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Lysogenic Transfer of Group A *Streptococcus* Superantigen Gene among Streptococci

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Abstract

A group A *Streptococcus*(GAS) isolate,serotypeM12,recovered from a patient with streptococcal toxic shock syndrome was analyzed for superantigen-carrying prophages, revealing ϕ149, which encodes superantigen SSA. Sequence analysis of the att-L proximal region of ϕ149 showed that the phage had a mosaic nature. Remarkably, we successfully obtained lysogenic conversion of GAS clinical isolates of various M serotypes (M1, M3, M5, M12, M19, M28, and M94), as well as of group C *Streptococcus equisimilis* (GCSE) clinical isolates, via transfer of a recombinant phage ϕ149::Km^r. Phageϕ149::Km^r from selected lysogenized GAS and GCSE strains could be transferred back to M12 GAS strains. Our data indicate that horizontal transfer of lysogenic phages among GAS can occur across the M-type barrier; these data also provide further support for the hypothesis that toxigenic conversion can occur via lysogeny between species. Streptococci might employ this mechanism specifically to allow more efficient adaptation to changing host challenges, potentially leading to fitter and more virulent clones.

Streptococcus pyogenes, or β-hemolytic group A *Streptococcus* (GAS), is a notorious human pathogen, responsible for a number of different infections that range from mild skin diseases to fulminant, severe, invasive syndromes. Since the mid-1980s, a remarkable increase in the incidence of the severe forms of GAS infections (in particular, necrotizing fasciitis and streptococcal toxic shock syndrome [STSS]) has been observed worldwide [1, 2]. Many hypotheses have emerged to explain this phenomenon; some focus on impaired host defense against specific strains or specific virulence determinants (e.g., immunogenetic background or predisposing factors), whereas others focus on changes in bacterial virulence itself [3–5].

Fluctuations in the severity and character of streptococcal virulence have also been linked to the plasticity of the streptococcal genome. Mobile genetic elements, including lysogenic bacteriophages, that have integrated into the GAS genome over time were found to be

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almost exclusively responsible for the genetic differences among strains [6–11]. Recent genomic analyses indicate that GAS is a polylysogenic organism in which prophage sequences constitute ~10% of the total genome [1, 3, 7, 12]. In GAS, prophages encode 1 or more putative or established virulence factors, including phospholipases, streptodornases, and superantigens, in addition to their essential viral proteins [1, 12]. It is now widely acknowledged that virulence gene transfer may occur among GAS when favorable conditions are encountered in the human host [13–18]. By means of lysogenic conversion, the toxin-encoding bacteriophages can convert their bacterial host from a nonpathogenic strain to a virulent strain or a strain with increased virulence [12, 13, 19]. Along these lines, prophages have been shown to play a key role in subclone diversification and to be largely responsible for the uniqueness of clinical isolates [3]. Consequently, GAS prophages play a critical role in determining the distinct disease pathologies associated with otherwise similar strains [1].

The critical role of superantigens in severe GAS infection underlines the clinical relevance of prophages [2, 20, 21]. In GAS, the majority of superantigen genes are located on prophage genomes. Experimental ex vivo toxigenic conversion by lysogeny has been demonstrated only for *speA* and *speC* and almost exclusively among strains belonging to the same M serotype. The suggested bacteriophage-mediated transfer of other superantigens is based only on indirect evidence: for example, their association with prophage sequences, their distribution pattern throughout the streptococcal population, or the inducible character of prophages carrying the respective genes [13, 15, 22–25]. Further, a number of studies indicate that M proteins could function, directly or indirectly, as barriers to horizontal gene exchange. A phenotypic correlation between resistance to bacteriophage infection and M-protein surface expression has been described [26, 27]. Nonrandom associations between exotoxin alleles and *emm* patterns were also observed, thus suggesting some direct or indirect biological interactions between M-protein surface structures and bacteriophage-associated properties [28–30]. In this study, we focus on the mobility of superantigen SSA, which has been associated with GAS isolated from patients with STSS and which has highly potent superantigenic activity that has been proven experimentally [31–33]. Presence of the *ssa* gene has been detected across the GAS population, as well as in clinical isolates of group C (GCS) and group G (GGS) streptococci (*Streptococcus dysgalactiae* subspecies *equisimilis* GCSE and GGSE, respectively), thus suggesting the possibility of interspecies *ssa* gene transfer [14, 29, 30, 34–36].

We report here the characterization of an inducible chimeric prophage, ϕ149, carrying the superantigen-encoding *ssa-3* allele from a GAS clinical isolate of serotype M12, RDN149. We describe the host range of this phage by investigating its transfer-ability to GAS strains of various M types, as well as to GCSE and GGSE. This study experimentally confirms previous indications that lysogenic toxin conversion can occur across the M-type barrier in GAS and between GAS and GCSE.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions

Streptococcal strains (table 1; [29, 37, 38]) were grown at 37°C with 5% CO_2 without agitation in Todd-Hewitt broth either with or without 0.2% yeast extract supplementation and on tryptic soy agar supplemented with 3% sheep blood or THY agar. Transformation of *Escherichia coli* and *S. pyogenes* with plasmid DNA was performed as described elsewhere [39, 40]. Whenever required, antibiotics were added to the media: erythromycin at 3 μg/mL for *S. pyogenes*; and kanamycin at 25 μg/mL for *E. coli* and at 300 μg/mL for streptococcal strains.

General DNA manipulation and analysis

Isolation of streptococcal DNA (genomic and phage) and general DNA manipulations were performed as described elsewhere [16, 39], with minor modifications. VBC-Biotech Services supplied the primers used in this study (table 2) and performed sequencing reactions. General sequence analysis was performed by use of the DNA* (DNAStar) software package and the Basic Local Alignment Search Tool (BLAST) algorithm.

Construction of ϕ149::Km^r

A kanamycin-resistance (Km^r) cassette, *aphIII*, was inserted into the *ssa* gene of phage ϕ149 in strain RDN149, thus generating strain EC516, which carried recombinant phage ϕ149::Km^r. For this purpose, an 856-bp fragment upstream of *ssa* and a 569-bp fragment downstream of *ssa* were amplified by use of RDN149 genomic DNA and primers (containing flanking restriction sites) OLEC154/OLEC155 and OLEC153/OLEC159, respectively. After digestion with appropriate restriction enzymes, fragments were ligated and cloned into pEC61, a suicide plasmid for GAS. The resulting plasmid, pEC97, was digested with *Sca*I (which interrupts the ampicillin-resistance cassette) and used to transform electrocompetent RDN149. Km^r clones were then selected and analyzed by polymerase chain reaction (PCR) and Southern blot analyses. This ensured that the recombination event by double crossover was correct and did not affect the DNA regions located upstream and downstream of *ssa*

Phage induction by mitomycin C treatment

Mitomycin C (Sigma) was added (0.2 μg/mL) to cultures grown to early–mid-logarithmic phase [24, 41]. After incubation for 3 hat 37°C, cells were removed by centrifugation and supernatants were sterile-filtered through 0.45-μm pores (Iwaki Glass). Possible bacterial contamination of filtrates was excluded by inoculating an aliquot of the filtrates in liquid and on solid media.

Detection of phage release in mitomycin C–treated bacterial cultures

Two approaches were used. (1) A phage indicator strain culture grown to mid-logarithmic phase was mixed with sterile water, poured over THY agar plates, and allowed to sediment. Superfluous bacterial suspension was carefully removed and plates were left to dry. Supernatants of mitomycin C–treated phage donor cultures were then spotted on the indicator lawn. (2) Supernatants of mitomycin C–treated phage donor cultures were mixed with cultures of phage indicator strain and added to molten top agar. The mixture was then poured over THY agar plates and allowed to dry. In both approaches, the final step was to incubate the plates at 37°C until plaques were observed.

Lysogenization of recipient strains with phage ϕ149

By use of the first approach described above, secondary growth colonies were recovered from the areas of lysis and analyzed for sensitivity to phage infection. Lysogenized strains for which no lysis was observed were then selected and analyzed by PCR and Southern blot analysis for the presence of the phage.

Determination of the host range of ϕ149::Km^r

Supernatants of mitomycin C–treated EC516 cultures were mixed with recipient cultures grown to early–mid-logarithmic phase and further incubated for 3 hours at 37°C. Aliquots of the mixture were then plated on tryptic soy agar that contained kanamycin. Selected Km^r-

positive colonies were analyzed by PCR and Southern blot analysis to determine whether the recombinant phage was present. Lysogenization was confirmed for 2–3 independent lysogens per infection experiment. Lysogenization experiments were performed independently 3–4 times.

RESULTS

Phage release after mitomycin C treatment of an M12 clinical isolate

The analysis of a type M12 clinical GAS isolate, RDN149, recovered from a patient with STSS, revealed the superantigen genes *ssa* and *speH* (table 1). Subsequent treatment of a bacterial culture of RDN149 with mitomycin C resulted in the release of phage particles in the culture supernatant, which could be demonstrated by plaque detection with the phage indicator strains CS24 and RDN151 (M12 types). The plaques obtained with both indicator strains were turbid, ~2 mm in diameter, and the bacteriophage titer was estimated at 7×10^4 pfu/mL (data not shown). The presence of phage particles in the supernatant of mitomycin C–induced RDN149 cultures was further confirmed by isolation of phage DNA. In contrast, no phage DNA could be detected in the supernatant of cultures that were not subjected to mitomycin C treatment (data not shown). Further characterization of the released prophage(s) by PCR analysis indicated the possible presence of 2 phages in the supernatant (figure 1*A*).

Ex vivo lysogeny of phage ϕ149 associated with ssa-toxigenic conversion

To investigate whether phage(s) released from strain RDN149 could convert *speH*-negative and *ssa*-negative strains into *speH*-positive and/or *ssa*-positive strains, lysogenization experiments involving the phage indicator strain CS24 with mitomycin C–induced supernatants of RDN149 were conducted. Each lysogenization experiment performed was positively controlled with ϕCS112 lysogenization of recipient strain CS24 with strain CS112 used as phage donor [24]. Lysogenic clones were obtained successfully and further analyzed for transfer of the superantigen genes. PCR analysis of genomic DNA from the phage donor strain (RDN149), phage indicator strain (CS24), and lysogenized CS24 strains (RDN312 and EC500), as well as purified phage DNA from RDN149, showed that only *ssa*, not *speH*, was transferred to the phage indicator strain (figure 1*A*).

These results, confirmed by Southern blot analysis, suggested that in RDN149, *speH* and *ssa* are encoded on 2 distinct prophages (figure 1*B*). The transferable, lysogenic, *ssa*-carrying phage was designated ϕ149. To further verify that lysogeny was responsible for the observed *ssa*-toxigenic conversion, we repeated the experiments with the second phage indicator strain, RDN151. Toxigenic conversion of RDN151 with ϕ149 was also observed (figure 1). Thus, we have identified *ssa*-carrying phage ϕ149 in strain RDN149, which can convert *ssa*-negative clinical strains of type M12 to *ssa*-positive strains via lysogenization.

Characterization of the ssa-carrying phage ϕ149

The superantigen SSA shares considerable homology with the *S. aureus* enterotoxins SEB and SEC [33, 42]. Three alleles of *ssa* have already been identified in natural GAS populations [35]. The alleles *ssa-1* and *ssa-3* differ by a single synonymous substitution in codon 94, and both encode SSA-1. The *ssa-2* allele is identical to *ssa-3* at codon 94, but has a nonsynonymous substitution at codon 28, which changes the second amino acid of the mature protein from serine to arginine [35]. PCR amplification of the *ssa* coding sequence in RDN149 with primers oliRN237 and OLEC156, followed by sequencing, revealed the *ssa-3*

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allele. To determine the attachment site of ϕ149 and its location on the chromosome of strain RDN149, we took advantage of the fact that, in all GAS prophages, the genes encoding virulence factors are located at one extremity of the prophage, between the phage lysis cassette and the phage attachment site [12]. At the time of the study, 2 GAS prophages, ϕ315.2 (strain MGAS315 [M3 type]) and ϕSPsP6 (strain SSI-1 [M3 type]), had already been described that carried the *ssa* gene (*ssa-1* allele) [7, 10].

Southern blot analysis of *Hin*dIII-digested genomic DNA of strain RDN149 revealed an *ssa*-hybridizing DNA fragment, the size of which (~2700 bp) was different from that expected in strains MGAS315 and SSI-1 (5090 bp) (figure 1). There are several possible explanations for this result: (1) ϕ149 is identical to ϕ315.2 or ϕSPsP6 but is integrated at a different attachment site; (2) ϕ149 is identical to ϕ315.2 or ϕSPsP6 and is integrated at the same attachment site, but the chromosomal region surrounding the attachment sites differs among strains; or (3) ϕ149 is a novel GAS prophage that has not yet been characterized.

We performed inverse PCR using religated, *Hin*dIII-digested genomic fragments of strain RDN149 as DNA templates and *ssa*-specific outward primers OLEC141 and OLEC142 (table 2; figure 2). The PCR fragment obtained was purified and used as a DNA template in PCR reactions with embedded primers OLEC166 and OLEC167 (table 2; figure 2). Sequence analysis of the resulting PCR fragment indicated that ϕ149 consisted of a chimeric GAS prophage. The downstream region of the *ssa* coding sequence, which includes a hypothetical phage protein referred to as paratox, the left ϕ149 attachment site, and the adjacent sequence on the bacterial chromosome showed identity to the DNA region upstream of the streptodornase gene (*mf3*)in4 GAS prophages that have already been described: ϕ370.2 (SF370 [M1]), ϕSPsP4 (SSI-1 [M3]), ϕ315.3 (MGAS315 [M3]), and ϕ370.2-like prophage (MGAS8232 [M18]) (figure 2) [7, 8, 10, 11].

All 4 prophages, and therefore also ϕ149, are integrated into the GAS bacterial genome at the same attachment site, which is located 341 bp upstream of a putative heavy metal–transporting, ATPase-encoding gene. Nucleotide sequence analysis of the upstream region of *ssa-3* in ϕ149 revealed 99% identity with the upstream region of *ssa-1* in ϕ315.2 (MGAS315 [M3]) and ϕSPsP6 (SSI-1 [M3]). The difference consists of a 34-bp insertion located 103 bp upstream of the *ssa-3* start codon, which is not present upstream of *ssa-1*. This 34-bp insertion comprises a 26-bp insertion element (5′-CTCTTTTAAAATTAAAACATTGATTT-3′), which doubles the sequence 5′-AATTTTAT-3′. The 26-bp insertion element was previously identified upstream of the insertion sequence IS*1239* and, on this basis, it was suggested that it may function as a target sequence [42]. It was shown to be more frequently associated with *ssa-3* than with *ssa-1* or *ssa-2* [35]. In addition, the presence of IS*1239* associated with the 26-bp insertion element was identified upstream of *ssa-3* in several serotypes—M4, M15, M23, M33 and M41—leading to the suggestion that IS*1239* might have contributed to the horizontal transfer of *ssa* among GAS [35]. Taken together, these observations suggest that recombination events may have taken place, leading to the mosaic nature of ϕ149.

Determination of host range of ϕ149 among clinical isolates of GAS

We were interested in the possibility that ϕ149 could be transferred ex vivo to clinical isolates of GAS. As described above, we showed transfer of ϕ149 from the donor strain RDN149 (type M12) to the phage indicator strains CS24 and RDN151 (type M12). To facilitate the detection of toxin-converted strains, a selective marker (the kanamycin-resistance cassette, *apiII*) was inserted into the *ssa-3* coding sequence of ϕ149, thus creating strain EC516. Strain EC516 was then used as a donor strain of ϕ149::Km^r in infection experiments involving other GAS clinical isolates of various M serotypes. Lysogenic strains were selected on the basis of their Km^r phenotype, which was verified by PCR scoring for the presence of the Km^r cassette flanked by the interrupted *ssa-3* sequence and further verified by subsequent Southern blot analysis. In this study, 34 clinical isolates, which represented 12 M types, were analyzed for lysogenic conversion. A total of 17 strains that comprised 7 different M types and were classified as pattern A-C/SOF- strains (M1, M3, M5, M12, and M19) and pattern E/SOF[H11001] strains (M28 and M94) [43, 44] were successfully lysogenized (table 3; figure 3). Further, the transfer of ϕ149::Km^r from selected M1, M12, and M94 lysogens back to clinical isolates of type M12 (RDN151 and RDN138) was obtained. This indicated that the recombinant phage was still functional once inserted in the chromosome of the lysogenic strains. In addition, infection experiments with UV-induced and hydrogen peroxide (H_2O_2)–induced EC516 culture supernatants led successfully to lysogenic clones among M12 strains (data not shown).

Ex vivo lysogenic transfer ϕ149 to other streptococcal species

Several studies report the presence of *ssa-3* among the emerging human pathogens GCSE and GGSE, thus suggesting a temporal dynamic for *ssa-3* exchange across streptococcal species [14]. Here, we asked whether ϕ149 could be lysogenically transferred ex vivo from GAS to other streptococcal species. Infection experiments were conducted that used the ϕ149::Km^r phage donor strain EC516 and 10 GCSE clinical isolates and 10 GGSE clinical isolates as recipient strains, by use of the same approach described above. Two GCSE strains were successfully lysogenized (table 3). ϕ149::Km^r was also transferred from selected GCSE lysogens back to clinical isolates of type M12 (RDN151 and RDN138).

Taken together, the ability of ϕ149:: Km^r to lysogenize GAS strains of different clonal lineages and different M types as well as GCSE isolates indicate that phage transfer most probably played a critical role in mediating the spread of *ssa* across the streptococcal population.

DISCUSSION

The recent reemergence of severe, invasive GAS diseases observed during the mid-1980s has encouraged many researchers to investigate this phenomenon. Some studies questioned whether enhanced virulence could be associated with a particular M type or with certain virulence factors. Reports show the existence of particular virulent and invasive clones that have successfully spread worldwide [3, 4, 7, 45]. However, despite the dominance of certain M types, such as M1, M3, M12, and M28, among endemic and invasive GAS isolates, no exclusive link between M type and specific diseases could be demonstrated. Only certain M types seem to exhibit a correlation with invasive diseases (e.g., M1 and M3) or postinfectious sequelae from superficial GAS infection (e.g., M18) [11, 29, 30, 45]. Furthermore, invasive GAS isolates do not seem to be characterized by a common toxin-

gene profile. Although reports show the dominance of 1 or 2 toxin-gene profiles in all M types, strains that did not share the predominant profile still showed nonrandom distribution of key toxin genes that were characteristic of the M type [29].

Among bacterial factors that can determine enhanced virulence are superantigen-carrying bacteriophages that can, via lysogenic transfer, convert a nontoxinogenic strain to a toxin-producing strain [19]. In this study, the analysis of a clinical GAS isolate, type M12, to determine its superantigen-encoding bacteriophage profile uncovered prophage ϕ149, which carried the allele *ssa-3* that encodes the streptococcal superantigen SSA. Sequence analysis of the att-L proximal region of ϕ149 indicated that the phage was a mosaic prophage that shared characteristics of 2 different types of prophages: ϕ370.2, ϕSPsP4, ϕ315.3, and ϕ370.2–like phages (already identified in M1, M3, and M18 strains) and ϕ315.2 and SPsP6 phages (M3 strains). This indicates that phage genomic rearrangements occurred, leading to genetic mosaicism in ϕ149. Recently, the complete genome sequence of an M4-type strain, MGAS10750, revealed that the strain harbors an *ssa-3*– carrying prophage, ϕ10750.3 [46], whose att-L proximal region is 100% identical to that of ϕ149 (figure 2). The contribution that the genetic mosaicism of GAS prophages makes to the emergence and diversification of the globally disseminated clonal M1T1 strain has already been demonstrated, indicating the exchange of genetic material among GAS prophages [1, 3, 12].

Superantigen SSA produced by GAS shares greater homology with staphylococcal enterotoxins SEB and SEC than it does with streptococcal superantigens. Several studies during the past decade have further documented the presence of the *ssa* gene among GAS isolates of different M types, as well as in emerging clinical isolates of GCSE and GGSE [14, 29, 30, 32–35]. In our study, we performed infection experiments on GAS clinical isolates of various M types, as well as experiments on GCSE and GGSE clinical isolates using mitomycin C–induced cell-free culture supernatants of strain EC516 harboring the selective recombinant phage ϕ149::Km^r. Our results show the ability of ϕ149:Km^r to lysogenize GAS clinical isolates of 7 different M types (M1, M3, M5, M12, M19, M28, and M94), as well as the GCSE clinical isolates. To our knowledge, this is the first direct experimental evidence for toxigenic conversion via lysogeny across a diverse range of strains of distinct M types and between GAS and GCSE. However, no lysogenic conversion of GGSE clinical isolates could be observed. We hypothesize that such events could take place, but the experimental conditions in our study might have limited their detection.

Although some studies indicate that M proteins could function as barriers to horizontal gene exchange, recent genomic sequence analysis strongly suggests that extensive lateral gene transfer occurred among GAS isolates of different M types [1]. Along these lines, lysogenic conversion of 1 T25-type strain with SpeC toxin originating from an M12-type isolate was described; however, the lysogenic transfer event was not confirmed by DNA analysis [31]. Reports on intergroup streptococcal bacteriophage transfer remain limited. Intergroup transduction of markers for streptomycin and bacitracin resistance between GCS and GAS using A-25 lytic phage and propagation of a temperate phage isolated from GGS on either GAS or GCS have been reported [47, 48]. GCSE and GGSE, although generally considered to be commensal organisms, have also been isolated from infections in humans that had clinical manifestations similar to those caused by GAS. In particular, reports of GCSE and GGSE isolates recovered from patients with severe forms of infection and of the presence of superantigen genes in these isolates have increased in recent years [49]. Our data strongly support the hypothesis that the emergence of superantigen genes in GCSE and GGSE may

occur by means of horizontal transfer from GAS via temperate phage. Accordingly, it has been suggested that housekeeping genes are exchanged among GCSE, GGSE, and GAS via lateral transfer with a strong net directionality of gene movement from GAS donors to GCS and GGS recipients, but only on the basis of phylogenetic studies [50]. Our experimental findings raise questions about the extent to which M type and streptococcal species are barriers to the horizontal transfer of lysogenic phages, as well as questions about the assumption of phage specificity within a given streptococcal group.

Given the critical role of temperate bacteriophages in toxi-genic conversion and in the mediation of strain diversity and evolution in GAS, it will be important to obtain additional knowledge of the molecular mechanisms underlying these events [19, 23]. Further investigation is also needed to unravel the complex interplay between strain genotype, in vivo toxin-gene transfer, regulation of toxin expression, and host factors (i.e., genetics and underlying conditions) that is responsible for the development of severe streptococcal disease.

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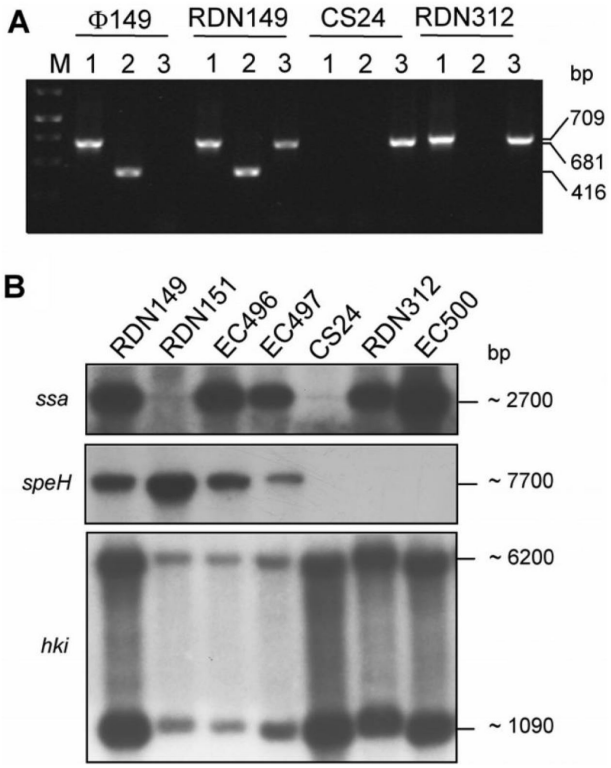

Figure 1.

Gel electrophoresis patterns demonstrating ex vivo lysogenic transfer of *ssa-3*– carrying phage Φ149 to group A *Streptococcus* (GAS) clinical isolates of type M12. *A*, Results of polymerase chain reaction analysis of isolated phage DNA from strain RDN149 and genomic DNA from phage donor strain RDN149, phage indicator strain CS24, and lysogenized strain RDN312 with primers specific to the *ssa* gene *(lane 1)*, the *speH* gene *(lane 2)* and the *hki* gene *(lane 3). hki* encoding a putative histidine kinase served as positive control for GAS genomic DNA. *M*, 1-kb DNA ladder (Fermentas Life Sciences). The 709-, 681-, and 416-bp DNA fragments correspond to the *ssa*-, *hki*-, and *speH*-specific amplified products, respectively. *B*, Results of Southern blot analysis of *Hin*dIII-digested genomic DNA of phage donor strain RDN149; phage indicator strains RDN151 and CS24; and lysogenized strains EC496, EC497, RDN312, and EC500, with α-^{32}P labeled DNA probes specific to the *ssa, speH*, and *hki* genes. The approximate sizes of the hybridizing fragments are indicated in bp.

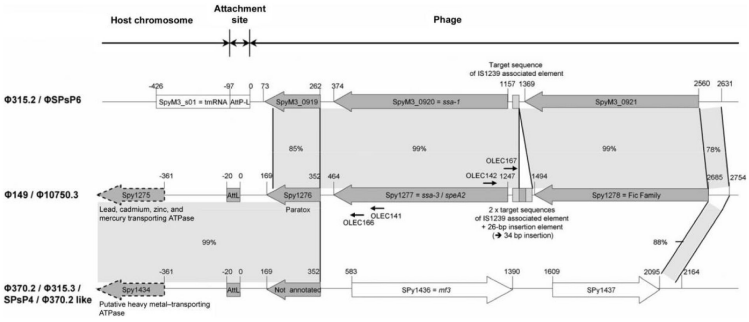

Figure 2.

Comparison of the att-L proximal region of *ssa-3*–carrying phage Φ149 with the att-L proximal regions of *ssa-1* –carrying phages Φ315.2 (MGAS315; M3) and SPsP6 (SSI-1, M3) and *mf3*-carrying phages Φ315.3 (MGAS315; M3), SPsP4 (SSI-1; M3), Φ370.2 (SF370; M1), and Φ370.2-like (MGAS8232; M18). The shaded areas indicate the regions of similarity with the percentage of nucleotide identity given. The 34 bp IS*1239*-related sequence located 103 bp upstream of the *ssa* coding sequence is shown. Annotated open reading frames refer to the genomes of MGAS315 strain (Φ315.2) and SF370 (Φ370.2). *Small, black arrows*, positions of primers.

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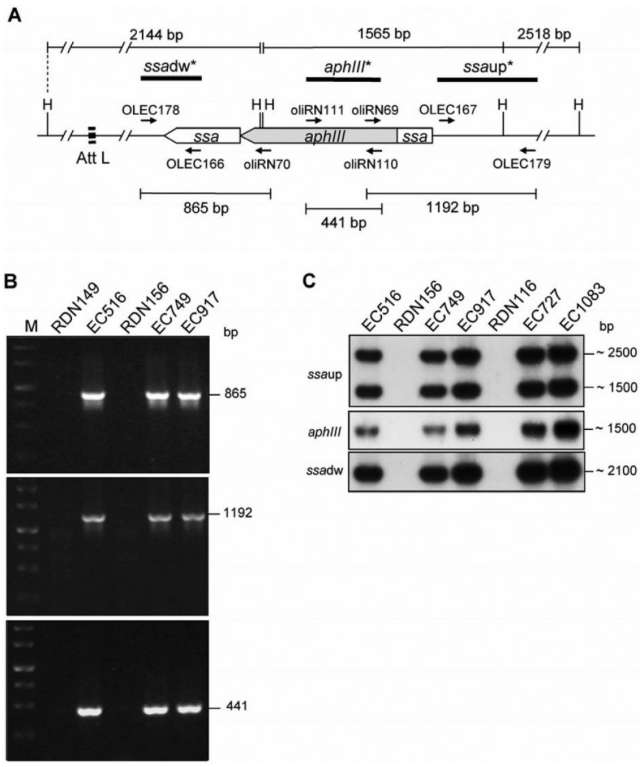

Figure 3.

Analysis of the broad host range of recombinant phage ϕ149::Km^r. *A*, Representative restriction map of the att-L proximal region of ϕ149::Km^r. Genes are drawn to scale. *Small, black arrows*, positions of primers. Sizes of PCR and hybridizing restriction fragments are indicated in bp. *B*, Results of polymerase chain reaction analysis of genomic DNA from ϕ149 donor strain RDN149, ϕ149::Km^r donor strain EC516, recipient strain RDN156 (M28), and lysogenized strains EC749 and EC917, with primers OLEC178 and oliRN70 *(top)*, oliRN69 and OLEC179 *(middle)*, and oliRN111 and oliRN110 *(bottom). M*, 1-kb DNA ladder (Fermentas Life Sciences). *C*, Results of Southern blot analysis of *Hin*dIII-digested genomic DNA from ϕ149::Km^r donor strain EC516, phage recipient strain RDN156 (M28) and corresponding lysogenized strains EC749 and EC917, phage recipient strain RDN116 (M94) and corresponding lysogenized strains EC727 and EC1083 with α-^{32}P labelled DNA probes specific to the *ssa* upstream fragment (*ssa*up), *aphIII* gene, and *ssa* downstream fragment (*ssa*dw).

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Table 1

Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strain		
E. coli	Host strain for cloning	Lab collection
DH5-α		
TOP10	Host strain for cloning	Lab collection
S. pyogenes		
RDN149	M12, ϕ149 donor, *ssa+*, *speH+*	[29]
EC516	RDN149 (ϕ149::Km^r)	This study
RDN151	M12, indicator strain for ϕ149, *ssa−*, *speH+*	[29]
EC627	RDN151 (pMSP3535)	This study
EC496	RDN151 lysogenized with ϕ149	This study
EC497	RDN151 lysogenized with ϕ149	This study
CS112	M12, ϕCS112 donor, *speC+*	[38]
CS24	M12, indicator strain for ϕCS112 and ϕ149, *speC−*, *ssa−*, *speH−*	[38]
RDN313	CS24 lysogenized with ϕCS112	This study
RDN312	CS24 lysogenized with ϕ149	This study
EC500	CS24 lysogenized with ϕ149	This study
RDN1	M1, clinical isolate	Lab collection
RDN2	M19, clinical isolate, recipient for ϕ149	Lab collection
RDN3	M1, clinical isolate	Lab collection
RDN4	M49, clinical isolate	Lab collection
RDN5	M3, clinical isolate, recipient for ϕ149	Lab collection

Strain or plasmid	Relevant characteristic(s)	Source or reference
RDN6	M3, clinical isolate, recipient for ϕ149	Lab collection
RDN8	M1, clinical isolate, recipient for ϕ149	Lab collection
RDN27	M12, clinical isolate, recipient for ϕ149	Lab collection
RDN29	M1, clinical isolate Lab	collection
RDN57	M49, clinical isolate	Lab collection
RDN60	M5, clinical isolate, recipient for ϕ149	Lab collection
RDN74	M89, clinical isolate	Lab collection
RDN78	M3, clinical isolate Lab	collection
RDN83	M89, clinical isolate	Lab collection
RDN86	M1, clinical isolate	Lab collection
RDN89	M3, clinical isolate	Lab collection
RDN100	M83, clinical isolate	Lab collection
RDN104	M81, clinical isolate	Lab collection
RDN106	M94, clinical isolate, recipient for ϕ149	Lab collection
RDN107	M1, clinical isolate	Lab collection
RDN114	M3, clinical isolate	Lab collection
RDN116	M94, clinical isolate, recipient for ϕ149	Lab collection
RDN119	M1, clinical isolate	Lab collection
RDN138	M12, clinical isolate, recipient for ϕ149	Lab collection
RDN139	M28, clinical isolate	Lab collection
RDN144	M28, clinical isolate, recipient for ϕ149	Lab collection
RDN145	M12, clinical isolate, recipient for ϕ149	Lab collection
RDN146	M12, clinical isolate, recipient for ϕ149	Lab collection
RDN147	M12, clinical isolate, recipient for ϕ149	Lab collection
RDN148	M12, clinical isolate, recipient for ϕ149	Lab collection

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Strain or plasmid	Relevant characteristic(s)	Source or reference
RDN152	M22, clinical isolate	Lab collection
RDN156	M28, clinical isolate, recipient for ϕ149	Lab collection
	RDN148 lysogenized with ϕ149::Km^r	This study
EC1092	RDN148 lysogenized with ϕ149::Km^r	This study
EC1093	RDN148 lysogenized with ϕ149::Km^r	This study
EC749	RDN156 lysogenized with ϕ149::Km^r	This study
EC917	RDN156 lysogenized with ϕ149::Km^r	This study
EC727	RDN116 lysogenized with ϕ149::Km^r	This study
EC1083	RDN116 lysogenized with ϕ149::Km^r	This study
S. dysgalactiae subsp. equisimilis		
EC783	Clinical isolate	Lab collection
EC788	Clinical isolate	Lab collection
Plasmid		
pUC19	ColE1ori, Amp^r, *lacZ*	Lab collection
pMSP3535	*ermB*, *repDEG*-pAMβ-1, *nisRK*, *ColE1ori*, *PnisA*	[37]
pEC61	pUC19Ω*aphIII*	This study
pEC96	pEC61Ω*ssa-up*	This study
pEC97	pEC96Ω*ssa-down*	This study

NOTE. Lab, laboratory.

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Table 2

Oligonucleotides used in this study.

Oligonucleotide	Direction	Sequence (5′ to 3′ direction)	Specific gene target
oliRN70	F	GCTATTTTTTGACTTACTGGG	*aphIII*
oliRN69	R	TCCGTATCTTTTACGCAGCGG	*aphIII*
oliRN110	F	CGCTGCGTAAAAGATACG	*aphIII*
oliRN111	R	AATGGAGTGTCTTCTTCC	*aphIII*
oliRN133	F	TATTCGCTTAGAAAATTAA	*emm*
oliRN134	R	GCAAGTTCTTCAGCTTGTTT	*emm*
oliRN39	F	AAAAATGACATTGCTTGGGC	*hki*
oliRN40	R	TTTAGTAGATATACTTTATTGGC	*hki*
oliRN233	F	AGATTGGATATCACAGG	*speH*
oliRN234	R	CTATTCTCTCGTTATTGG	*speH*
oliRN237	F	GTGTAGAATTGAGGTAATTG	*ssa*
oliRN238	R	TAATATAGCCTGTCTCGTAC	*ssa*
OLEC141	F	AACAAGTCACTGTTCAAG	*ssa*
OLEC142	R	GCTACAACGAGTATTCTT	*ssa*
OLEC166	F	GTACGAGACAGGCTATAT	*ssa*
OLEC211	F	TGCACAATTATTATCGATTAG	*ssa*
OLEC153	F	TAACTTGGATCCGTTCAATAGTAAAGATTTAGCTGC	*Insertion of aphIII into ss*
OLEC154	R	TTCTTACTGCAGGCTGACCTGTGGATCTTACATTAG	*Insertion of aphIII into ss*
OLEC155	F	AAGTAAGCATGCACCCAATATTTTTCGTTAGTGAAG	*Insertion of aphIII into ss*
OLEC159	R	TATGAAGGTACCTTAACTAGTATCATGGATTTGATAC	*Insertion of aphIII into ss*
OLEC179	F	TTGCTCATGCGCAATTCG	Spy1278
OLEC497	R	AATATTGGGTGTTTTAGAAGCC	Spy1278
OLEC178	R	ATCGCTTGCTTAAACTCG	*prx*
OLEC395	F	ATGGAGAAGTTGTGACTAAGG	*prx*
OLEC396	F	TGACAATGGATATATCGCAGG	*prx*
OLEC156	R	TTAACTAGTATCATGGAT	Intergenic *ssa-prx*
OLEC167	R	CAATTACCTCAATTCTAC	Intergenic *ssa-Spy*1278
OLEC267	R	AAGAACCTTGTCGCTATC	Intergenic *attR-Spy*1275
OLEC325	R	GATGACTGACCATTATAG	Intergenic *attR-Spy*1275

Oligonucleotide	Direction	Sequence (5′ to 3′ direction)	Specific gene target
OLEC324	R	GCTACAAAATAACGAAAC	Intergenic *attR-prx*
OLEC500	F	AGAAGCAACTGAATTGA	Spy1329

NOTE. The underlined sequences indicate restriction sites. Spy1275, Spy1278, and Spy1329 refer to annotated open reading frames in strain MGAS10750F. F, forward; *prx*, paratox gene; R, reverse.

Table 3

Ex vivo lysogenic transfer of ϕ149::Kmr between streptococcal species.

Group, M type	Clinical isolates, no.	Serum opacity factor	Lysogenization[a] with ϕ149::Km^r	
			Positive	Negative
Group A *Streptococcus*				
M1	7	−	1	6
M3	5	−	2	3
M5	1	−	1	0
M12	8	+	8	0
M19	1	−	1	0
M22	1	+	0	1
M28	3	+	2	1
M49	2	+	0	2
M81	1	+	0	1
M83	1	−	0	1
M89	2	+	0	2
M94	2	+	2	0
Group C *Streptococcus*	10	…	2	8
Group G *Streptococcus*	10	…	0	10

[a]Each lysogenization experiment was performed at least 3 times independently. Lysogens were analyzed by polymerase chain reaction and Southern blot analysis for the presence of the recombinant phage.