Microbiological and Molecular Assessment of Bacteriophage ISP for the Control of Staphylococcus aureus

Katrien Vandersteegen¹, Wesley Mattheus¹, Pieter-Jan Ceyssens¹, Florence Bilocq⁴, Daniel De Vos⁴, Jean-Paul Pirnay⁴, Jean-Paul Noben³, Maia Merabishvili⁴,⁵, Urszula Lipinska², Katleen Hermans², Rob Lavigne¹*

¹ Division of Gene Technology, Katholieke Universiteit Leuven, Heverlee, Belgium, ² Department of Pathology, Bacteriology and Poultry Diseases, Ghent University, Merelbeke, Belgium, ³ Hasselt University, Biomedical Research Institute and Transnational University Limburg, School of Life Sciences, Diepenbeek, Belgium, ⁴ Laboratory for Molecular and Cellular Technology, Burn Centre, Queen Astrid Military Hospital, Brussels, Belgium, ⁵ Eliava Institute of Bacteriophage, Microbiology and Virology, Tbilisi, Georgia

Abstract

The increasing antibiotic resistance in bacterial populations requires alternatives for classical treatment of infectious diseases and therefore drives the renewed interest in phage therapy. Methicillin resistant Staphylococcus aureus (MRSA) is a major problem in health care settings and live-stock breeding across the world. This research aims at a thorough microbiological, genomic, and proteomic characterization of S. aureus phage ISP, required for therapeutic applications. Host range screening of a large batch of S. aureus isolates and subsequent fingerprint and DNA microarray analysis of the isolates revealed a substantial activity of ISP against 86% of the isolates, including relevant MRSA strains. From a phage therapy perspective, the infection parameters and the frequency of bacterial mutations conferring ISP resistance were determined. Further, ISP was proven to be stable in relevant in vivo conditions and subcutaneous as well as nasal and oral ISP administration to rabbits appeared to cause no adverse effects. ISP encodes 215 gene products on its 138,339 bp genome, 22 of which were confirmed as structural proteins using tandem electrospray ionization-mass spectrometry (ESI-MS/MS), and shares strong sequence homology with the ‘Twort-like viruses’. No toxic or virulence-associated proteins were observed. The microbiological and molecular characterization of ISP supports its application in a phage cocktail for therapeutic purposes.

Introduction

The scientific reappraisal of the use of bacteriophages in the treatment of bacterial infections is reflected by hundreds of phage therapy-related publications in the last decade. However, so far, no phage preparation has been approved for market authorization. In 2009, Merabishvili et al. [1] evaluated the safety and efficacy of bacteriophage therapy with the standardized quality-controlled small-scale production of the phage cocktail BFC-1. This cocktail contains two phages, 14/1 and PNM, infecting Pseudomonas aeruginosa, and one phage infecting Staphylococcus aureus, both frequent pathogens in burn wound infections. The S. aureus-infecting phage in BFC-1 is ISP, a member of the Myoviridae and closely related to phage G1 [2]. ISP was originally isolated in the 1920s from an unknown source in Tbilisi (Georgia) by the Eliava Institute of Bacteriophage, Microbiology and Virology and was selected as a therapeutic phage based on a host range study on burn wound isolates. The physicochemical properties and the pyrogenicity of the phage cocktail, and hence of the ISP preparation, are conform to the European Pharmacopeia standards and show no cytotoxicity towards human neonatal foreskin keratinocytes. The quality control of BFC-1 also confirmed the absence of temperate bacteriophages and verified the presence of the expected virion morphology as well as the specific interaction with the target bacteria [1].

In this paper, we present the complete microbiological and molecular examination of this therapeutically important phage, which includes stability assays, genome and virion analysis and an extensive host range screening.

Analysis

ISP host range screening and analysis of the Staphylococcus host collection

High-titer ISP stocks were obtained through amplification in liquid Mueller Hinton medium using S. aureus subsp. aureus Rosenbach ATCC 6538 (further referred to as ‘strain ATCC
Phage ISP was subjected to a host screening involving 86 S. aureus strains and nine S. haemolyticus isolates (Table S1). These isolates have a different origin, ranging from human and animal isolates to propagation strains for typing phages. All isolates were typed using automated repetitive sequence-based PCR (rep-PCR) DNA fingerprinting. Therefore, bacterial DNA was isolated with the UltraClean™ Microbial DNA Isolation Kit (MO Bio Laboratories, Carlsbad, USA) and rep-PCR was performed using the DiversiLab™ DNA Staphylococcus fingerprinting Kit (bioMérieux, Brussels, Belgium). In a next step, rep-PCR profiles were obtained using the microfluidic DNA chips (DiversiLab™ LabChip, bioMérieux) and an Agilent 2100 BioAnalyzer (Agilent Technologies, USA) according to the manufacturer’s instructions. The resulting rep-PCR fingerprinting profiles were compared using the web-based DiversiLab software (bioMérieux), version 3.3. The S. aureus and S. haemolyticus isolates were clustered into 22 genotypes (90% similarity) (Figure 1). The S. aureus and the S. haemolyticus isolates are clearly clustered separately. Further, the human clinical S. aureus isolates (N = 34) and the phage propagation strains (N = 31) were quite randomly distributed over 14 clusters. The horse (N = 6), pig (N = 3) and poultry (N = 5) isolates grouped into two closely related clusters and the rabbit (N = 7) isolates clustered together with two phage propagation strains.

For a subset of human and animal isolates, the presence of genes encoding resistance to commonly used antimicrobial agents was determined with DNA array hybridization using the StaphyType96 Kit (Alere Technologies GmbH, Jena, Germany) (Table S1). All tested isolates carry tetracycline resistance genes and all but one isolate encode methicillin resistance and penicillinase genes. Further, genes encoding resistance to MLS (macrolide-lincosamide-streptogramin) antimicrobials and aminoglycosides are present in 14 and 19 isolates, respectively. These resistance profiles illustrate the clinical relevance of the target strains. DNA array hybridization also revealed the presence of bacterial toxin genes, for example toxic shock toxin, leukocidins and enterotoxins.

The subset of analyzed isolates includes members of Epidemic MRSA (EMRSA) clones Rhine-Hesse EMRSA/UK-EMRSA-3 (K56, K57), North German/Iberian EMRSA (U9, UG10, UG11, UG12), Lyon Clone/UK-EMRSA-2 (K89, K91) and Berlin EMRSA (K51, K52, K53, K54, K55, K510, K511, K514). These EMRSA clones are widespread and represent hospital-acquired, carrying ‘staphylococcal chromosomal cassette’ (SCC) mec element types I, II and III, as well as community-acquired, carrying SCCmec types IV and V, MRSA [4,5]. In addition, all pig, poultry and horse isolates are members of the emerging MRSA clonal complex CC398, the most prevalent strain of livestock-associated MRSA [6,7].

The host range was examined by spotting a tenfold serial dilution of an ISP stock on a Mueller Hinton soft agar lawn containing the potential host. The results were confirmed by a plaque assay [8], which permitted to assess the efficiency of plating, the relative phage titer on a bacterial strain compared to the maximum titer observed. ISP infects 86% of the S. aureus strains and none of the S. haemolyticus strains. All 34 human S. aureus isolates were infected by ISP, while nine phage propagation strains are insensitive to ISP infection. Interestingly, none of the pig strains, but all other animal strains are sensitive to ISP infection. Moreover, all but one of the S. aureus strains isolated from humans...
and rabbits were infected by ISP with a high efficiency of plating, whereas ISP displays a moderate efficiency of plating on six of the sensitive phage propagation strains. In addition, the efficiency of plating varies among the horse strains and is mainly low on the poultry strains.

No correlation was observed between the 22 genotypes and ISP sensitivity nor between the subset of characterized EMRSA clones and ISP sensitivity. This is consistent with previous observations made for fifteen Pseudomonas aeruginosa phages and their infection patterns on 94 AFLP-typed host strains [9]. Nonetheless, the lytic activity of ISP on all S. aureus strains isolated from patients is promising for phage therapy purposes.

**ISP infection parameters and emergence of bacterial resistance**

In addition to infectivity on agar plates, the capability of phage ISP to adsorb on and subsequently infect S. aureus in a liquid culture at 37°C was examined. The optical density at 600 nm of...
two *S. aureus* strains, phage propagation strain 47 and strain ATCC 6538, infected at different multiplicities of infection (MOI, the ratio of phage particles to host cells) was monitored during a certain time period. Both infection curves suggest a lytic cycle of approximately 40 minutes. Interestingly, these killing curves progress differently in time, as shown in Figure 2. Phage ISP is able to completely lyse a culture of phage propagation strain 47 at MOI 10 and for two hours this bacterial culture remains stable. In contrast, *S. aureus* subsp. *aureus* Rosenbach ATCC 6538 is only attenuated by ISP. At MOI 0.1, 1 and 10 the optical density at 600 nm stabilizes at 0.8, 0.5 and 0.3, respectively. Monitoring of the number of free phages, adsorbed phages and viable bacteria during the experiment hints at a lysis inhibition phenomenon (data not shown). However, this long-delayed lysis mechanism for virion yield maximizing could not be substantiated, since no increased burst size was observed. For adsorption experiments, samples were taken at fixed intervals and transferred to Mueller Hinton medium supplemented with chloroform to lyse the remaining bacteria [8,10]. Titration of the amount of nonadsorbed or reversibly adsorbed phages showed that ISP adsorption occurs quite rapidly. Approximately 50% of the ISP particles were bound within 1 minute and after 25 minutes a maximal adsorption of 85% was reached (Figure S1).

For five human isolates, the rate at which ISP-resistant *S. aureus* cells arise, was determined using the method of Beale [11] with small adjustments. Therefore, 300 µl of bacterial cultures grown in liquid Mueller Hinton medium was mixed with an excess of ISP particles and plated out. Following 48 hours of incubation at 37°C, resistant colonies were counted. As with the *P. aeruginosa* phage phiKMV [12], resistant colonies of various sizes were observed. Interestingly, the ISP mutation rate (Table S2) varies greatly between the different human isolates. *S. aureus*-resistant colonies were observed a hundred times less frequent than phage-resistant mutants of *Streptomyces erythreus* and *Streptococcus thermophilus* in a similar experiment [13,14]. Additionally, ten randomly selected resistant colonies of each human isolate were purified two times by single-colony isolation and challenged again with ISP, to verify their resistance. 44 out of 50 (88%) colonies appeared to have acquired stable ISP resistance and hence the presented mutation rates are slightly overestimated. The appearance of phage-resistant mutants can be countered by using a complementary phage cocktail [15].

**Biophysical stability and in vivo safety of ISP**

To assess the stability of phage ISP for storage and manipulation of the phage preparation as well as future animal testing and therapeutic application, the survival of ISP was examined at different temperatures and in the complete pH range. Phage ISP was shown to be stable at 16°C, 37°C and 42°C, while freezing at −20°C and heating to 50°C reduced the phage titer with more than two logarithmic units (Figure S2 A). Consequently, ISP should be suited for the treatment of humans and animals, including those with fever, and the decontamination of hospital and farm environments. At pronounced acid (pH 1–3) or alkaline (pH 11–13) conditions, ISP is completely inactivated (Figure S2 B). A pH of 4 resulted in a significant logarithmic reduction of the titer, while ISP was stable between pH 5 and 9. This implies that ISP will not survive in gastric acid (pH 1–2) and therefore cannot

Figure 3. Summary of the bio-informatical analysis of the phage ISP genome. (A) Representation of the genome of phage ISP. Predicted proteins are indicated as bars, putative promoters are depicted as arrows and putative terminators as hair pins and the 4 tRNA’s are indicated in light blue. The DNA polymerase and the endolysin, both interrupted by endonucleases, are colored red and orange respectively. Genes which were not annotated in phage G1 are colored purple and genes mistakenly annotated in phage G1 in dark blue, additional genes in phage ISP are framed in red. (B, C and D) Pairwise DNA homology of ISP and G1, ISP and K and ISP and Twort, compared using a sliding window of 50 bp. doi:10.1371/journal.pone.0024418.g003
be administered orally without manipulation. Blood has a neutral pH and might thus provide a suitable environment for phages. To exclude any negative effects of blood components on the viability of phages in an ex vivo situation, ISP was incubated in rabbit blood which was collected using a syringe rinsed with heparin and transported in blood collection tubes (Microvette 500 Lithium Heparin, SARSTEDT AG & Co., Nümbrecht, Germany) containing heparin to prevent blood clotting. No loss of infectivity was observed, enabling potential intravenous phage administration.

In a preliminary study, the safety of ISP application was assessed in hybrid albino rabbits following approval of the ethical committee from the Faculty of Veterinary Medicine of Ghent University (EC 2008_112, EC 2009_063). Nasal administration in both nasal cavities as well as subcutaneous injection (1 mL syringe and 26 G×1/2″ needle, Terumo International, Leuven, Belgium) of 10⁶ ISP particles was evaluated in three rabbits. In a parallel experiment, three rabbits first received 3 mg/kg ranitidine (Zantac®, Glaxo Smith Kline, Genval, Belgium) to inhibit gastric acid secretion, followed by an oral dose of 10⁶ ISP particles. Subsequent daily clinical observation of the rabbits for at least 5 days revealed no adverse effects: the rabbits had a healthy appetite, a normal rectal temperature and general condition and no symptoms of illness. For ISP detection purposes after oral administration, blood samples were obtained from the lateral ear vein at several time points from 5 minutes till 12 hours after phage administration using a 24 G×3/4″ catheter (Terumo International). Following euthanasia with 0.5 mL/kg T61 (Intervet, Brussels, Belgium), the faeces were collected and tissue samples were taken from the liver, kidneys and spleen. ISP could not be detected in the blood, the organs or the faeces of the rabbits.

Analysis of the ISP genome

ISP DNA was extracted from a CsCl-purified stock solution (>10⁹ pfu/mL) according to Sambrook and Russell [3]. Initial sequencing was performed by shotgun sequencing of a library of phage DNA in a pUC18 vector (Fermentas GmbH, St. Leon-Rot, Germany) combined with primer walking until a single contig was obtained. The linear double-stranded DNA of ISP with terminally redundant ends and containing 138,339 bp (EMBL: FR852584) was sequenced with an average redundancy of 6.7 and a coverage redundant ends and containing 138.339 bp (EMBL: FR852584) was sequenced with an average redundancy of 6.7 and a coverage of each base position with at least three sequencing reactions. ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/), GeneMarkTM [16] and BLASTs [17] were used to predict 215 open reading frames (ORFs) (Figure 3), followed by manual verification. Further, the predicted protein sequences were compared with known proteins and putative functions were assigned using BLASTp [17] and HHpred [18]. The nucleotide positions and additional information of all predicted genes are shown in Table S3. Finally, four tRNA’s, encoding Asp-tRNA, Phe-tRNA, Trp-tRNA and Met-tRNA, were predicted in two non-coding regions of the genome using tRNAscan-SE [19] and ARAGORN [20].

K [21], G1 and Twort [2] are the best-known representatives of the ‘Twort-like viruses’ [22], myoviruses infecting gram-positive, low G+C content bacteria, and are assumed to show promising therapeutic potential. DNA homology with these phages was examined with EMBoss stretcher [23,24]. The genome of ISP is 99.5% identical to the 138,715 bp genome of phage G1 (Figure 3). The mutual differences comprise small sequence deletions and insertions which result in three extra hypothetical genes in ISP: ORF92, ORF126 and ORF147. Seven genes of G1 were chosen not to be annotated (ORF216, ORF247, ORF285, ORF309, ORF311, ORF388 and ORF450), due to their gene location integral in other genes or their questionable or absent Shine-Dalgarno sequence. In contrast, five additional genes which were not annotated in G1, ORF89, ORF103, ORF104, ORF140 and ORF205, were added to the protein-coding part of the ISP genome. At the nucleotide level, ISP displays 90.6% and 56.1% homology with K (127,395 bp) and Twort (130,706 bp) respectively, as shown in Figure 3. The differences with K are mainly confined to a region of 25 kb containing only hypothetical genes, while the variation between ISP and Twort is found throughout their genomes. An overview of their homologous genes is presented in Figure S3. In addition, several ISP genes were found to be homologous with predicted genes of Listeria phage A511 and Enterococcus phage phiEF24C, a ‘Twort-like virus’ and an orphan of the Spounavirinae, respectively [22].

As described earlier for the phages K [21] and Twort [2], ISP has its genes organized in three major modules encoding structural proteins, components of the replication and transcription machinery, and proteins responsible for cell lysis. Further, these phages are typified by an overall conserved gene organization, insertions and deletions, and the presence of unrelated genes between...
conserved genes [2]. For the purpose of phage therapy, the ISP genome encodes no potential gene products resembling known virulence or toxic proteins.

Phage ISP does not encode an RNA polymerase, implying its dependency on the host RNA polymerase for transcription. In this context, phage ISP encodes a sigma factor-binding polypeptide, Gp47, first identified in phage G1 by Dehbi et al. [25]. This polypeptide, which displays a growth-inhibitory effect when overexpressed in S. aureus, is considered to be one of the crucial components in the phage strategy to redirect the host RNA polymerase to transcription of phage DNA.

Conserved regulatory motifs in the ISP genome were searched using Nostradamus [26], PHIRE [27] and MEME/MAST [28] and resulted in the prediction of 64 host-specific promoters (Table S4). These putative promoters contain a variety of conserved motifs and a spacer region with a length of 17 or 18 nucleotides. The −35 box sequence is well-conserved, whereas more sequence variation is observed in the −10 box. Both the −35 box and the −10 box comprise six nucleotides and their conservation is graphically analyzed with WebLogo [29] (Figure S4). In addition, Palindrome [23] was used to detect 31 potential factor-independent terminators, while the free energy of their secondary structures was computed with Mfold [30] (Table S5). In addition, Palindrome [23] was used to detect 31 potential factor-independent terminators, while the free energy of their secondary structures was computed with Mfold [30] (Table S5). The promoters and the terminators are equally distributed over the ISP genome.

### Proteome analysis of the ISP particle

In a next step, electrophoretic and mass spectrometric techniques were used to elucidate the composition of the ISP virion. Extraction of phage proteins from a CsCl-purified ISP stock solution (>10^2 pfu/ml) was performed by methanol/ chloroform extraction (1:1:0.75, v/v/v). Following gel electrophoresis, five major protein bands were clearly visualized with Simply Blue™ Safe Stain (Invitrogen Ltd, Paisley, UK) (Figure 4). Nonetheless the whole SDS-PAGE gel was fragmented and subjected to tandem electrospray ionization-mass spectrometry (ESI-MS/MS) on a LCQ Classic (ThermoFinnigan) equipped with a nano-PLC column switching system as described by Lavigne et al. [31]. In 25 gel pieces, peptides of 22 phage proteins were identified (Table 1). Thirteen of these proteins were already listed as virion components and the structural function of Gp30, Gp38, Gp40, Gp72 and Gp186 was confirmed by Eyer et al. in 2007 [32]. Six additional proteins, Gp2, Gp13, Gp16, Gp28, Gp31 and Gp83, can be conferred to the group of structural proteins. However, nine proteins, identified by Eyer et al. [32] using one-dimensional and two-dimensional approaches, were not found in the present study. Generally, the identified proteins were localized in a major (Gp8–Gp40) and a minor (Gp70–Gp72) structural region in the ISP genome.

Several ISP proteins were found throughout the entire SDS-PAGE gel, illustrating their abundance in the phage virion. The

### Table 1. Characteristics of the phage ISP ESI-MS/MS identified proteins.

| ORF | Band number | Molecular mass (kDa) | Number of identified peptides | Sequence coverage (%) | HHpred search | e-value | K/G1 homologue1 | Identification method1 |
|-----|-------------|---------------------|-------------------------------|----------------------|--------------|--------|----------------|---------------------|
| Gp2 | 19          | 30.6                | 1                             | 8.4                  | /            | /      |               |                     |
| Gp8 | 8           | 64.0                | 12                            | 27.4                 | 6.10E-41 ORF 41 (K) | 2-DE   |               |                     |
| Gp11| 1, 2, 4–13, 15–25 | 51.2 | 15 | 63.9 | Capsid protein (HK97) | 5.60E-01 ORF 44 (K) | SDS-PAGE, 2-DE |
| Gp13| 14          | 34.1                | 3                             | 12.9                 | 1.60E+02 /      | /      |               |                     |
| Gp16| 16          | 31.8                | 4                             | 21.9                 | 1.50E+02 /      | /      |               |                     |
| Gp18| 1, 2, 4–11, 13, 15–25 | 64.4 | 21 | 62.7 | Tail sheath protein (T4) | 0 ORF 49 (K) | SDS-PAGE, 2-DE |
| Gp19| 1, 22–25    | 15.9                | 5                             | 54.2                 | 1.00E+01 ORF 50 (K) | SDS-PAGE, 2-DE |
| Gp26| 1           | 143.9               | 1                             | 1.4                  | 3.10E-04 ORF 55 (K) | SDS-PAGE |
| Gp28| 15          | 34.6                | 2                             | 10.5                 | 4.60E-11 /      | /      |               |                     |
| Gp30| 16          | 29.3                | 4                             | 24.3                 | ORF 59 (K) 2-DE |       |               |                     |
| Gp31| 21          | 19.9                | 1                             | 6.3                  | /            | /      |               |                     |
| Gp32| 16          | 26.6                | 4                             | 25.6                 | 2.00E-19 /      | /      |               |                     |
| Gp33| 10, 15      | 39.1                | 3                             | 6.9                  | 3.90E-42 ORF 62 (K) | SDS-PAGE, 2-DE |
| Gp35| 21, 22, 25  | 19.2                | 5                             | 44.5                 | 3.10E-04 ORF 64 (K) | SDS-PAGE, 2-DE |
| Gp36| 1–8, 15, 17, 24 | 129.1 | 33 | 40.5 | / | ORF 65 (K) | SDS-PAGE, 2-DE |
| Gp38| 7, 8, 15–17 | 72.6                | 20                            | 41.7                 | ORF 66 (K) 2-DE |       |               |                     |
| Gp40| 17          | 50.4                | 2                             | 7.0                  | ORF 68 (K) SDS-PAGE, 2-DE |
| Gp70| 1, 15–25    | 23.2                | 9                             | 67.1                 | 5.20E+01 ORF 95 (K) | SDS-PAGE, 2-DE |
| Gp71| 18, 21      | 17.8                | 3                             | 42.4                 | 4.60E-21 ORF 96 (K) | SDS-PAGE, 2-DE |
| Gp72| 25          | 17.8                | 1                             | 7.9                  | /            | /      |               |                     |
| Gp83| 23          | 17.8                | 1                             | 7.9                  | /            | /      |               |                     |
| Gp186| 25         | 10.1                | 1                             | 17.2                 | ORF 172 (G1) 2-DE |       |               |                     |

1 Adjusted from Eyer et al. (2007).
For each gene product (Gp) the band number(s) (according to Figure 4), the molecular weight in kDa, the number of identified peptides (>99% protein identification probability with manual validation), the protein sequence coverage in percent, the putative protein function according to HHpred and the corresponding e-value are shown. Further, earlier identified homologous proteins of phage K or G1 and the corresponding identification method (SDS-PAGE/2-DE) are listed.

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Discussion

By describing the composition, laboratory-based production and quality control of an experimental phage cocktail containing *S. aureus* phage ISP, Merabishvili et al. [1] support the practical re-introduction of phage therapy in Western medicine. The microbiological and molecular characterization of phage ISP in the present report is a necessary step in the medical exploitation of the antibacterial potential of BFC-1. The presented host range screening including clinically relevant MRSA strains, the determination of the infection parameters and the assessment of the ISP virion stability (temperature, pH, blood components) contribute to the further development of the therapeutic application of ISP. Moreover, phage ISP can be safely administered orally, in the nose and through subcutaneous injection. In case of oral application, ISP did not reach the blood, the faeces or the organs. In addition, the genome analysis of ISP, which revealed no toxic or virulence-associated genes, emphasizes the mutual similarity between the ‘Twort-like viruses’, which remains the only known genus of virulent *S. aureus*-infecting viruses so far.

Supporting Information

Figure S1 Adsorption curve of phage ISP on *S. aureus* subsp. *aureus* Rosenbach ATCC 6538. The proportion of the amount of non-adsorbed phages to the amount of phages used for infection, based on three independent experiments, is shown and standard deviations are indicated. (TIF)

Figure S2 Biophysical stability of phage ISP. The logarithmic drop in infectious ISP particles after 24 hours of incubation at different pH levels (A) and at different temperatures (B) is shown. Three independent experiments were performed, standard deviations are indicated. (TIF)

Figure S3 Summary of the common genes of the phages ISP, K and Twort. The genome of ISP contains 83 genes which have homologous counterparts in K and Twort. Further, ISP shares 85 and 1 additional homologous genes with K and Twort respectively. In contrast, K and Twort have no additional common genes. Forty-one ISP genes absent in phage K are generally organized in three major blocks and situated downstream the DNA replication and transcription module. The differences between ISP and Twort are located throughout their whole genome sequence. ISP and Twort have 41 and 109 unique genes respectively and their common genes are about 70% homologous. (TIF)

Figure S4 Sequence logo of the conserved motifs of the promoters of phage ISP. The conservation of the −35 box (A) and the −10 box (B) of the 65 promoters predicted in the genome of ISP is depicted. The height of each stack designates the sequence conservation at that position (measured in bits), while the height of the symbols within each stack indicates the relative frequency of the nucleotide at that position. (TIF)

Table S1 Overview of the host range of *Staphylococcus phage* ISP. The clarity of the lysis zones after spotting several phage dilutions was scored as clear and turbid and the dilution on which lysis spots and individual plaques appeared was taken into account. These observations in combination with a verifying plaque assay were used to differentiate between sensitive (+) and insensitive (−) to ISP infection on one hand and a low, moderate and high efficiency of plating on the other hand. For each isolate, the species, the origin, the sensitivity to ISP infection and the efficiency of plating is given. For 33 isolates the presence (+) or absence (−) of genes encoding resistance to methicillin, MLS3 antimicrobials, aminoglycosides and tetracycline, as well as the presence or absence of penicillinase is shown. (DOCX)

Table S2 Mutation rate for ISP resistance. For five human *S. aureus* isolates, the mutation rate conferring ISP resistance was calculated by dividing the number of resistant colonies by the number of bacterial cells at the time of ISP application. Based on five individual experiments, the mean values and corresponding standard deviations are indicated. (DOCX)

Table S3 Features of the predicted ORFs of phage ISP. For each predicted ORF the start and stop position in the genome, the length of the corresponding gene product in amino acids, the reading frame, the start and stop codon, the putative protein function and the corresponding prediction program and e value are shown. (DOCX)

Table S4 Predicted host-specific promoters of phage ISP. For each promoter the strand, the start and stop position in the genome, the −35 box, the spacer region, the −10 box and the length of the spacer region are given. (DOCX)
Table S5 Predicted factor-independent terminators of phage ISP. For each terminator the strand, the start and stop position in the genome, the free energy of their secondary structure and the sequence of the regulatory element (the palindromic sequence is underlined) are given.

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Author Contributions

Conceived and designed the experiments: KV WM JP UL KH RL. Performed the experiments: KV WM FB,JN UL. Analyzed the data: KV WM DD JP JN UL KH RL. Contributed reagents/materials/analysis tools: KV WM JP JN UL KH RL. Wrote the paper: KV PC DD JP MM UL KH RL.

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