staphylococcus* phage phiIBB-SEP1 (5e-	100% (100%)	AGR48274.1	

							19)			
154	115396	115635	240	79	9.13 (7.96)	Hypothetical protein	Hypothetical protein SEP1_148 [*Staphylococcus* phage phiIBB-SEP1 (2e-49)	100% (100%)	AGR48275.1	
155	115639	115785	147	48	5.42 (9.61)	TreN	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (1e-23)	100% (100%)	AGR48276.1	
156	115953	116282	330	109	13.11 (3.70)	Hypothetical protein	Hypothetical protein SEP1_151 [*Staphylococcus* phage phiIBB-SEP1 (7e-66)	96% (97%)	AGR48278.1	
157	116456	116719	264	87	10.46 (4.12)	Tre protein	Hypothetical protein SEP1_152 [*Staphylococcus* phage phiIBB-SEP1 (1e-52)	100% (100%)	AGR48279.1	
158	116791	117264	474	157	18.53 (3.60)	Hypothetical protein	Hypothetical protein SEP1_153 [*Staphylococcus* phage phiIBB-SEP1 (2e-102)	99% (98%)	AGR48280	
159	117376	117645	270	89	10.44 (4.94)	Hypothetical protein				
160	117704	117841	138	45	5.18 (4.32)	Hypothetical protein	Hypothetical protein SEP1_154 [*Staphylococcus* phage phiIBB-SEP1] (7e-19)	91%(95%)	AGR48281.1	
161	117911	118189	279	92	11.18 (3.87)	Hypothetical protein	Hypothetical protein SEP1_155 [*Staphylococcus* phage phiIBB-SEP1] (4e-31)	93% (96%)	AGR48282.1	
162	118527	119954	1428	475	56.41 (9.26)	Hypothetical protein	Hypothetical protein SEP1_156 [*Staphylococcus* phage phiIBB-SEP1] (0.0)	98% (98%)	YP_007005520.1	
163	120441	120013	429	142	16.49 (4.18)	Hypothetical protein	ORF062 [*Staphylococcus* phage Twort] (4e-11)	31% (56%)	YP_238669.1	
164	121022	120516	507	168	20.44 (4.76)	Hypothetical protein	Hypothetical protein SEP1_157 [*Staphylococcus* phage phiIBB-SEP1 (5e-103)	99% (100%)	AGR48284.1	
165	121335	121081	255	84	10.34 (4.59)	BofL	Hypothetical protein SEP1_158 [*Staphylococcus* phage phiIBB-SEP1 (1e-51)	99% (98%)	AGR48285.1	
166	121922	121338	585	194	23.29 (4.81)	Hypothetical protein	Hypothetical protein SEP1_159 [*Staphylococcus* phage phiIBB-SEP1 (1e-129)	98% (98%)	AGR48286.1	
167	122270	121962	309	102	12.19 (8.65)	Hypothetical protein	Hypothetical protein SEP1_161 [*Staphylococcus* phage phiIBB-SEP1] (3e-66)	97% (98%)	AGR48288.1	
168	122721	122359	363	120	14.19 (5.06)	Staphylococcal nuclease	Nuclease [*Staphylococcus* phage phiIBB-SEP1] (2e-81)	100% (100%)	AGR48289.1	Staphylococcal nuclease homologues (1.40e-16)
169	123080	122802	279	92	9.67 (4.03)	Tail protein	Hypothetical protein SEP1_163 [*Staphylococcus* phage phiIBB-SEP1] (4e-57)	100% (100%)	AGR48290.1	
170	124126	123548	579	192	22.38 (4.63)	Hypothetical protein	Hypothetical protein SEP1_164 [*Staphylococcus* phage phiIBB-SEP1] (1e-	100% (100%)	AGR48291.1	

							136)			
171	124487	124146	342	113	26.48 (10.25)	Tail protein	Hypothetical protein SEP1_165 [*Staphylococcus* phage phiIBB-SEP1] (8e-74)	100% (100%)	AGR48292.1	
172	125186	124503	684	227	15.62 (10.16)	GIY-YIG homing endonuclease	Intron-associated endonuclease [*Staphylococcus* phage vB_SepS_SEP9] (2e-22)	41% (61%)	YP_009007670.1	SSF82771 GIY-YIG endonuclease (1.5e-11)
173	125347	125186	162	53	6.39 (10.63)	Resolvase	Resolvase [*Lactobacillusequi*] (3e-15)	66% (86%)	WP_023859949.1	IPR000551 MerR-type HTH domain (1.3e-10)
174	126136	125474	663	220	23.67 (6.27)	Transglycosylase	Transglycosylase-like domain protein [*Staphylococcus* phage phiIBB-SEP1] (1e-155)	97% (98%)	AGR48293.1	IPR008258 Lytic transglycosylase-like SLT domain (1.1e-07)
175	126411	126259	153	50	5.86 (5.76)	RinB	Transcriptional activator RinB [*Staphylococcus* phage phiIBB-SEP1] (9e-22)	90% (94%)	AGR48294.1	
176	126732	126415	318	105	12.50 (5.69)	Hypothetical protein	Hypothetical protein SEP1_168 [*Staphylococcus* phage phiIBB-SEP1] (1e-63)	93% (97%)	AGR48295.1	
177	127141	126725	417	138	16.24 (5.13)	Hypothetical protein	Hypothetical protein SEP1_169 [*Staphylococcus* phage phiIBB-SEP1] (4e-91)	99% (100%)	AGR48296.1	IPR021739 Bacteriophage T7, Gp1.7 (1.8e-08)
178	127566	127264	303	100	10.99 (4.68)	NTP pyrophosphohydrolase	Pyrophosphatase [*Solibacillus silvestris* StLB046] (8e-39)	67% (82%)	YP_006461979.1	IPR004518 NTP pyrophosphohydrolase MazG, putative catalytic core (1.5e-07)
179	127780	127613	168	55	6.57 (4.99)	Hypothetical protein	Hypothetical protein SEP1_172 [*Staphylococcus* phage phiIBB-SEP1] (2e-29)	96% (100%)	AGR48299.1	
180	128169	127903	267	88	9.95 (5.13)	Hypothetical protein	Hypothetical protein SEP1_173 [*Staphylococcus* phage phiIBB-SEP1] (6e-41)	94% (98%)	AGR48300.1	
181	128785	128150	636	211	23.63 (9.63)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (3e-138)	97% (99%)	AGR48301.1	
182	129158	128856	303	100	11.25 (9.15)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-59)	93% (97%)	AGR48302.1	
183	129777	129160	618	205	23.37 (4.21)	Nucleoside-2-deoxyribosyltransferase	nucleoside 2-deoxyribosyltransferase [*Staphylococcus* phage phiIBB-SEP1] (3e-80)	63% (74%)	AGR48303.1	IPR007710 Nucleoside 2-deoxyribosyltransferase (2.8e-16)
184	130051	129791	261	86	9.84 (10.13)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (7e-34)	69% (79%)	AGR48305.1	
185	130308	130066	243	80	9.73 (6.74)	Hypothetical protein	Hypothetical protein SEP9_060 [*Staphylococcus* phage vB_SepS_SEP9] (6e-47)	98% (100%)	YP_009007729.1	
186	131139	130363	777	258	31.03 (9.86)	HNH homing endonuclease	HNH endonuclease [*Staphylococcus* phage vB_SepS_SEP9] (3e-179)	97% (98%)	YP_009007730.1	HNH_3[pfam13392], HNH endonuclease (7.35e-03)
187	131879	131151	729	242	27.83 (5.29)	PhoH-related protein	PhoH-related protein [*Staphylococcus* phage vB_SepS_SEP9] (1e-169)	94% (97%)	YP_009007731.1	IPR003714 PhoH-like protein (2.3e-25)

188	132419	131907	513	170	19.55 (4.63)	Hypothetical protein	Hypothetical protein SEP1_181 [*Staphylococcus* phage phiIBB-SEP1] (5e-114)	99% (99%)	AGR48308.1	
189	132850	132434	417	138	15.81 (8.99)	Ribonuclease H	Ribonuclease H [*Staphylococcus* phage phiIBB-SEP1] (3e-94)	100% (100%)	AGR48309.1	RNase_HI_bacteria_HBD[cd09277], Bacterial RNase HI containing a hybrid binding domain (HBD) at the N-terminus (2.36e-41)
190	133028	132840	189	62	7.33 (9.16)	Hypothetical protein	Hypothetical protein SEP1_183 [*Staphylococcus* phage phiIBB-SEP1] (2e-35)	100% (100%)	AGR48310.1	
191	133647	133051	597	198	22.72 (4.11)	Hypothetical protein	Hypothetical protein SEP1_184 [*Staphylococcus* phage phiIBB-SEP1] (1e-129)	99% (98%)	AGR48311.1	
192	133861	133640	222	73	8.70 (5.38)	Transcriptional regulator	Putative transcriptional regulator [*Staphylococcus* phage phiIBB-SEP1] (2e-41)	99% (98%)	AGR48312.1	IPR001387 Cro/C1-type helix-turn-helix domain (3.8e-13)
193	134092	133871	222	73	8.80 (10.41)	Hypothetical protein	Hypothetical protein SEP1_186 [*Staphylococcus* phage phiIBB-SEP1] (5e-33)	97% (100%)	AGR48313.1	
194	135717	134263	1455	484	55.01 (9.78)	Endolysin	Endolysin [*Staphylococcus* phage phiIBB-SEP1] (0.0)	99% (99%)	AGR48314.1	Amidase_2[pfam01510], N-acetylmuramoyl-L-alanineamidase (1.68e-14) CHAP[pfam05257], CHAP domain (2.70e-24) H3_5[pfam08460], Bacterial SH3 domain (1.01e-10)
195	136280	135720	561	186	20.03 (4.27)	Holin	Holin [*Staphylococcus* phage phiIBB-SEP1] (8e-128)	99% (100%)	AGR48316	Phage_holin_1[pfam04531], Bacteriophage holin (1.18e-19)
196	136958	136593	366	121	14.08 (9.98)	Hypothetical protein	Hypothetical protein SEP9_085 [*Staphylococcus* phage vB_SepS_SEP9] (3e-59)	77% (89%)	YP_009007753.1	
197	138169	137948	222	73	9.02 (10.09)	Hypothetical protein	Hypothetical protein SEP1_194 [*Staphylococcus* phage phiIBB-SEP1] (1e-45)	99% (100%)	AGR48320.1	
198	138833	138624	210	69	78.97 (7.74)	Hypothetical protein	Hypothetical protein SEP1_196 [*Staphylococcus* phage phiIBB-SEP1] (2e-41)	100% (100%)	AGR48322.1	
199	139176	138844	333	110	12.82 (4.73)	Hypothetical protein	Hypothetical protein SEP1_197 [*Staphylococcus* phage phiIBB-SEP1] (2e-67)	99% (100%)	AGR48323.1	
200	139513	139187	327	108	12.72 (6.51)	Hypothetical protein	Hypothetical protein SEP1_198 [*Staphylococcus* phage phiIBB-SEP1] (2e-67)	98% (99%)	AGR48324.1	
201	139924	140283	360	119	13.97 (5.42)	Membrane protein	Membrane protein [*Staphylococcus* phage phiIBB-SEP1] (2e-75)	99% (100%)	AGR48326.1	

202	140264	140527	264	87	10.01 (10.19)	Membrane protein	Hypothetical protein SEP1_200 [*Staphylococcus* phage phiIBB-SEP1] (2e-55)	99% (100%)	AGR48327.1	
203	140532	140951	420	139	15.67 (4.08)	Hypothetical protein	Hypothetical protein SEP1_201 [*Staphylococcus* phage phiIBB-SEP1] (3e-92)	99% (100%)	AGR48328.1	

Table S2: Features of bacteriophage phiIPLA-C1C *orfs*, gene products (gp) and functional assignments.

Promoter	**Strand**	**Sequence**	**Nt position**	**Spacer**	**TG**	**Gene**
phiIPLA-RODI						
P1	-	TTGACATTCTAATTAATATCCTTTATACT	1964-1992	17	-	Orf5
P2	-	TTGACTTTTTTTACTAAGTATGGTAAGAT	6909-6937	17	+	Orf16
P3	-	TTGACAAAATAGAAAAAGTAGTGTATAGT	11380-11408	17	+	Orf25
P4	-	TTGACAAATGAAAATACTTGTATTATAAT	15732-15760	17	-	Orf33
P5	-	TTGACAAATATGACTTACTATGATATGAT	19495-19523	17	+	Orf39
P6	-	TTGACAAACCTCCTTAGTTATGGTATACT	22318-22346	17	+	Orf45
P7	-	TTGACTTCATAAGTTAACTATGCTATAAT	25531-25559	17	+	Orf52
P8	-	TTGACAAAATTAAATACATAGTGTATAGT	27631-27659	17	+	Orf55
P9	-	TTGACTTATTTATCAATATAGTATATAGT	32469-32497	17	-	Orf60
P10*	+	TTGACCTATTATTTCTAGAACTTTTAGATT	34122-34151	18	-	Orf64
P11	+	TTGACAAATTAAAACTAATAAATTATAAT	55708-55736	17	-	Orf91
P12	+	TTGACAGAAAGTTAATAATATGGTATACT	73596-73624	17	+	Orf103
P13	+	TTGACTTAGGGAGCATTATGTGGTATACT	81631-81659	17	+	Orf109
P14	+	TTGACATTTTATATGTTAGGTGGTATAAT	86518-86546	17	+	Orf112
P15	+	TTGACAAGATTTAAAATATATGGTATAGT	98834-98862	17	+	Orf128
P16	+	TTGACAATATGTTTAACTTATGTTATACT	102120-102148	17	+	Orf129
P17	+	TTGACAAATATAAAAAACTATGTTATAAT	102944-102972	17	+	Orf131
P18	+	TTGACAAGAACAAATAAGTGTAGTATAGT	118027-118055	17	-	Orf154
P19	+	TTGACAAATCATTTTATATAGTGTATAGT	118747-118775	17	-	Orf157
P20	+	TTGACACTTCTAAACTTTTGTATTATACT	121451-121479	17	-	Orf165
P21	+	TTGACAAATGAGTGTGCATAGGTTATACT	122469-122497	17	-	Orf168
P22	+	TTGACAAAGGGAGTTTTTTATTGTATAGT	126557-126585	17	+	Orf175
P23	+	TTTACATTTAGTAAGTAATATGGTAATATT	127212-127241	18	-	Orf177
P24	+	TTGACAACGAAGGAAACATGTAGTATAGT	128429-128457	17	-	Orf181
P25	+	TTGACAACAAATAAACCATGTAGTATAAT	129202-129230	17	-	Orf183
P26	+	TTGACAATAGTAAAGTAATAGGTTATAAT	130829-130857	17	-	Orf187
P27	+	TTGACATGGATAGAGTTTTACTATATACT	131544-131572	17	-	Orf188
P28	+	TTGACAATCATGAAGCGGTATGTTATAAT	133272-133300	17	+	Orf192
P29*	-	TTGACTTCTGAATAACTATACTGTAATAT	137054-137082	17	-	Orf202
P30	+	TTGACTTTATTATCATATGGTAGTAATAT	137177-137205	17	-	Orf203
P31	+	TTGACACCTTACAAGATACATGTTATTAT	137687-137715	17	+	Orf204
P32	-	TTGACATTAAGACCGAATTATTATATAAT	139360-139388	17	-	Orf206
P33	+	TTGACTTATGTTTATTTCTATAGTAATAT	139434-139362	17	-	Orf207
P34	+	TTGACAACCTATAAACAACGTGTTAATAT	139655-139683	17	+	Orf208
P35	+	TTGACAGTCACTTGAAACCATGATATTAT	139969-139997	17	+	Orf209
P36	+	TTGACTTCCAAGCCCTAGCATGTTATTAT	140373-140401	17	+	Orf210
P37	+	TTGACAACCTTCCAACTACATGTTATTAT	141244-141272	17	+	Orf211
P38	+	TTGACAAACTAACCGCTTCATGATAATAT	141728-141756	17	+	Orf212
P39	+	TTGACATTTAGCCCCTTAGATGTTATTAT	141972-142000	17	+	Orf213
P40	+	TTGACATCCTAGCAAATAGATGGTAATAT	142290-142318	17	+	Orf1
phiIPLA-C1C						
P1*	+	TTGACAAAATAATAATATATGATATAAT	372-399	16	+	Orf2
P2	+	TTGACAACCATCAAGGGTTAAATTATAAT	17452-17480	17	-	Orf21
P3	+	TTGACAAACTAATAAAGAGAAGATATAAT	18306-18334	17	-	Orf24
P4	+	TTGACTTATTAAAAGTTAAGTGGTATAAT	53130-53158	17	+	Orf49
P5	+	TTGACTTATCAAAAGTTAAGTGGTATAAT	55110-55138	17	+	Orf52
P6*	+	TTGACAAACTATATCTATTTATGATATAGT	71274-71303	18	+	Orf71
P7*	+	TTGACAAATATATACATATATGTTATAGT	77949-77975	17	+	Orf80
P8*	+	TTGACAAAAAAGTTATTATAGTTTATAGT	89480-89508	17	-	Orf100
P9	+	TTGACAGGGGTCTTTTTTTTATGTTATAGT	93681-93710	18	+	Orf105

P10	+	TTGACACTGGGTGTTTTTGTTATATACT	95823-95851	17	-	Orf109
P11	+	TTGACAACTGTATAAATATAGTGTATATT	104207-104235	17	-	Orf132
P12	-	TTGACAGGAGGTCTTTTCTATGATATACT	108967-108995	17	+	Orf141
P13	-	TTGACAACTAATATTACTTATGCTATAAT	109378-109406	17	+	Orf142
P14*	-	TTGACTATTGCTTTTTAATGCAGATTAATAT	112897-112927	19	-	Orf148
P15	+	TTGACACCTTATAAGAAACATGTTAATAT	113600-113628	17	+	Orf150
P16	+	TTGACAATATTATATTAAAAATGCTATAAT	114425-114454	18	+	Orf152
P17	+	TTGACAACTTAAACACAACATGTTATTAT	115114-115142	17	+	Orf153
P18	+	TTGACAATCAACCCCTACACATGTTATTAT	115333-115362	18	+	Orf154
P19	+	TTGACAATCTCTTCACTATTTGATATTAT	115788-115816	17	+	Orf156
P20	+	TTGACAGTAATTTGAAACTATGATAATAT	116298-116326	17	+	Orf157
P21	+	TTGACAACGTAACTAGAACATGATATTAT	116730-116758	17	+	Orf158
P22	+	TTGACAGTTTCTATGTTATAATGTTATAAT	117850-117879	18	+	Orf161
P23	-	TTGACAGATAAAGTTATCTATGGTATACT	122302-122330	17	+	Orf167
P24	-	TTGACAAAAATGAATAGCTATGGTATACT	127601-127629	17	+	Orf178
P25	-	TTGACAACTTAAGTAGTAAATGTTATAAT	127855-127883	17	+	Orf179
P26	-	TTGACTTTTAAGTTTATATGTGTTATAAT	134125-134153	17	+	Orf193
P27	-	TTGACATTTATCAAAAATAAGATTATAAT	138211-138239	17	+	Orf197

Table S3: Putative promoters of phages phiIPLA-RODI and phiIPLA-C1C. -10 and -35 boxes are underlined. Nucleotide positions and presence of the TG dinucleotide were also indicated. * Promoters without AT-rich upstream sequences.

Terminator	Strand	Sequence (5’-3’)	Nt position	Stem lenght	ΔG	After gene
phiIPLA-RODI						
T1	+	GUUUAGACUAAGAGGG**AAUAAAA**UCCCUCUUUUAUUUUUAU	1304-1344	18	-9.8	Orf4
T2	-	UGAACUAGUUGGAGGGG**AGUUAAC**UCCCUCUCUUUUUUUUUU	1608-1649	19	-8.4	Orf5
T3	-	UUAAAUGAUAAACACCUAU**UAAAUUA**AUAGGUGUUUUUUUAUUGACU	6932-6978	23	-8.6	Orf17
T4	-	AUUAAUUCUUAGGCUACU**UUAAUU**AGUAGCCUUUUUUUGUUGA	11405-11447	20	-11.2	Orf26
T5	-	UAGGUACAGAAGCAGACUU**UUAAU**AAGUCUGCUUUUCUCUUAUAU	12171-12215	21	-11.8	Orf28
T6	-	CUUUCCUUUUUCACCUUGC**UUGUAUCCAA**GCAGGGUGUUUUUUUAUAUA	16604-16652	26	-11.7	Orf36
T7	-	AUAUUGACAAACCUCCUUA**GUUAUGGUAUACUUACUUUAUAAUAAC**UAAGGAGGaUUUUUUUAUGAAU	22282-22349	43	-10.6	Orf46
T8	-	UAAUAUAUUAAGACUAAGA**UUAAUU**UCUUAGUCUUUUUUGUAUAUU	25564-25609	22	-10.5	Orf53
T9	-	AAUAAUAAAUUAGAGAGGU**UAAU**ACCUCUCUUUUUUUUGUCUUUA	26457-26501	20	-11.6	Orf54
T10	-	AAUAGUAAUUUAGACGGAU**UUUAA**AUCCGUCUaUUUUUUUUGCAAA	27666-27711	21	-11	Orf56
T11	+	AUAAAACUGAAGAGGAGU**AAUU**ACUCCUCUUUUUUGUUUGC	40917-40957	18	-10.8	Orf72
T12	+	AUUAAUUAAUAAGCCUAGA**AUAAA**UCUAGGCUUUGUUUAUUUUUU	43443-43487	21	-11.3	Orf75
T13	+	ACAAGAGAAUAGGGAUAAA**CUUAGGG**UUUAUCCCUUUUUUAUUAAAA	46911-46957	23	-10.2	Orf78
T14	+	UUUCUUAUUAAGACCUAAC**AAUAAAA**GUUAGGUCUUUUUUUAUUGA	53676-53721	23	-11.4	Orf86
T15	+	GUAUAUGUAAAGGGUGGUA**GGUGAUAC**UACCAUCCUUAUUUUUUUAA	57242-57288	24	-12.1	Orf93
T16	+	UUUAAUAUUAAAGACCUAU**UAAU**UUAGGUCUUUUUUUAGUUGUA	67310-67353	20	-9	Orf97
T17	+	UGAAUAAACUAGAGGGGUU**GAUU**GACCCCUCUUUAUUUAAUAA	77692-77734	20	-14.4	Orf104
T18	+	AAUAUGCCAUAGACUAGGA**AACUUA**UCCUAGUCUUUUUUUCUUGA	81590-81634	22	-12.1	Orf108
T19	+	GACUUAAUGAAGAAGAGAA**AUAA**UUCUCUUCUUUUUUUAUUGACA	98795-98839	20	-9.3	Orf127
T20	+	UAUAAGAUAUAGAGUGC**CUUAGA**GCACUCUUUUAUUUGAGA	104276-104316	18	-9.2	Orf131
T21	+	GUAAAAGAAUCUUUGGGGA**AUGCAAAU**UCCUCAGAUGUUCUUCCUUU	105633-105679	24	-8.2	Orf132
T22	+	AUAAUAAUUAAGACCAACU**AAAA**AGUUGGUCUUUUUUUAUUGA	108136-108178	20	-11.5	Orf137
T23	+	GAUUUCUUAUAGAGUCAAG**UCUUUA**CUUGACUCUUUUUACUAUAU	111166-111210	22	-12.1	Orf142
T24	+	GAACAGUGAUUGAGUCAAG**UUAAUU**CUUGACUCUCUUUUUGUUUU	117159-117203	22	-11.5	Orf151
T25	+	AUAAAUCUUAAACUCCCUA**UUGACA**AAGGGAGUUUUUUAUUGUAUA	126538-126583	22	-10.2	Orf174
T26	+	AAAAACUUUGACUCUAUCU**AUUGACAU**GGAUAGAGUUUUACUAUAUA	131524-131570	24	-9.5	Orf187
T27	+	AAAAAUAAAUACACUAGGA**UAUUAU**UCCUAGUGUAUUAUAUAAUU	136582-136626	22	-12.3	Orf201
T28	-	AAUUAUAUAAUACACUAGG**AAUAAUAU**CCUAGUGUaUUUAUUUUUGCGG	136578-136626	24	-12.2	Orf202
T29	+	AAUUAUACAAUUCCCUAGG**AUUAAAUU**CCUAGGGAUUUUUAUUUGUU	138352-138398	24	-14.6	Orf205
T30	-	ACAAAUAAAAAUCCCUAGG**AAUUUAAU**CCUAGGGAaUUGUAUAAUUUUU	138349-138397	24	-14.9	Orf206
T31	+	AAAAAUUAAAAUAAGGGGU**UGACAUUUA**GCCCCUUAgaUGUUAUUAUUAA	141954-142003	25	-11.5	Orf213
T32	+	AUAAAUCUUAAACUCCCUA**UUGACA**AAGGGAGUUUUUUAUUGUAUA	126538-126583	22	-10.2	Orf174

T33	+	AAAAACUUUGACUCUAUCU**AUUGACAU**GGAUAGAGUUUUACUAUAUA	131524-131570	24	-9.5	Orf187
T34	+	AAAAAUAAAUACACUAGGA**UAUUAU**UCCUAGUGUAUUAUAUAAUU	136582-136626	22	-12.3	Orf201
T35	-	AAUUAUAUAAUACACUAGG**AAUAAUAU**CCUAGUGUaUUUAUUUUUGCGG	136578-136626	24	-12.2	Orf202
T36	+	AAUUAUACAAUUCCCUAGG**AUUAAAUU**CCUAGGGAUUUUUAUUUGUU	138352-138398	24	-14.6	Orf205
T37	-	ACAAAUAAAAAUCCCUAGG**AAUUUAAU**CCUAGGGAaUUGUAUAAUUUUU	138349-138397	24	-14.9	Orf206
T38	+	AAAAAUUAAAAUAAGGGGU**UGACAUUUA**GCCCCUUAgaUGUUAUUAUUAA	141954-142003	25	-11.5	Orf213
T39	+	AUAAAUCUUAAACUCCCUA**UUGACA**AAGGGAGUUUUUUAUUGUAUA	126538-126583	22	-10.2	Orf174
T40	+	AAAAACUUUGACUCUAUCU**AUUGACAU**GGAUAGAGUUUUACUAUAUA	131524-131570	24	-9.5	Orf187
T41	+	AAAAAUAAAUACACUAGGA**UAUUAU**UCCUAGUGUAUUAUAUAAUU	136582-136626	22	-12.3	Orf201
T42	-	AAUUAUAUAAUACACUAGG**AAUAAUAU**CCUAGUGUaUUUAUUUUUGCGG	136578-136626	24	-12.2	Orf202
T43	+	AAUUAUACAAUUCCCUAGG**AUUAAAUU**CCUAGGGAUUUUUAUUUGUU	138352-138398	24	-14.6	Orf205
T44	-	ACAAAUAAAAAUCCCUAGG**AAUUUAAU**CCUAGGGAaUUGUAUAAUUUUU	138349-138397	24	-14.9	Orf206
T45	+	AAAAAUUAAAAUAAGGGGU**UGACAUUUA**GCCCCUUAgaUGUUAUUAUUAA	141954-142003	25	-11.5	Orf213
T46	+	AUAAAUCUUAAACUCCCUA**UUGACA**AAGGGAGUUUUUUAUUGUAUA	126538-126583	22	-10.2	Orf174
T47	+	AAAAACUUUGACUCUAUCU**AUUGACAU**GGAUAGAGUUUUACUAUAUA	131524-131570	24	-9.5	Orf187
T48	+	AAAAAUAAAUACACUAGGA**UAUUAU**UCCUAGUGUAUUAUAUAAUU	136582-136626	22	-12.3	Orf201
T49	-	AAUUAUAUAAUACACUAGG**AAUAAUAU**CCUAGUGUaUUUAUUUUUGCGG	136578-136626	24	-12.2	Orf202
T50	+	AAUUAUACAAUUCCCUAGG**AUUAAAUU**CCUAGGGAUUUUUAUUUGUU	138352-138398	24	-14.6	Orf205
T51	-	ACAAAUAAAAAUCCCUAGG**AAUUUAAU**CCUAGGGAaUUGUAUAAUUUUU	138349-138397	24	-14.9	Orf206
T52	+	AAAAAUUAAAAUAAGGGGU**UGACAUUUA**GCCCCUUAgaUGUUAUUAUUAA	141954-142003	25	-11.5	Orf213
phiIPLA-C1C						
T1	+	UAAGUAAUUUAAAGGGUAG**AUAUA**CUACCCUUUUUUUGCAUGUUAU	3632-3676	21	-10.6	Orf5
T2	+	AAAAUUUUAUAGAGGGUAA**ACUUUGU**UUAUCCUCUUUUUUUAUUAU	9623-9668	23	-8.7	Orf11
T3	+	UUCUAAGUAAAUAGACCAG**GAUUAAAUU**CUUGGUCUAUUUAACUUGACA	16407-16455	26	-9	Orf19
T4	+	UUAAUACAAAACCUAUACU**AUCUGUUAUAUUAAUUAUGAAUAAUUAAAU**AGUAUAGGUUUAUUUUACGUU	18696-18765	46	-7.31	Orf24
T5	+	UAUAUAUGUAGGGUGGUAG**GAUGUUA**CUACCACCUUUUUUUAAAGU	19728-19773	23	-14.2	Orf26
T6	+	GAGUUUAAUAAGGUGGUU**UAUA**AACCACCUUUUAUACAUAU	25289-25329	18	-10.1	Orf30
T7	+	UUAAUUUAUAAGGCGGU**UUUAU**ACCGUCUUUUUUAUUAUG	31717-31756	17	-8.5	Orf33
T8	+	UAUCUAUUUAAGACUAAGU**UAAAA**ACUUAGUCUUUUUUUAUUUGCA	455639-45684	21	-9.3	Orf44
T9	+	AAAGACUUAGAGAGACAGA**UUAAAAAA**UUUGUCUCUUUUUUUAUUGA	66853-66899	24	-9.2	Orf67
T10	+	AUUUAAUAUUAGAGUGC**UUAA**GCACUCUUUUAUUUGAUU	73137-73174	16	-9.7	Orf72
T11	+	ACCGCUUUUUAGACUAGGA**UUAAAU**UCCUAGUCUUUUUUUUAUUG	77907-77951	22	-12.9	Orf79
T12	+	AUUAAGAAAAAGUAGAGGC**AUAUUU**GCUUCUACUUUAAUUAUAUG	80619-80663	22	-11.9	Orf83
T13	+	CUUUUUUUAAGCAGUCGAU**ACUAAAAAGU**AUUGACUGUUUUUUGCUUUU	87110-87158	26	-7.4	Orf94
T14	+	AAUUUUUUACUGACCCCU**AUUGAC**AGGGGUCUUUUUUUUAUGU	93662-93704	20	-12.7	Orf104

T15	+	UAAAUUAUAAAAACACCCU**UGACAC**UGGGUGUUUUUUGUUAUAUAC	95805-95849	22	-10.3	Orf108
T16	+	GAUGAAAUGGGGGAGUAGG**GAAA**CUUACUCCUUUUUUUUAUUUA	101395-101437	20	-15	Orf123
T17	-	AAUAGUUGAGUACCGCUUC**AGUAAUAUAG**AAGCGGUaUUUUUUUUUGUUU	108276-108325	25	-12.2	Orf140
T18	-	AAUAAUUACUAGACCUCUU**GAC**AGGAGGUCUUUUCUAUGAUAU	108970-109012	19	-10.3	Orf142
T19	+	UAUAAUAACAAACCGCUUC**AGAUUAAAUUCUC**GAAGCGGUcUUAUUUUUUUAGG	111294-111347	29	-11.3	Orf146
T20	-	AUAAUCAAUUAGACUAGGG**UUUU**CCCUAGUCUUUUUAUGUUCUA	112122-112165	20	-14	Orf147
T21	+	UAAUUUAAAAUUAGGGGUU**GCAAUCAA**AACCCCUAUUUGCUAUAAUA	113490-113536	24	-12.5	Orf149
T22	+	AAUAAUAUUAAGCCUAGGA**UUAAAU**UCCUAGGUUUUUUUUUUAUUUU	114326-114371	22	-12.6	Orf151
T23	+	GUAAGAGAGUACAAAGGGG**AAUAAU**UCCCUUUGUAUUUUUUUGUU	118166-118210	22	-10.1	Orf161
T24	+	AUAAUUUAAAAAGCAAGAG**AUAAAAA**CUCUUGCUUUUAUUUAUAUAGA	119932-119979	23	-10.2	Orf162
T25	-	AAAGAUUUAAAAGACUAGU**UACAA**ACUAGUCUUUUUUUAUUUAUA	122332-122376	21	-9	Orf168
T26	-	AAUAAUUACUAGGACUAGG**AUUUAUU**CCUAGUCCUUAUUUUUUUAGG	122731-122777	23	-14.3	Orf169
T27	-	AUUAGUAAUAAACAAGGGA**UAAA**ACCCUUGUUUAUUUUUUUUAC	125421-125464	20	-9.5	Orf174
T28	-	UCUUUCCUUUUCCCUUAUU**ACUUUUGU**AGUAAGGGUUUUAUUUUAUA	127155-127201	24	-8.5	Orf178
T29	-	ACUAUUACUACAGAAGAAC**UUUAAAAA**GUUCUUCUUUUUUUUUAUUGACA	127878-127926	24	-9	Orf180
T30	-	AUUUAUUUUUAGACUAGGA**UUAAAU**UCCUAGUCUUUUUUUUAUUGA	134211-134255	22	-12.9	Orf194
T31	-	AUAUAGAAUAACCACCUAU**UUAU**GUAGGUGGUUUUCUUAUAUU	136347-136389	20	-12	Orf196
T33	-	GAAUUAUUAAAGGCUAAC**UUAU**GUUAGUCUUUUUUUUAUAUA	138593-138633	18	-8.3	Orf198

Table S4: Putative terminators of phages phiIPLA-RODI and phiIPLA-C1C. The underline sequence corresponds to the terminator stem. Lowercase letter in RNA motifs indicates the spacer element between the stem-loop and T-rich region.

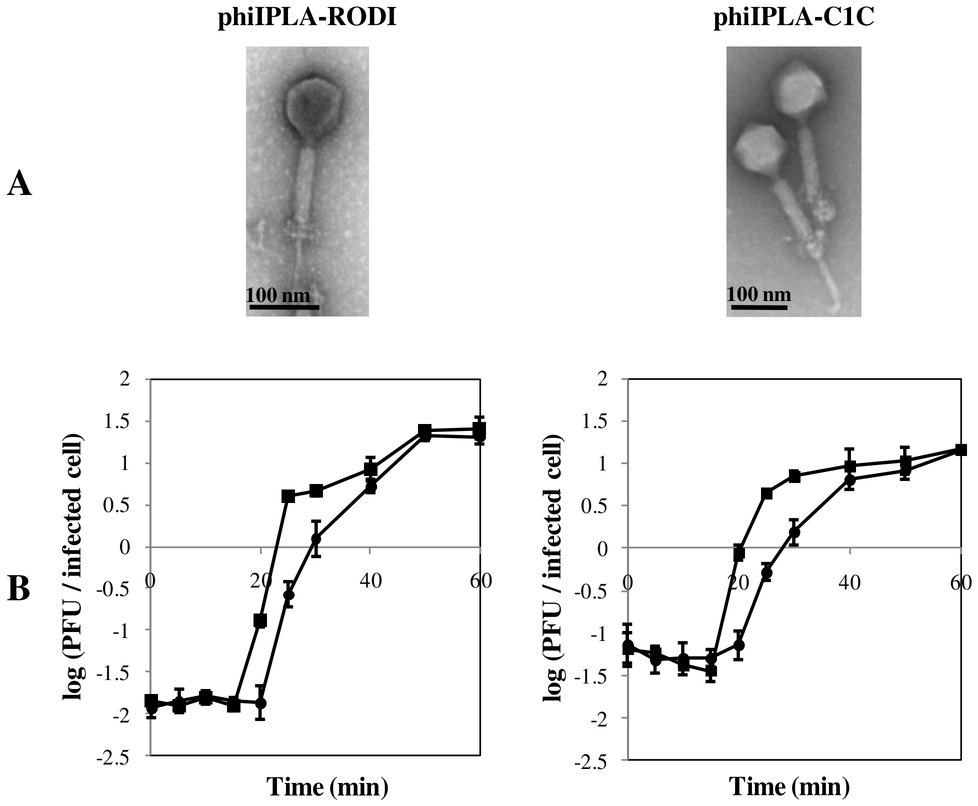

Figure 1. A) Transmission electron microphotographs of phages phiIPLA-RODI and phiIPLA-C1C; scale bars correspond to 100 nm. B) One-step growth curves of phage phiIPLA-RODI in *S. aureus* IPLA16 and phiIPLA-C1C in *S. epidermidis* LO5081. Values correspond to the number of PFU per infected cell in chloroform-treated cultures (■) and in untreated cultures (●). Each data point is the mean ± standard deviation of three independent experiments.

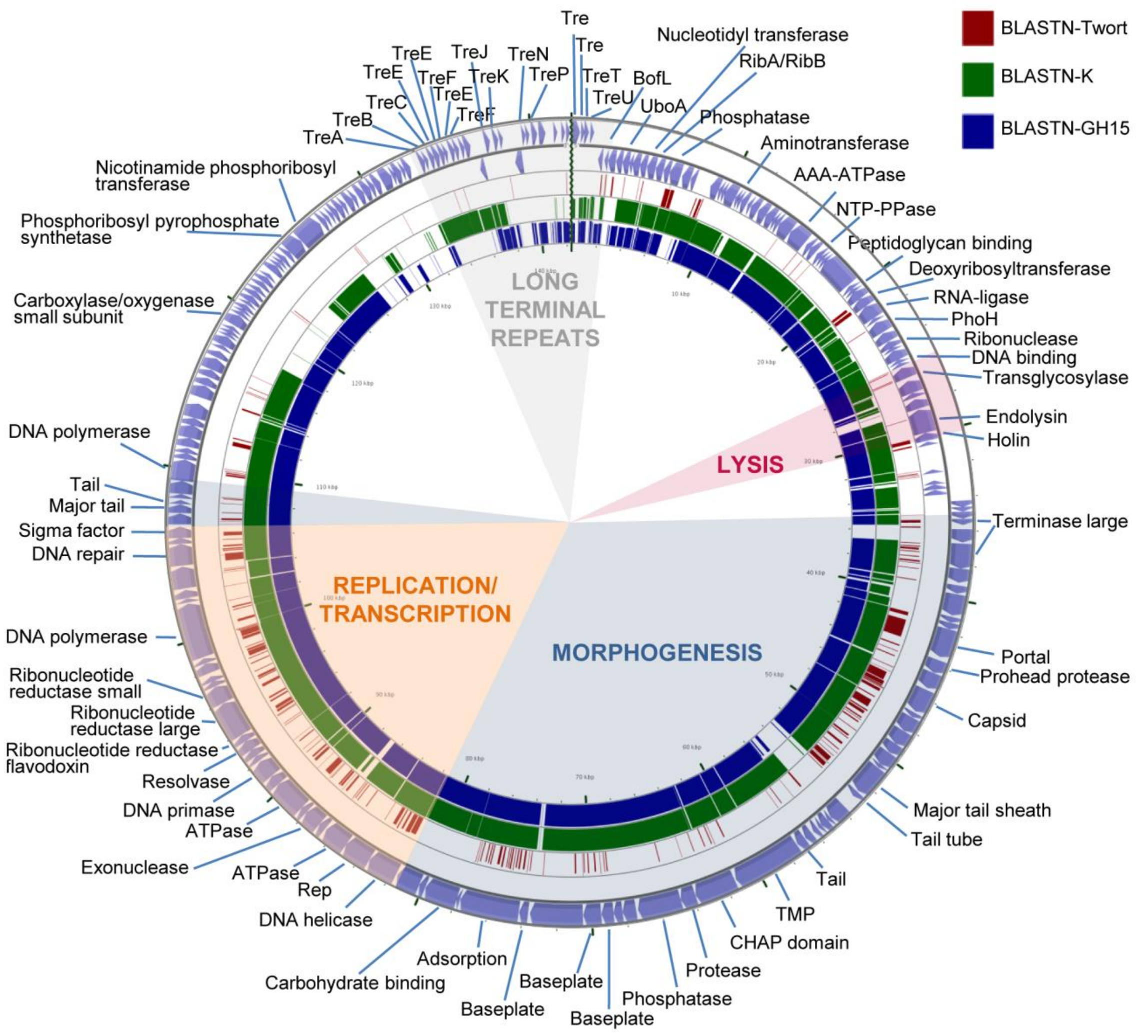

Figure 2. Genome organization of phage phiIPLA-RODI and BLASTN comparison. The outer ring with the arrows represents the *orf*s of the circularized phage. The predicted gene functions are also indicated. The different functional modules in the genome are shown as a coloured shadow. BLASTN, that is represented by each inner ring, was performed with the representative phage Twort (pink), the phage K (green) and the most similar GH15 (light blue).

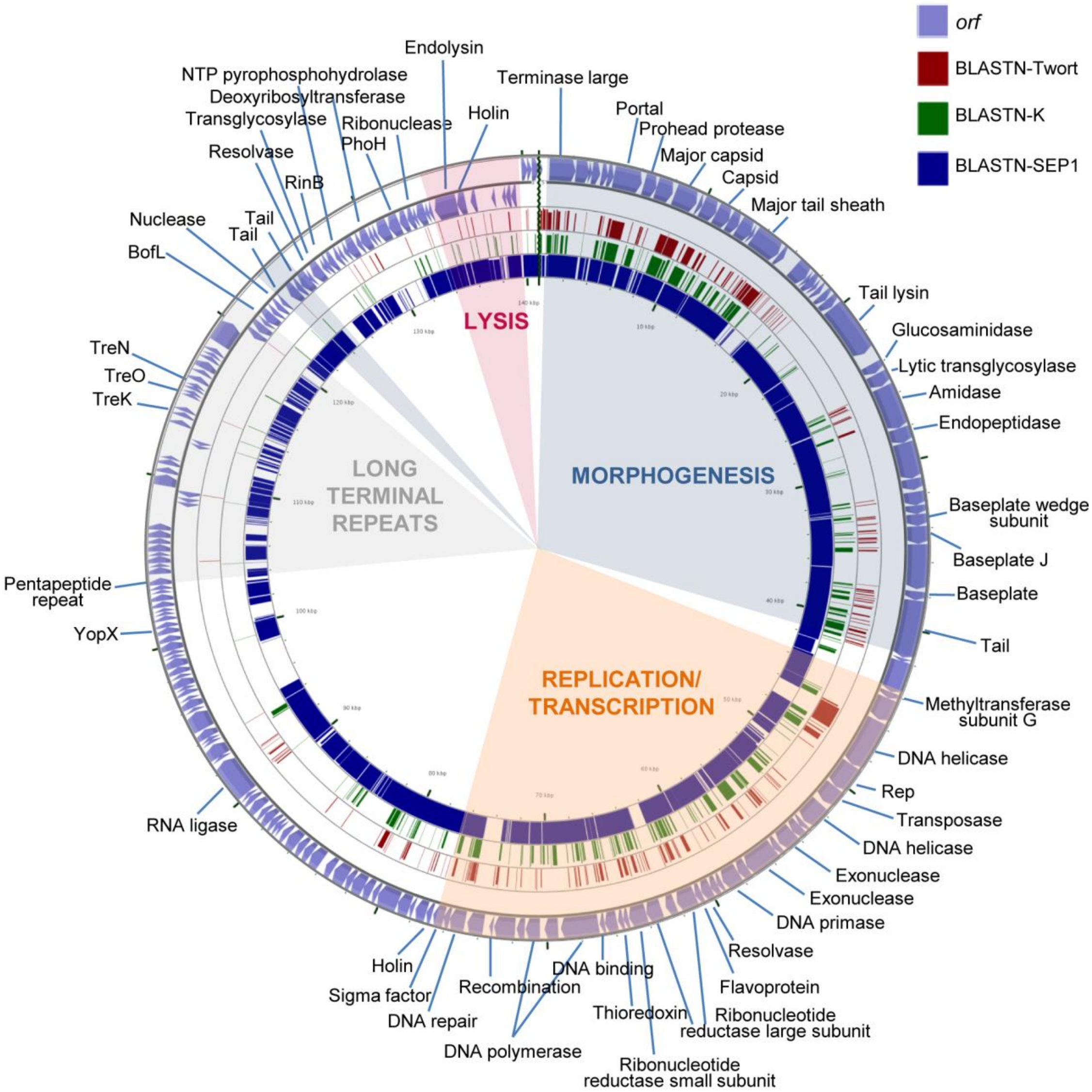

Figure 3. Genome organization of phage phiIPLA-C1C and BLASTN comparison. The outer ring with the arrows represents the *orf*s of the circularized phage. The predicted gene functions are indicated. The different functional modules in the genome are shown as a coloured shadow. BLASTN, that is represented by each inner ring, was performed with the representative phages Twort (pink), and K (green) and the most similar phiIBB-SEP1 (light blue).

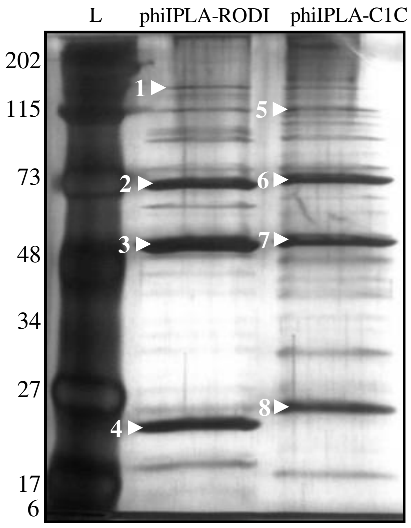

Figure 4. Analysis by SDS-PAGE electrophoresis and silver staining of the structural proteins of phages phiIPLA-RODI and phiIPLA-C1C. Protein molecular size markers (kDa) are shown on the left (Lane L). Bands marked with a white arrow were identified by mass-spectrophotometry: In phage phiIPLA-RODI (1) adsorption-associated tail protein (*orf104*), (2) major tail sheath protein (*orf85*), (3) capsid protein (*orf78*) and (4) major tail protein (*orf136*). In phage phiIPLA-C1C, (5) tail protein (*orf40*), (6) major tail sheath (*orf18*) (7) major capsid protein (*orf11*) and (8) hypothetical protein (*orf85*).

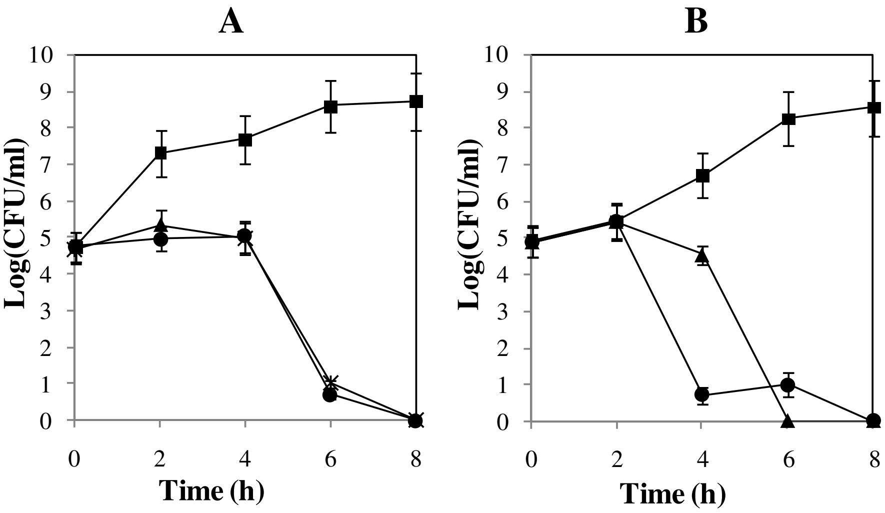

Figure 5. Susceptibility of the strains (A) *S. aureus* IPLA16 and (B) *S. epidermidis* LO5081 to phages phiIPLA-RODI and phiIPLA-C1C. Cell counts of control cultures (■) and treated cultures with phiIPLA-RODI (▲) and phiIPLA-C1C (●) are represented as log (CFU/ml). Each value corresponds to the mean ± standard deviation of three independent experiments.

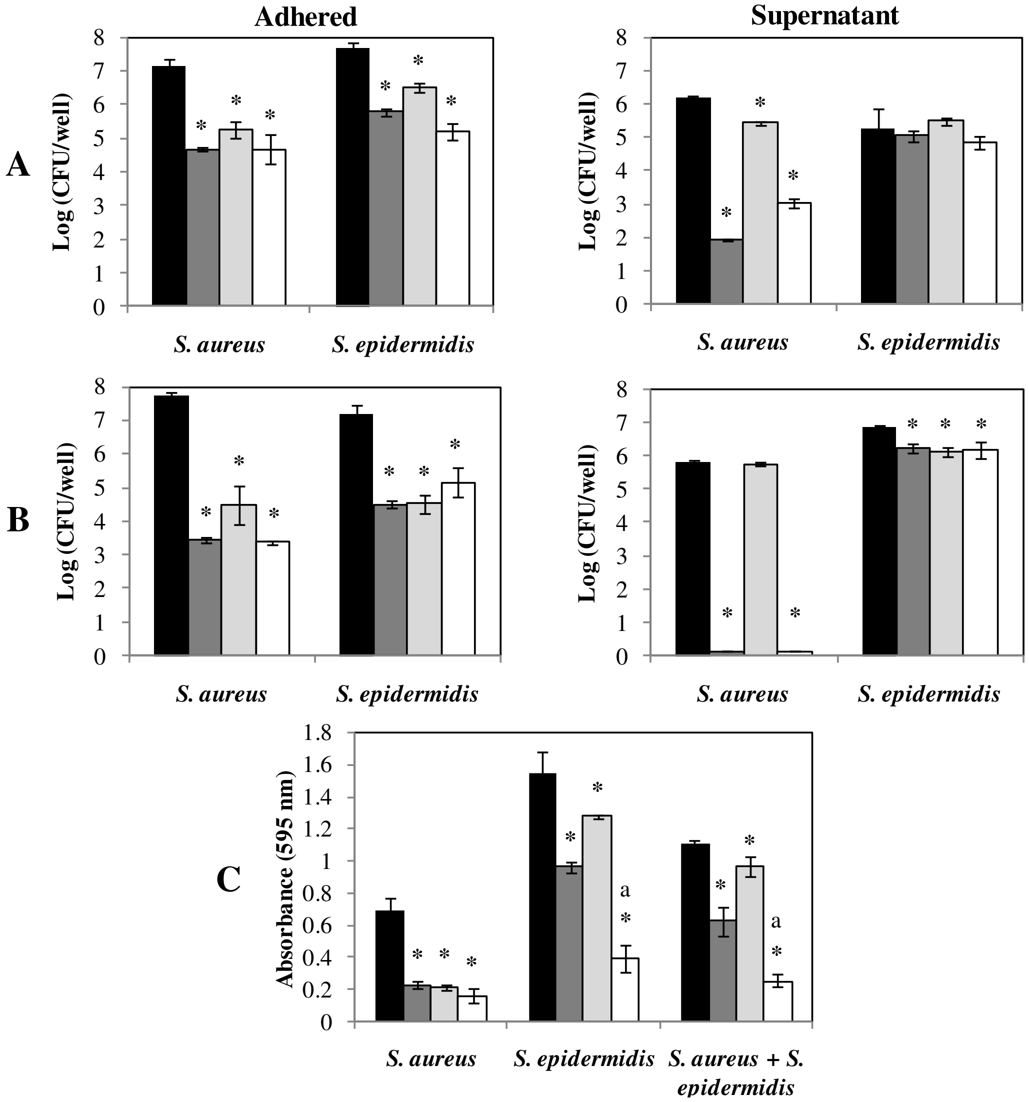

Figure 6. Bacteriophage mediated removal of 24 h-old *S. aureus* and *S. epidermidis* biofilms. (A) Mono- or (B) dual-species biofilms of *S. aureus* IPLA16-rif^R and *S. epidermidis* LO5081 were treated with phage phiIPLA-RODI (dark grey), phage phiIPLA-C1C (light grey) or with a mixture of both phages (white), for 4 h. Control biofilms are presented in black. Adhered cell counts and supernatant cell counts were expressed as Log (CFU/well). Bacteria detection threshold [<10 Log (CFU/ml)]. Alternatively, biomass was calculated by crystal violet staining of adhered cells after phage treatment (C). Absorbance was measured at a wavelength of 595 nm. Means and standard deviations were calculated from three biological replicates. Bars having an asterisk are significantly different from the control (ANOVA; $P<0.05$) and bars with a lower case ‘a’ indicates a significantly different decrease in biomass between the treatment with the mixture of phages and the individual treatment either with phiIPLA-RODI or phiIPLA-C1C (ANOVA; $P<0.05$).