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An Enterotoxin-Bearing Pathogenicity Island in *Staphylococcus epidermidis*▽†

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Cocolonization of human mucosal surfaces causes frequent encounters between various staphylococcal species, creating opportunities for the horizontal acquisition of mobile genetic elements. The majority of *Staphylococcus aureus* toxins and virulence factors are encoded on *S. aureus* pathogenicity islands (SaPIs). Horizontal movement of SaPIs between *S. aureus* strains plays a role in the evolution of virulent clinical isolates. Although there have been reports of the production of toxic shock syndrome toxin 1 (TSST-1), enterotoxin, and other superantigens by coagulase-negative staphylococci, no associated pathogenicity islands have been found in the genome of *Staphylococcus epidermidis*, a generally less virulent relative of *S. aureus*. We show here the first evidence of a composite *S. epidermidis* pathogenicity island (SePI), the product of multiple insertions in the genome of a clinical isolate. The taxonomic placement of *S. epidermidis* strain FRI909 was confirmed by a number of biochemical tests and multilocus sequence typing. The genome sequence of this strain was analyzed for other unique gene clusters and their locations. This pathogenicity island encodes and expresses staphylococcal enterotoxin C3 (SEC3) and staphylococcal enterotoxin-like toxin L (SElL), as confirmed by quantitative reverse transcription-PCR (qRT-PCR) and immunoblotting. We present here an initial characterization of this novel pathogenicity island, and we establish that it is stable, expresses enterotoxins, and is not obviously transmissible by phage transduction. We also describe the genome sequence, excision, replication, and packaging of a novel bacteriophage in *S. epidermidis* FRI909, as well as attempts to mobilize the SePI element by this phage.

Common genetic processes drive the evolution of both pathogens and commensals in response to selective environmental pressures. Thus, instances where two related species in a common environmental niche evolve toward divergent lifestyles (one as a pathogen and the other as a commensal) are especially significant for understanding of the evolution of pathogenicity.

Staphylococcus aureus and *Staphylococcus epidermidis* are Gram-positive colonizers of the human mucosal surfaces and frequently encounter each other during mixed infections at various sites. The two species share a core genome of 1,681 genes (14) but differ significantly in their accessory genomes, composed of the flexible gene pool that is found only in some strains of a species and usually contributes an evolutionary benefit in specific environments (13, 20). Sequenced *S. epidermidis* genomes lack several genes common to the accessory genome of *S. aureus*, including those encoding superantigen toxins, certain proteases, and staphylokinase (20). The accessory genome and mobile genetic elements of *S. epidermidis* have been poorly characterized thus far, with only a few recent reports of conjugative plasmids and bacteriophages (9, 15). *S. aureus*, however, encodes more than 400 accessory genes, present on genomic islands, bacteriophages, and transposons, that account for the majority of toxins, resistance genes, and virulence determinants identified in the species (14). Antibiotic resistance genes are typically transferred by conjugative plasmids and recombination events, whereas phage-mediated transduction is the most common mechanism for the mobilization of superantigen toxins (27).

Lateral transfer of superantigen genes by transduction of *S. aureus* pathogenicity islands (SaPIs) among several strains of *S. aureus* is a well-characterized event (26, 30, 32), as is lateral transfer of such genes to related species, such as *Staphylococ-*

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cus xylosus and *Listeria monocytogenes* (3). SaPIs, which are chromosomal mobile elements with a conserved genome organization, typically comprise flanking site-specific attachment (*att*) sequences, one or more toxin genes, and a unique set of genes that enable their mobilization in conjunction with temperate phages such as ϕ11, ϕ13, and 80α (27).

The genetic processes that enable *S. aureus* to selectively stabilize and extensively exchange virulence determinants are poorly understood. From an evolutionary standpoint, toxin genes are equally relevant to the *S. aureus* host and to the persistence of the pathogenicity islands that transmit them among host genomes (13). Although recent reports have identified SaPI-like structures (lacking enterotoxins) in the sequenced genomes of *Staphylococcus haemolyticus* (27, 29) and *Staphylococcus saprophyticus* (18), and other studies have shown the presence of enterotoxin genes in several species of coagulase-negative staphylococci (CoNS) (6, 7, 33), there is no evidence so far to suggest that mobile elements in these staphylococcal commensals exchange virulence-associated genes as promiscuously as is seen in the *S. aureus* accessory genome.

In 2007, we presented the first evidence of a SaPI-like pathogenicity island and the expression of enterotoxins in a strain identified as *S. epidermidis* strain FRI909 (20a). The crystal structure of staphylococcal enterotoxin C3 (SEC3) from this strain has been reported previously as well (4). We report here the draft genome sequence of *S. epidermidis* strain FRI909, the complete sequence of a superantigen-bearing genomic island in strain FRI909, the transcriptional and translational expression of SEC3 and staphylococcal enterotoxin-like toxin L (SElL), and the sequence, excision, and packaging of a novel bacteriophage, ϕ909.

(Part of this work was presented at the 107th General Meeting of the American Society for Microbiology in 2007.)

MATERIALS AND METHODS

Bacterial strains and culture. All bacterial strains used in this study were grown overnight at 37°C on brain heart infusion (BHI) agar medium. Broth cultures were grown aerobically in BHI broth at 37°C. *S. epidermidis* strain FRI909 was obtained from Merlin Bergdoll, formerly of the Food Research Institute, Madison, WI. *S. epidermidis* strain RP62a is a methicillin-resistant, biofilm-producing isolate whose genome is one of the two sequenced *S. epidermidis* genomes (14). *S. epidermidis* 414, referred to as HER1292 in this study, is commonly used as a phage recipient strain of *S. epidermidis* (8, 9) and was kindly provided by Vincent Fischetti of the Rockefeller University. Taxonomic identification of *S. epidermidis* FRI909 was initially conducted by biochemical assays (API Staph ID test), determination of the absence of coagulase production and its gene, PCR-restriction fragment length polymorphism (PCR-RFLP) of the *gap* and *tuf* genes, and multilocus sequence typing (MLST). Complete genome sequencing was subsequently used to confirm the genetic identity of the strain.

DNA methods. Staphylococcal cells were lysed by lysostaphin treatment at 37°C for 30 min, and genomic DNA was isolated using the Qiagen (Valencia, CA) DNeasy kit. General DNA manipulations were performed by standard procedures (1).

Whole-genome sequencing and annotation of *S. epidermidis* FRI909. FRI909 was sequenced by 454 FLX pyrosequencing (Roche Applied Science, Indianapolis, IN) to 20-fold coverage across the genome. Sequence assembly was completed using GS De Novo Assembler software (Roche) and resulted in 69 contigs greater than 500 bp, ranging from 558 bases to 293 kbp. The genome sequences linking the contigs comprising the *S. epidermidis* pathogenicity island (SePI) and ϕ909 were confirmed by Sanger dideoxy sequencing (see the footnotes to Tables 1 and 2). Genome annotation of *Staphylococcus epidermidis* FRI909 was performed at The Institute for Genomic Sciences (IGS), University at Maryland School of Medicine, using the IGS Annotation Engine (http://ae.igs.umaryland.edu/cgi/index.cgi). The genomes of *Staphylococcus epidermidis* ATCC 12228 and RP62A were downloaded from GenBank. Coding sequences with at least 50 amino acids were extracted from GenBank files, and orthologs and recent paralogs were determined using OrthoMCL with the default parameter set (i.e., a BLAST E value cutoff of 1e−5 and an inflation parameter of 1.5) (19). Briefly, all-against-all BLASTp searches were first performed to reveal putative pairs of orthologs or recent paralogs based on reciprocal BLAST. Recent paralogs are defined as genes within the same genome that are reciprocally more similar to each other than to any sequence from another genome. BLAST *P* values were converted to a normalized similarity matrix by OrthoMCL. The matrix was analyzed by MCL, a Markov Cluster algorithm based on probability and graph flow theory (http://www.micans.org/mcl/), to generate a set of clusters. Each cluster contained a set of orthologs and/or recent paralogs.

Identification of a SEC-encoding genetic fragment. *Staphylococcus epidermidis* FRI909 was originally isolated from a human source and was identified as an enterotoxin C-producing CoNS (22). The presence of a *sec* gene in FRI909 was confirmed by PCR amplification and DNA sequencing with primers corresponding to *sec3* (17). To identify the chromosomal region containing *sec*, FRI909 genomic DNA was digested with HindIII and was analyzed by Southern blotting using an alkaline phosphatase-labeled 500-nucleotide (nt) internal *sec3* fragment (17). The sequence of the 10-kb genomic restriction fragment containing the *sec* gene was determined by sequencing a series of deletion products generated using the Erase-A-Base system (Promega, Madison, WI). The remaining sequence of the SePI element was determined after genome sequencing of FRI909 by 454 FLX pyrosequencing.

PCR screen of *S. epidermidis* clinical isolates for SePI. *S. epidermidis* clinical isolates recovered in cases of bacteremia were obtained from hospitals in the Kaleida Health System of Western New York. Two hundred isolates were from monomicrobial *S. epidermidis* infections. Six isolates were from mixed *S. epidermidis-S. aureus* infections. The isolates were screened for the presence of a SePI-like island by multiplex PCR amplification of nine *S. aureus* enterotoxin genes (including *sec3* and *sell*) and three genes within the core of the SePI element: (i) the SePI integrase (GSEF_0619), (ii) a SaPI-like packaging protein (GSEF_0617), and (iii) a hypothetical SaPI protein (GSEF_0613) homologous to MW0756, which is found within the SaPImw3 of MW2.

RNA extraction and RT-PCR. Bacteria were grown overnight in BHI and were diluted 1:100 in fresh medium. Culture aliquots were removed at specified time points and were added to RNAProtect Bacteria reagent (Invitrogen, Carlsbad, CA). Total RNA was isolated from samples by bead-beating cultures in lysing matrix B tubes (MP Biomedicals, Solon, OH) with Trizol (Invitrogen), followed by chloroform extraction from the aqueous phase and precipitation in isopropanol. Extracted RNA was treated with RNase-free DNase (Ambion, Austin, TX) in order to remove any contaminating genomic DNA and was purified on RNeasy columns (Qiagen). Complete removal of contaminating DNA was confirmed by PCR. Clean RNA was quantified spectrophotometrically and was subsequently used for quantitative reverse transcription-PCR (qRT-PCR) using the MyiQ SYBR green detection system (Bio-Rad, Hercules, CA). *S. aureus* N315 was used as a positive control for SEC3 production. For each time point, fold changes in gene expression were calculated relative to the expression of the internal control genes for 16S rRNA and DNA gyrase A.

Generation of an anti-SEC3 antibody. To generate anti-SEC3 sera, SEC3 was purified from cultures by using preparative isoelectric focusing as described previously (10). New Zealand White rabbits (Simonsen Laboratories, Gilroy, CA) were immunized biweekly with SEC3. One week after the fourth boost, sera were harvested and pooled.

Western blotting. Bacterial cultures were grown as for the RT-PCR experiments described above. Ten-milliliter samples from cultures were pelleted (16,000 × *g*, 10 min) at the time points specified in Fig. 3C. Supernatants were sterilized with 0.45-μm-pore-size filters (Millipore, Bedford, MA), and were concentrated to a 100-μl volume with Amicon Ultra-15 concentration units (Millipore) by centrifugation at 5,000 × *g* for 15 min. Samples were boiled in sodium dodecyl sulfate (SDS) sample buffer, subjected to SDS-polyacrylamide gel electrophoresis (PAGE) (10% polyacrylamide gels), and transferred to Immobilon-P membranes (GE Healthcare, Piscataway, NJ). After being blocked with 2% skim milk in TTBS (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.05% Tween 20), membranes were incubated with anti-SEC3 (see above) or anti-SEL sera, followed by a horseradish peroxidase-conjugated anti-rabbit IgG (GE Healthcare). Proteins were visualized using a chemiluminescence detection kit according to the manufacturer's directions (Pierce, Rockford, IL).

Bacteriophage induction, transduction, and DNA extraction. Bacteria were grown to an optical density at 600 nm (OD_{600}) of 0.45 and were induced by the addition of mitomycin C (Sigma-Aldrich, St. Louis, MO) at a final concentration of 5 μg/ml. Cultures were grown aerobically at 37°C for 16 h in order for complete lysis to occur. The resultant lysates were used for electron microscopy. Lysates were filter sterilized (with 0.45-μm-pore-size filters) and were used to

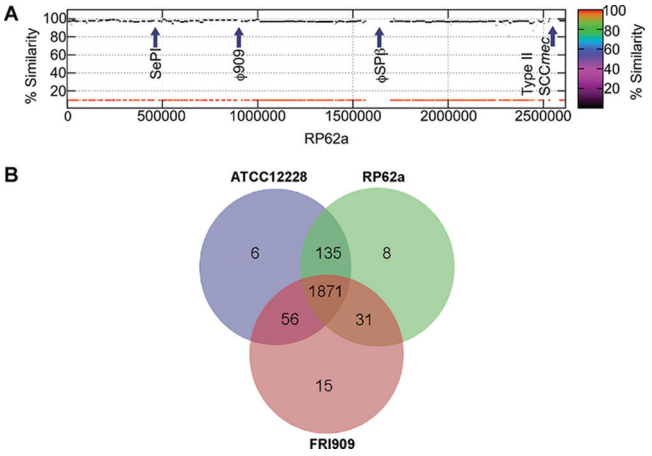

FIG. 1. (A) Genome alignment and ortholog comparison of *S. epidermidis* FRI909 with *S. epidermidis* RP62a and ATCC 12228, whose genomes have been completely sequenced. (A) Alignment of the *S. epidermidis* FRI909 and RP62a genomes shows the positions of FRI909 SePI and ϕ909 relative to each other and to RP62a ϕSPβ and type II SCC*mec*. The SePI is inserted into the tmRNA region of FRI909. (B) Venn diagram showing the number of orthologous groups in each genome, as well as the common and unique clusters identified.

transduce recipient strain *S. epidermidis* HER1292 as described in the literature (25). Phage DNA was extracted by standard methods (1). Briefly, purified phage particles were treated with DNase to remove any contaminating genomic DNA, followed by phenol-chloroform extraction and isopropanol precipitation of phage DNA. PCR to test for the presence of SePI-1 in this DNA was performed using primers for *gyrA* (to confirm the absence of genomic DNA), the portal gene (phage), and SEC3.

Electron microscopy. Bacteriophages were pelleted by centrifugation at 70,000 × *g* for 1 h, washed twice with ammonium acetate solution (100 mM), and resuspended in 4 mM $CaCl_2$–1 mM $MgCl_2$. The sample was adsorbed onto a carbon grid and was negatively stained using 2% sodium phosphotungstate (pH 7.0). The sample was air dried and examined at a magnification of ×85,000 on a JEOL 100 CX2 transmission electron microscope.

Nucleotide sequence accession numbers. The results of this Whole Genome Shotgun project have been deposited at DDBJ/EMBL/GenBank under accession number AENR00000000. The version described in this paper is the first version, AENR01000000. Nucleotide sequences and annotation for the *S. epidermidis* FRI909 SePI element and the ϕ909 bacteriophage are included in this accession.

RESULTS

Taxonomic placement of strain FRI909. Because *S. epidermidis* FRI909 was isolated from a human source (22) and previous studies have raised concerns about the species identity of clinical CoNS isolates based on phenotypic characteristics (16, 24), we confirmed the genotype of *S. epidermidis* strain FRI909 by PCR-RFLP of *gap*, sequencing of *tuf*, and multilocus sequence typing (MLST) (21).

Comparison of the *S. epidermidis* FRI909 genome to those of sequenced strains. Contigs assembled from genome sequencing were annotated using the IGS annotation engine. The alignment of the FRI909 draft genome sequence with the genome sequence of *S. epidermidis* RP62a and the locations of SePI, ϕ909, ϕSPβ, and the type II staphylococcal cassette chromosome *mec* element (SCC*mec*) are indicated in Fig. 1A. SePI and ϕ909 are unique to *S. epidermidis* FRI909. ϕSPβ and the type II SCC*mec* are unique to *S. epidermidis* RP62a. Families of orthologous gene groups were identified by comparison to the two completely sequenced *S. epidermidis* genomes, those of ATCC 12228 and RP62a. As shown in the Venn diagram in Fig. 1B, FRI909 contains 15 unique orthologous groups (see Table S1 in the supplemental material) not present in the other two genomes. Several hypothetical proteins were identified in these groups, along with clusters of mobilization proteins that appear to be chromosomal remnants of a plasmid that may have integrated into the genome and undergone rearrangements. Interestingly, some of these mobilization proteins also flank a putative CRISPR locus identified in the genome. The only other significant unique group consists of the genes present on SePI-1, corresponding to the enterotoxins and SaPI-like elements identified on this pathogenicity island. The bacteriophage present in *S. epidermidis* FRI909 is clustered with orthologous phage genes from the bacteriophage present in *S. epidermidis* RP62a.

Sequence analysis and identification of a genomic island. Multiplex PCR was used to screen the FRI909 genome for nine staphylococcal superantigens (see Table S2 in the supplemental material). The strain tested positive only for *sec* and *sell* (data not shown). Sequencing and additional PCR with primers specific to *sec3* and *sell* confirmed the presence of these two toxins. Because these two toxin genes are typically associated with mobile pathogenicity islands flanked by *att* sites in *S. aureus*, the sequences of regions flanking the toxin genes were determined. We identified a novel genomic sequence of about 20.5 kb inserted at the transfer-messenger RNA (tmRNA) gene of *S. epidermidis* FRI909, just downstream of the SsrA binding protein (identical in sequence to the corresponding region of the sequenced genome of *S. epidermidis* RP62a; SERP0451 is the locus identification [ID] for *ssrA*, and SERP_SetmRNA1 is the locus ID for tmRNA, in RP62a, while GSEF_0621 is the locus ID for *ssrA* in FRI909). The tmRNA region is associated with the insertion of foreign genetic elements in the genomes of several bacteria, and the insertion of the pathogenicity island μSaα3mw (called SaPImw2 by Novick et al. [27]) in *S. aureus* MW2 occurs in an identical region (2). As described below, the presence of multiple transposases and repeat sequences, along with the mixed *S. aureus* and *S. epidermidis* sequence in this region, suggests that more than one insertion and recombination event have led to the formation of this SePI. Considering the gene organization of this composite genomic island, we propose that it comprises two distinct regions, as shown in Fig. 2A. The first of these is SePI-1, a classical pathogenicity island that shows significant homology to the enterotoxin-bearing SaPIs, and the second is SeCI-1 (*S. epidermidis* chromosomal insertion 1), an IS*1272*-like region flanked by 73-bp direct repeats, which contains multiple transposases and several exported *S. aureus* proteins.

Chromosomal integration site and comparison to known SaPIs. SePI-1 is a 9.6-kb region flanked by direct repeats that encompasses the two superantigen genes. The 14-bp direct repeats (DR1) defining the ends of SePI-1 (Fig. 2A) are identical to the *att* sequences of SaPImw2 in *S. aureus* MW2 and to those of three other known SaPIs (2, 27). The map of SePI-1 includes 11 open reading frames (ORFs) identified on the basis of homology to known sequences (Fig. 2A; Table 1). The putative SEC3 gene identified bears 95% identity to previously sequenced SEC3 genes from *S. aureus*. It differs at 12 amino acid residues from the SEC3 gene of *S. aureus* Mu3. However, none of these residues lie in the Zn-binding or T-cell receptor

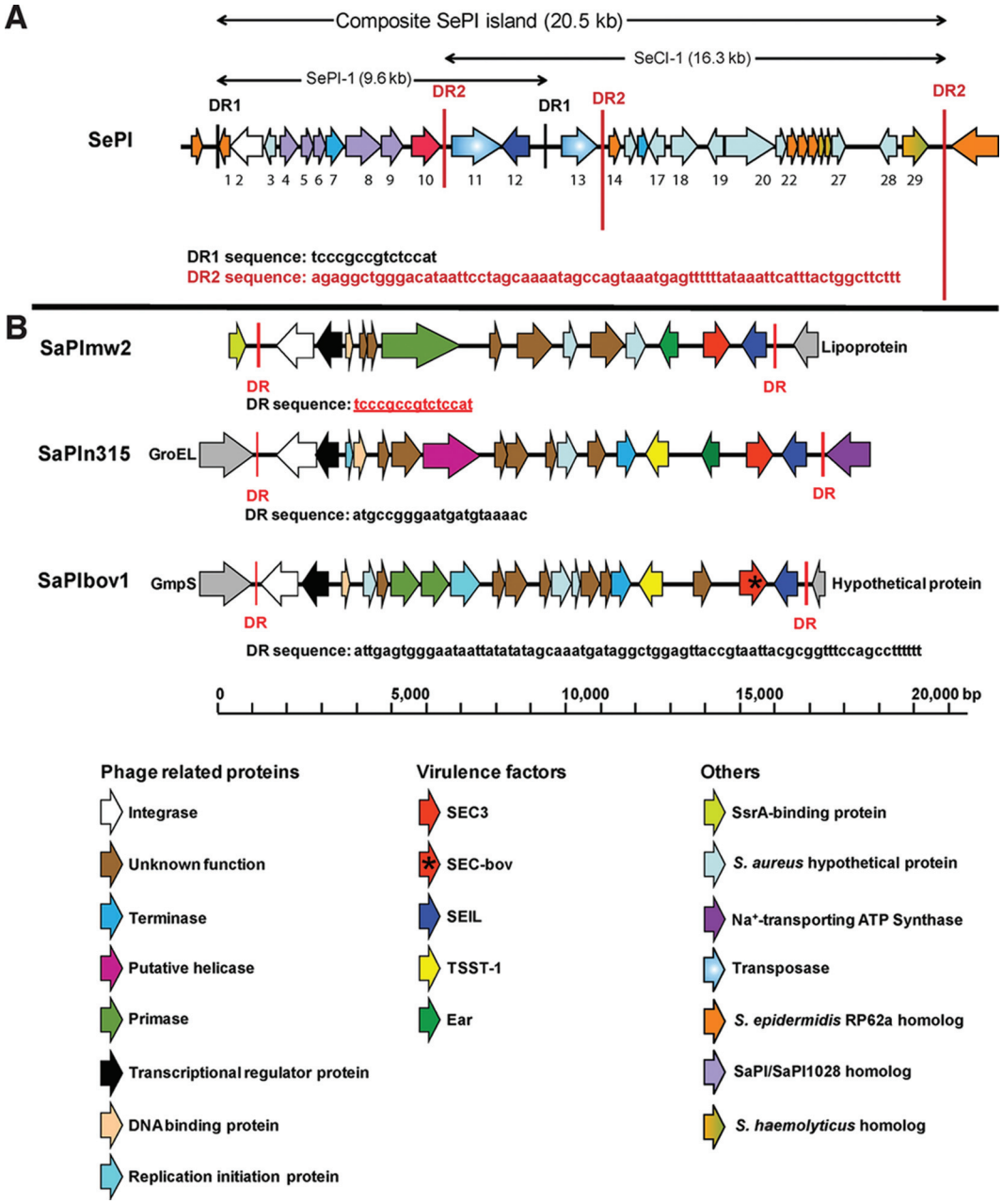

FIG. 2. Sequence and ORF map of the composite SePI and comparison with sequenced SaPIs. (A) The 20.5-kb mobile genetic element is inserted into the tmRNA region of *S. epidermidis* FRI909, between the SsrA-binding protein (GSEF_0621) and a Na^+-transporting ATPase (GSEF_0143). This island appears to be the product of two independent insertional events, one resulting in the insertion of SePI-1 (flanked by 14-bp *att*L and *att*R direct-repeat sequences) and the other in a transposon-mediated insertion. The second insertion, SeCI-1, is flanked by 73-bp direct-repeat sequences and comprises several *S. aureus* proteins not found in sequenced *S. epidermidis* genomes. A list of ORFs and predicted functions is presented in Table 1. (B) For comparison to the composite SePI, ORFs in SaPImw2, SaPIn315, and SaPIbov1, which have been functionally characterized (31, 32), are shown.

binding domains, suggesting that functional differences are unlikely. The crystal structure of FRI909 SEC3 has also been described previously (4). The SEIL gene is 97% identical to known *S. aureus* SEIL genes, and its product differs at 6 amino acid residues from the *S. aureus* gene product.

An integrase at the left-hand junction of this putative pathogenicity island is 92% identical to the integrase in genomic island SaPImw2, present in *S. aureus* strain MW2 (2). Although SePI-1 ORF3 occurs in a context and at a location similar to those of the regulatory *stl* gene in SaPIs, it bears no sequence homology to *stl*. Other genes within the island encode a terminase and four of the packaging proteins associated with sequenced SaPIs, as shown in Table 1. For purposes of comparison with the SePI ORFs, the ORFs of sequenced SaPIs are shown in Fig. 2B.

The presence of another set of direct-repeat sequences of 73

TABLE 1. ORFs identified in genomic islands and the SePI[a] in *S. epidermidis* FRI909

ORF[b]	Locus ID	Size (bp)	Size (kDa)	Predicted function	Most significant database match (E value)	% aa identity (similarity)
1	GSEF_0620	231	9.12	Unknown	Hypothetical protein SERP0452, *S. epidermidis* RP62a (1e−36)	99 (99)
2	GSEF_0619	945	44.5	Integrase	Integrase, *S. aureus* MW2 genomic island Saα3mw (5e−173)	92 (97)
3	GSEF_0618	297	12.7	Unknown	No significant match	
4	GSEF_0617	477	26	Packaging protein, SaPI	ORF007, *S. aureus* SaPI1028 (7e−25)	38 (62)
5	GSEF_0616	336	21	Packaging protein, SaPI	ORF010, *S. aureus* SaPI1028 (4e−25)	58 (78)
6	GSEF_0615	342	13.8	Packaging protein, SaPI	ORF014, *S. aureus* SaPI1028 (2e−14)	35 (62)
7	GSEF_0614	492	22.7	Terminase, small subunit	ORF009, *S. aureus* SaPI1028 (6e−54)	64 (83)
8	GSEF_0613	999	39	Hypothetical *S. aureus* protein	Hypothetical protein MW0756, *S. aureus* MW2 (4e−60)	37 (64)
9	GSEF_3000	582	22.3	Hypothetical *S. aureus* protein	Hypothetical protein MW0757, *S. aureus* MW2 (5e−23)	50 (71)
10	GSEF_0163	801	31.9	Enterotoxin C3	Enterotoxin type C3, *S. aureus* Mu50 (9e−141)	95 (97)
11	GSEF_0162	1,423	67	Transposase	Putative transposase, *S. epidermidis* ATCC 12228 (0.0)	97 (99)
12	GSEF_0161	723	28.8	Enterotoxin L	Extracellular enterotoxin L, *S. aureus* Mu50 (1e−122)	97 (98)
13	GSEF_0160	1,014	40.4	Transposase	ISSep-1-like transposase, *S. aureus* (0.0)	98 (98)
14	GSEF_0159	60	2.4	Hypothetical protein	Hypothetical protein, *S. epidermidis* (0.061)	90 (95)
15	GSEF_0158	309	18.8	Hypothetical *S. aureus* protein	Hypothetical protein, *S. aureus* USA300 (1e−18)	52 (68)
16	GSEF_0157	261	22.7	Terminase, small subunit	Terminase small subunit, *S. aureus* MRSA252 (5e−23)	69 (82)
17	GSEF_0156	438	17.7	Hypothetical *S. aureus* protein	Hypothetical protein SAR1890, *S. aureus* MRSA252 (6e−31)	56 (75)
18	GSEF_0155	747	64.3	Hypothetical *S. aureus* protein	Hypothetical protein SAR1895, *S. aureus* MRSA252 (3e−46)	49 (68)
19	GSEF_0154	429	17.8	Hypothetical *S. aureus* protein	Hypothetical protein SAR1894, *S. aureus* MRSA252 (3e−19)	38 (64)
20	GSEF_0153	1,527	64.3	Hypothetical *S. aureus* protein	Hypothetical protein SAR1895, *S. aureus* MRSA252 (1e−77)	46 (66)
21	GSEF_0152	300	11.8	Hypothetical *S. aureus* protein	Hypothetical protein SAR1896, *S. aureus* MRSA252 (1e−17)	47 (72)
22	GSEF_0151	426	17.04	Hypothetical protein	Conserved hypothetical protein, *S. epidermidis* W23144 (1e−32)	48 (69)
23	GSEF_0150	339	13.56	Hypothetical protein	Conserved hypothetical protein, *S. epidermidis* W23144 (2e−30)	75 (84)
24	GSEF_0149	405	16.2	Conserved hypothetical protein	Conserved hypothetical protein, *S. epidermidis* W23144 (1e−38)	96 (99)
25	GSEF_0148	168	6.72	Unnamed protein product	Hypothetical protein SH1800, *S. haemolyticus* JCSC1435 (5e−20)	87 (94)
26	GSEF_0147	141	5.64	Unnamed protein product	Hypothetical protein SH1800, *S. haemolyticus* JCSC1435 (2e−13)	88 (95)
27	GSEF_0146	354	18.8	Hypothetical *S. aureus* protein	Hypothetical protein, *S. aureus* USA300 (6e−13)	62 (75)
28	GSEF_0145	468	19.1	Hypothetical *S. aureus* protein	Hypothetical protein MW0372, *S. aureus* MW2 (1e−23)	46 (60)
29	GSEF_0144	738	29.5	Amidohydrolase	2-pyrone-4,6-dicarboxylic acid hydrolase, *S. haemolyticus* (9e−07)	45 (64)

[a] The SePI is encoded on two separate contigs: (i) GSEF_144_163 (contig 00004) and (ii) GSEF_0613-620 (contig 00011). The sequence (GSEF_3000) linking contigs 00004 and 00011 was confirmed by Sanger sequencing.
[b] ORFs not in consecutive numerical order were manually reannotated after initial automated annotation.

bp (DR2) flanking transposase elements and several exported proteins from *S. aureus*, suggests the occurrence of at least one other insertion event in this region of the FRI909 chromosome (Fig. 2A). The element flanked by DR2, termed SeCI-1, is similar to IS*1272*, which is also flanked by direct repeats and contains transposase genes, but bears no further similarity to known pathogenicity-associated or other genomic islands. Together, SePI-1 and SeCI-1 form the composite genomic island seen in strain FRI909.

Searches of the two sequenced *S. epidermidis* genomes reveal that strain ATCC 12228 contains 19 copies of the 73-bp repeat flanking SeCI-1 and that 1 of these is at the same genomic location (upstream of Na^+-transporting ATP synthase) as that in FRI909. The short 14-bp direct repeat is also found in ATCC 12228, at the same genomic location as that in FRI909 (downstream of the SsrA-binding protein).

Expression of enterotoxins SEC3 and SElL. Although previous studies have identified enterotoxin genes in CoNS (5, 7), the potential regulatory mechanisms and expression of these genes in non-*S. aureus* strains have not been examined. Ex-

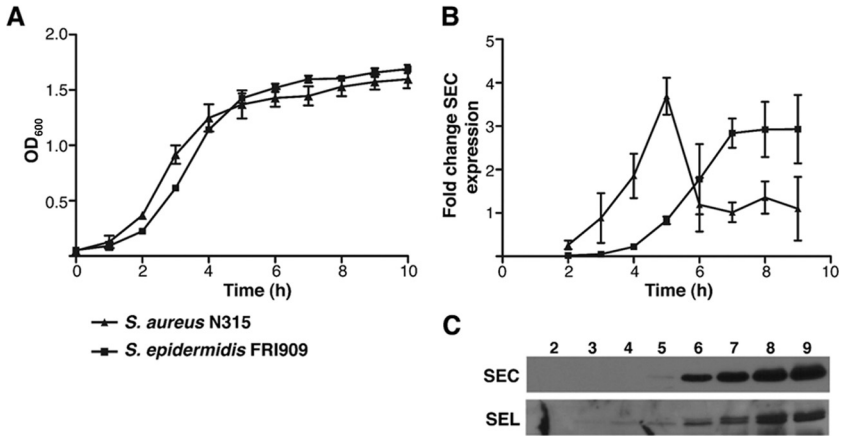

FIG. 3. Analysis of SEC3 expression by qRT-PCR and Western blotting. (A) Growth curves measured by the OD_{600} for *S. aureus* N315 and *S. epidermidis* FRI909. (B) Expression of enterotoxin SEC3 was evaluated by qRT-PCR using *gyrB* as a standard and the SEC3-producing strain *S. aureus* N315 as a positive control. (C) Similar levels of expression from the two strains were observed by Western immunoblotting. Lane labels indicate time (h).

pression of the SEC3 gene from this region was confirmed by Northern blotting (data not shown), and qRT-PCR was performed to identify possible growth-dependent regulation (Fig. 3B). SEC3 expression appears to increase significantly toward late-log phase and early-stationary phase (Fig. 3C), slightly later than the peak of expression observed with a control *S. aureus* strain, N315. Similar results were obtained with SElL (data not shown), and transcriptional expression was corroborated by translational studies with Western immunoblotting for both enterotoxins (Fig. 3C). While the regulation and significance of this pattern of toxin expression have yet to be elucidated, the virulence potential of this genomic island is evident in the expression of the two toxin genes present.

Mobilization. Both transduction and conjugation are known to function in the horizontal transmission of accessory genes in *S. aureus*. Since superantigen toxins and SaPIs are most frequently mobilized by bacteriophage, there is a strong possibility that interspecies transfer also occurs by similar mechanisms. We tested the phage-related excision and packaging of SePI-1 by mitomycin C treatment. A previously unknown *S. epidermidis* bacteriophage was identified in the culture lysate. Purified phage particles studied by transmission electron microscopy revealed symmetrical icosahedral heads (diameter, approximately 60 nm), flexible banded tails, and end plates (Fig. 4B). All induced phage particles were identical in size, suggesting that even if packaging of SePI-1 occurs in conjunction with this temperate phage, the small phage heads characteristically observed in SaPI transduction (30) are unlikely to be formed. The insertion of SeCI-1 interrupts the SePI-1 sequence with an *S. epidermidis* transposase, disrupting the SaPI ORFs implicated in the formation of smaller infectious SaPI phage particles (30, 32). This does not rule out the possibility that SePI-1 may be excised and packaged at a lower frequency in normal phage particles. PCR was performed on purified phage DNA using primers designed to amplify both the SePI-1 SEC3 gene and the phage-specific portal gene as a positive control. The absence of contaminating genomic DNA was confirmed by using *gyrA* as a negative control. Phage DNA gave a 600-bp product only for the portal gene, suggesting that SePI-1 is not packaged as part of the phage DNA (Fig. 4C). PCR to test SePI-1 excision and circularization, and Southern blotting to check for SePI-1-specific bands upon phage excision, gave negative results (data not shown), suggesting that SePI-1 is unlikely to be mobilized by a temperate phage in the SaPI-like excision-replication-packaging mechanism (32). Although we also identified plasmids in *S. epidermidis* strain FRI909, conjugative transfer of genomic elements to recipient strains RP62a and HER1292 was not seen at any discernible frequency (data not shown).

Phage genome. Since the 9.6-kb SePI-1 element is interrupted by a transposon not typically seen in SaPI sequences, it is possible that this genomic island was mobilized by a bacteriophage from *S. aureus* into *S. epidermidis* FRI909 but can no longer be mobilized due to the transposase interruption. The complete genome sequence of the endogenous prophage in *S. epidermidis* FRI909 was compared to those of known *S. epidermidis* bacteriophage and SaPI-associated temperate phages from *S. aureus*. Overall, this previously unidentified phage bears greater sequence homology to *S. aureus* phages than to the two phages in sequenced *S. epidermidis* genomes (9), as evidenced by the database matches in Table 2. The 30,124-bp genome contains 29 putative ORFs and has an AT-rich genome (GC content, 28%), which is comparable to other staphylococcal phage genomes, both in *S. aureus* and in *S. epidermidis*. A complete list of genes and their closest database matches appears in Table 2. The phage genome appears modular, organized as genes involved in lysis, lysogeny, and DNA replication and modification, followed by structural genes for capsid formation and assembly and for head and tail morphogenesis (Fig. 4A). Despite the similarity of the FRI909 phage to *S. aureus* temperate phages, its genome sequence indicates that it is not one of the many temperate phages with a well-defined relationship to the SaPIs.

Screening for SePI-like elements in *S. epidermidis* clinical isolates associated with bacteremia. To determine if acquisition of the SePI element by FRI909 was an isolated or rare event, we screened more than 200 *S. epidermidis* bacteremia isolates for the presence of nine *S. aureus* enterotoxins as well

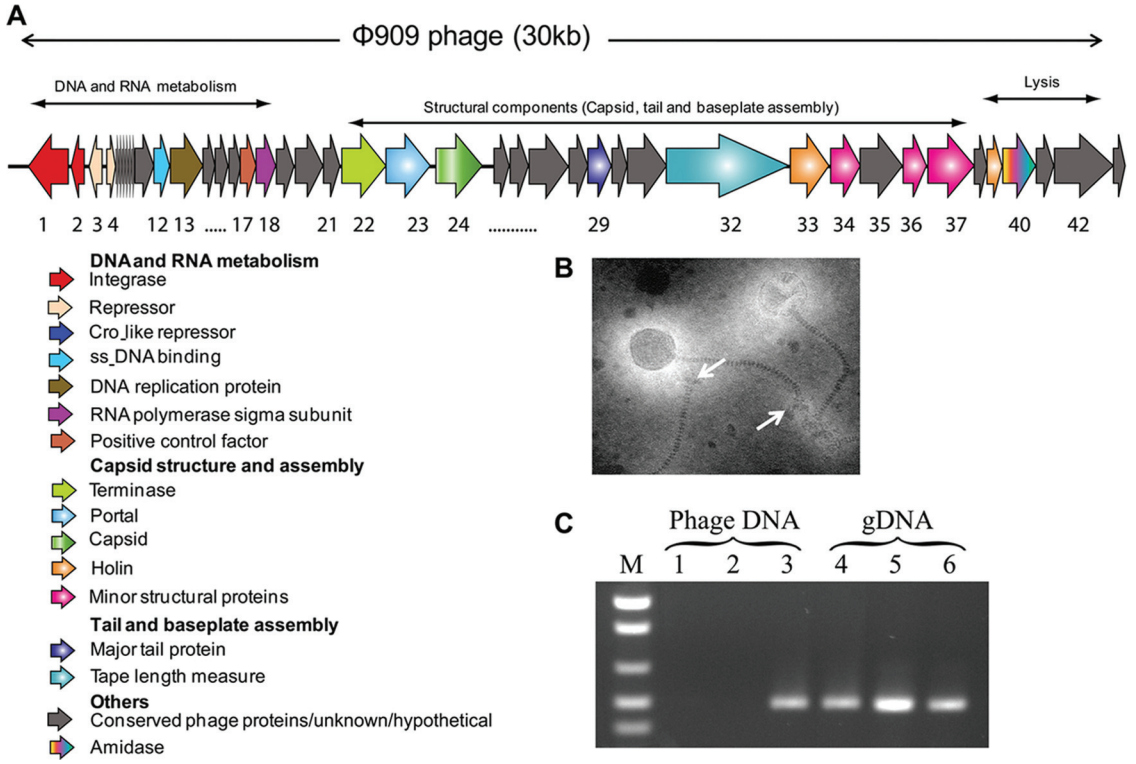

FIG. 4. Sequence and ORF analysis of the temperate bacteriophage ϕ909 in *S. epidermidis* FRI909. (A) The ϕ909 bacteriophage insertion is found immediately downstream of the FemC/glutamine synthetase gene (SERP0876 in RP62a; GSEF_0257 in FRI909), a site distinct from the composite genomic island containing SePI-1. The genome shows conserved regions corresponding to those seen in *S. aureus* phages. (B) Ultrastructure of the bacteriophage showing intact phage particles. Transmission electron microscopy reveals isometric heads (diameter, 60 nm) with long, banded tails and end plates. Arrows mark end plates on tails. (C) PCR amplification of toxin genes from phage DNA and bacterial genomic DNA (gDNA). Lanes 1 and 4, *gyrA*; lanes 2 and 5, the SEC3 gene; lanes 3 and 6, *porF*; lane M, marker.

as the SePI integrase (GSEF_0619), a SaPI-like packaging protein (GSEF_0617), and a hypothetical SaPI protein (GSEF_0613) homologous to MW0756, which is found within SaPImw3 of MW2 (see Table S2 in the supplemental material).

Two hundred of these isolates were from patients with monomicrobial *S. epidermidis* infections. Six were from patients with mixed *S. epidermidis*-*S. aureus* infections, an environment likely to promote the transfer of pathogenicity islands between these two species. None of the isolates contained enterotoxins, the SePI integrase, or the SaPI-like packaging proteins. The absence of *sec3* and *sell* in the isolates suggests that the SePI element we describe is unique to FRI909. The absence of the SePI integrase and SaPI-like packaging proteins in the bacteremia isolates suggests that SePI-like elements that do not encode these enterotoxins occur rarely in *S. epidermidis*.

DISCUSSION

Early reports of the presence of superantigen toxins in CoNS have been based primarily on phenotypic tests for coagulase production and subsequent identification of the toxin genes (5). Later studies contradicting these results suggest that coagulase production is not an adequate means of classification for the staphylococci (16). More-recent reports of enterotoxins and other superantigens in CoNS have also based the classification of clinical isolates predominantly on phenotypic criteria rather than on genotypic characterization and MLST. Since toxin-containing coagulase-negative *S. aureus* strains have been reported in the literature (24), and it is known that clinical isolates of bacteria tend to show greater phenotypic variability than laboratory strains, MLST or another method of genotypic characterization is of great importance to any studies of toxin gene distribution in staphylococcal species.

Although previous studies have identified superantigen genes in CoNS, their genomic location in other species has not been analyzed so far. Our results suggest that they can occur in more diverse genetic contexts than just the specialized pathogenicity islands exclusive to *S. aureus*. The SaPIs, as described thus far, all contain specific ORFs to hijack temperate phage function for the structural proteins required for SaPI packaging. They do not encode functions beyond these 20 or so ORFs, *att* sequences, and toxin genes. Little is known about the evolutionary processes that have resulted in the current composition of SaPIs, and they are usually regarded as highly specialized temperate phages. So far, there have been no reports of superantigen gene mobilization by means other than transduction or by mobile elements other than phages.

Compared to the specialized transfer mechanism of SaPIs, other virulence-associated accessory genes in staphylococci and the related enterococci are transmitted by genomic elements that are more variable, both in their structures and in their sources. Antibiotic resistance genes, for example, are

TABLE 2. Genome organization of *S. epidermidis* FRI909 temperate bacteriophage ϕ909[a]

ORF	Locus ID	Size (bp)	Predicted function	Most significant database match (E value)	% aa identity (similarity)
1	GSEF_0256	1,125	Integrase	*Bacillus cereus* E33L, integrase (9e−66)	39 (58)
2	GSEF_0255	372	Unknown	*Macrococcus caseolyticus* JCSC5402 integrase, MCCL_0938 (8e−86)	43 (65)
3	GSEF_0254	351	Repressor	*S. aureus* phage 80α, CI-like repressor (3e−22)	67 (85)
4	GSEF_0253	237	Repressor	*S. aureus* phage ϕPVL108, *cro*-like repressor (5e−29)	83 (88)
5	GSEF_0252	279	Unknown	*S. epidermidis* M23864:W1 unknown, HMPREF0793_2053 (1e−33)	72 (89)
6	GSEF_0251	156	Hypothetical	No significant match	0
7	GSEF_0250	204	Hypothetical	No significant match	0
8	GSEF_0249	165	Hypothetical	No significant match	0
9	GSEF_0248	210	Unknown	*S. aureus* phage 96, ORF086 (2.4e−20)	68 (80)
10	GSEF_0247	192	Membrane protein	*S. epidermidis* W23144, unknown, HMPREF0791_1646 (5e−12)	55 (77)
11	GSEF_0246	267	Unknown	*S. aureus* A9635, unknown, SALG_01528 (2e−30)	49 (70)
12	GSEF_0245	396	ssDNA binding protein[b]	*S. aureus* phage 80α, ssDNA binding protein (8e−32)	57 (79)
13	GSEF_0244	903	DNA replication	*S. aureus* USA300, DnaD DNA replication protein, HMPREF0776_0149 (3e−53)	43 (58)
14	GSEF_0243	312	Unknown	*S. aureus* phage 80a, gp26 (0.52)	30 (50)
15	GSEF_0242	300	Membrane protein	*S. epidermidis* W23144, unknown, HMPREF0791_1646 (5e−12)	55 (77)
16	GSEF_0241	225	Unknown	*Clostridium botulinum* strain E3, acetyltransferase, CLH_0023 (7.9)	37 (56)
17	GSEF_0240	252	Positive control factor	*Bacillus* sp. positive control sigma-like factor (1.3e−7)	42 (57)
18	GSEF_1849	144	RNA polymerase	*Bacillus pumilus* ATCC 7061, RNA polymerase sigma factor (6e−19)	33 (53)
19	GSEF_1848	393	Unknown	*Nitrobacter winogradskyi* Nb-255, dehydrogenase, Nwi_0422 (2.5)	26 (39)
20	GSEF_1847	357	HNH endonuclease	*S. aureus* phage ϕSLT, ϕSLTp37 (2e−21)	57 (68)
21	GSEF_1846	402	Unknown	*Bacillus anthracis* conserved hypothetical protein (3e−16)	53 (79)
22	GSEF_1845	1,716	Terminase	*B. anthracis* prophage LambdaBa01, terminase large subunit (3e−144)	61 (78)
23	GSEF_1844	1,227	Portal protein	Phage portal protein, *Clostridium botulinum* (6e−80)	39 (62)
24	GSEF_1843	1,251	Major capsid protein	Phage protein, *Clostridium perfringens* (2e−61)	33 (53)
25	GSEF_1842	288	Unknown	Hypothetical protein SA1771, *S. aureus* phage ϕN315 (2e−08)	32 (59)
26	GSEF_1841	339	DNA packaging	QlrG family phage protein (6e−07)	40 (61)
27	GSEF_1840	1,557	Unknown	*S. epidermidis* RP62a, unknown, SERP2279 (7.4e−273)	97 (98)
28	GSEF_1839	414	Unknown	Hypothetical protein SA1770, *S. aureus* phage ϕN315 (2e−16)	37 (59)
29	GSEF_1838	660	Tail protein, structural	Major tail protein, *S. aureus* phage ϕSLT (5e−30)	35 (61)
30	GSEF_1837	351	Unknown	*Bacillus subtilis*, conserved hypothetical protein (4e−04)	33 (57)
31	GSEF_1836	1,509	Glycosyl transferase	*S. epidermidis* ATCC 12228, SE2243, lipopolysaccharide biosynthesis protein (2.5e−265)	97 (99)
32	GSEF_1835	4,491	Tail tape length measure	Tail tape length measure protein, *S. aureus* USA300_TCH1516 (0.0)	41 (59)
33	GSEF_1834	825	Putative holin	Holin-like protein, *S. aureus* phage ϕ12 (8e−47)	37 (54)
34	GSEF_1833	1,179	Structural protein	Minor structural protein, *Listeria monocytogenes* phage (3e−74)	40 (62)
35	GSEF_1832	597	Conserved phage protein	*S. aureus* phage 187, ORF019 (7e−60)	53 (64)
36	GSEF_1831	1,017	Acylhydrolase, putative structural component	*S. aureus* phage 187, putative protein (3e−93)	55 (71)
37	GSEF_1830	1,278	Minor capsid protein	*S. aureus* phage 187, ORF006 (1e−21)	49 (70)
38	GSEF_1829	177	Putative membrane protein	*S. epidermidis* ATCC 12228, SE2234 (2.9e−24)	100 (100)
39	GSEF_1828	246	Holin	*S. epidermidis* phage lysis holin (1e−13)	50 (72)
40	GSEF_1827	918	Amidase	*Bacillus* sp., *N*-acetylmuramoyl-L-alanine amidase (8e−24)	37 (54)
41	GSEF_1826	774	Unknown	*Staphylococcus* phage 52A, ORF013 (3e−25)	41 (58)
42	GSEF_1825	2,859	Phage infection protein	*S. epidermidis* RP62a, SERP2262 (0e−0)	99 (99)
43	GSEF_1824	331	Unknown	Hypothetical protein SAB1174, *S. aureus* RF122 (1e−56)	77 (90)

[a] The phage is encoded on two separate contigs: (i) GSEF_1824 to GSEF_1849 (contig 00040) and (i) GSEF_0240 to GSEF_0256 (contig 00006). The sequence linking contigs 00040 and 00006 was confirmed by Sanger sequencing.
[b] ssDNA, single-stranded DNA.

known to be mobilized between *S. aureus* strains by chromosomal elements, from *S. epidermidis* to *S. aureus* by conjugative plasmids, and from enterococci to *S. aureus* by conjugative processes (12, 15, 34). Vancomycin resistance elements (VRE) are mobilized from *Enterococcus* spp. to *S. aureus* by conjugative plasmids (34). Interestingly, although the VRE in *Enterococcus* spp. bear the hallmarks of a classical excision-replication-integration pathway (*att* sequences, integrase, terminase), none of these features are involved in the process of mobilization into *S. aureus* (28). The conjugative process that transfers these resistance genes into the *S. aureus* genome operates exclusively between enterococci and *S. aureus*.

Similarly, the arginine catabolic mobile element (ACME) reported in the *S. aureus* USA300 genome is actually more prevalent in *S. epidermidis* strains and is mobilized only from *S. epidermidis* to *S. aureus*, not between *S. aureus* strains (11). Like the

VRE, this 57-kb composite island is interspersed with direct and inverted repeats and is a result of the insertion of three separate pieces of foreign DNA into the same region of the *S. epidermidis* genome.

Further, *S. aureus* itself is known to contain large nonmobile genomic islands encoding enterotoxin-like genes. Even though these are widespread among *S. aureus* strains, they are typically not taken into account in discussions of the mobilization of superantigen genes, since their horizontal transfer has not been demonstrated.

The stability and transmissibility of the accessory gene pool of a species may be significantly impacted by core genomic factors. Natural competence in many species is a function of, and is regulated by, the core genome. A recent report by Marraffini and Sontheimer also shows that sequence-directed immunity against bacteriophages can reduce conjugative gene acquisition in *S. epidermidis* (23). Other research has demonstrated that SaPI transduction to *S. epidermidis* occurs much less frequently than to *L. monocytogenes* (3).

Our results are the first conclusive evidence of the stable horizontal acquisition of virulence-associated mobile elements in an *S. epidermidis* genome. Although we have screened more than 200 other clinical *S. epidermidis* isolates, we have found no evidence of enterotoxins or SePI-like genomic elements in these other strains. In this context, the multiple insertions of chromosomal pathogenicity islands, truncated plasmids, transposases, and bacteriophage in *S. epidermidis* FRI909 are especially significant, and a closer examination of the whole genome may provide insights into the evolutionary pressures and core genomic components that have enabled the stabilization of the staphylococcal pathogenicity island SePI-1 in this genome.

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REFERENCES

1. **Ausubel, F. M., et al.** 2007. Current protocols in molecular biology, 5th ed. John Wiley and Sons, Inc., New York, NY.
2. **Baba, T., et al.** 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet **359:**1819–1827.
3. **Chen, J., and R. P. Novick.** 2009. Phage-mediated intergeneric transfer of toxin genes. Science **323:**139–141.
4. **Chi, Y. I., et al.** 2002. Zinc-mediated dimerization and its effect on activity and conformation of staphylococcal enterotoxin type C. J. Biol. Chem. **277:** 22839–22846.
5. **Crass, B. A., and M. S. Bergdoll.** 1986. Involvement of coagulase-negative staphylococci in toxic shock syndrome. J. Clin. Microbiol. **23:**43–45.
6. **Crass, B. A., and M. S. Bergdoll.** 1986. Involvement of staphylococcal enterotoxins in nonmenstrual toxic shock syndrome. J. Clin. Microbiol. **23:** 1138–1139.
7. **Cunha Mde, L., L. M. Rugolo, and C. A. Lopes.** 2006. Study of virulence factors in coagulase-negative staphylococci isolated from newborns. Mem. Inst. Oswaldo Cruz **101:**661–668.
8. **Curtin, J. J., and R. M. Donlan.** 2006. Using bacteriophages to reduce formation of catheter-associated biofilms by *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **50:**1268–1275.
9. **Daniel, A., P. E. Bonnen, and V. A. Fischetti.** 2007. First complete genome sequence of two *Staphylococcus epidermidis* bacteriophages. J. Bacteriol. **189:**2086–2100.
10. **Deringer, J. R., R. J. Ely, C. V. Stauffacher, and G. A. Bohach.** 1996. Subtype-specific interactions of type C staphylococcal enterotoxins with the T-cell receptor. Mol. Microbiol. **22:**523–534.
11. **Diep, B. A., et al.** 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. Lancet **367:**731–739.
12. **Forbes, B. A., and D. R. Schaberg.** 1983. Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. J. Bacteriol. **153:**627–634.
13. **Gal-Mor, O., and B. B. Finlay.** 2006. Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell. Microbiol. **8:**1707–1719.
14. **Gill, S. R., et al.** 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. J. Bacteriol. **187:**2426–2438.
15. **Hurdle, J. G., A. J. O'Neill, L. Mody, I. Chopra, and S. F. Bradley.** 2005. In vivo transfer of high-level mupirocin resistance from *Staphylococcus epidermidis* to methicillin-resistant *Staphylococcus aureus* associated with failure of mupirocin prophylaxis. J. Antimicrob. Chemother. **56:**1166–1168.
16. **Kreiswirth, B. N., P. M. Schlievert, and R. P. Novick.** 1987. Evaluation of coagulase-negative staphylococci for ability to produce toxic shock syndrome toxin 1. J. Clin. Microbiol. **25:**2028–2029.
17. **Kuroda, M., et al.** 2001. Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. Lancet **357:**1225–1240.
18. **Kuroda, M., et al.** 2005. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc. Natl. Acad. Sci. U. S. A. **102:**13272–13277.
19. **Li, L., C. J. Stoeckert, Jr., and D. S. Roos.** 2003. OrthoMCL: identification of ortholog groups for eukaryotic genomes. Genome Res. **13:**2178–2189.
20. **Lindsay, J. A., and M. T. Holden.** 2006. Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. Funct. Integr. Genomics **6:**186–201.
20a. **Madhusoodanan, J., K. S. Seo, J. Y. Park, A. L. Gill, J. Waterhouse, B. Remortel, G. Bohach, and S. R. Gill.** 2007. Abstr. 107th Gen. Meet. Am. Soc. Microbiol., abstr. B-420. American Society for Microbiology, Washington, DC.
21. **Maiden, M. C., et al.** 1998. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. U. S. A. **95:**3140–3145.
22. **Marr, J. C., et al.** 1993. Characterization of novel type C staphylococcal enterotoxins: biological and evolutionary implications. Infect. Immun. **61:** 4254–4262.
23. **Marraffini, L. A., and E. J. Sontheimer.** 2008. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science **322:** 1843–1845.
24. **Matthews, K. R., J. Roberson, B. E. Gillespie, D. A. Luther, and S. P. Oliver.** 1997. Identification and differentiation of coagulase-negative *Staphylococcus aureus* by polymerase chain reaction. J. Food Prot. **60:**686–688.
25. **Novick, R. P.** 1991. Genetic systems in staphylococci. Methods Enzymol. **204:**587–636.
26. **Novick, R. P.** 2003. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of *Staphylococcus aureus*. Plasmid **49:**93–105.
27. **Novick, R. P., and A. Subedi.** 2007. The SaPIs: mobile pathogenicity islands of *Staphylococcus*. Chem. Immunol. Allergy **93:**42–57.
28. **Shankar, N., A. S. Baghdayan, and M. S. Gilmore.** 2002. Modulation of virulence within a pathogenicity island in vancomycin-resistant *Enterococcus faecalis*. Nature **417:**746–750.
29. **Takeuchi, F., et al.** 2005. Whole-genome sequencing of *Staphylococcus haemolyticus* uncovers the extreme plasticity of its genome and the evolution of human-colonizing staphylococcal species. J. Bacteriol. **187:**7292–7308.
30. **Tallent, S. M., T. B. Langston, R. G. Moran, and G. E. Christie.** 2007. Transducing particles of *Staphylococcus aureus* pathogenicity island SaPI1 are comprised of helper phage-encoded proteins. J. Bacteriol. **189:**7520–7524.
31. **Ubeda, C., P. Barry, J. R. Penades, and R. P. Novick.** 2007. A pathogenicity island replicon in *Staphylococcus aureus* replicates as an unstable plasmid. Proc. Natl. Acad. Sci. U. S. A. **104:**14182–14188.
32. **Ubeda, C., et al.** 2008. SaPI mutations affecting replication and transfer and enabling autonomous replication in the absence of helper phage. Mol. Microbiol. **67:**493–503.
33. **Valle, J., S. Vadillo, S. Piriz, and E. Gomez-Lucia.** 1991. Toxic shock syndrome toxin 1 (TSST-1) production by staphylococci isolated from goats and presence of specific antibodies to TSST-1 in serum and milk. Appl. Environ. Microbiol. **57:**889–891.
34. **Weigel, L. M., et al.** 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. Science **302:**1569–1571.