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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†

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***Staphylococcus aureus* is an opportunistic pathogen and the major causative agent of numerous hospital- and community-acquired infections. *Staphylococcus epidermidis* has emerged as a causative agent of infections often associated with implanted medical devices. We have sequenced the ~2.8-Mb genome of *S. aureus* COL, an early methicillin-resistant isolate, and the ~2.6-Mb genome of *S. epidermidis* RP62a, a methicillin-resistant biofilm isolate. Comparative analysis of these and other staphylococcal genomes was used to explore the evolution of virulence and resistance between these two species. The *S. aureus* and *S. epidermidis* genomes are syntenic throughout their lengths and share a core set of 1,681 open reading frames. Genome islands in nonsyntenic regions are the primary source of variations in pathogenicity and resistance. Gene transfer between staphylococci and low-GC-content gram-positive bacteria appears to have shaped their virulence and resistance profiles. Integrated plasmids in *S. epidermidis* carry genes encoding resistance to cadmium and species-specific LPXTG surface proteins. A novel genome island encodes multiple phenol-soluble modulins, a potential *S. epidermidis* virulence factor. *S. epidermidis* contains the *cap* operon, encoding the polyglutamate capsule, a major virulence factor in *Bacillus anthracis*. Additional phenotypic differences are likely the result of single nucleotide polymorphisms, which are most numerous in cell envelope proteins. Overall differences in pathogenicity can be attributed to genome islands in *S. aureus* which encode enterotoxins, exotoxins, leukocidins, and leukotoxins not found in *S. epidermidis*.**

The staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteremia. In spite of large-scale efforts to control their spread, they persist as a major cause of both hospital- and community-acquired infections worldwide. In the hospital setting alone, they are responsible for upwards of one million serious infections per year (41). The two major opportunistic pathogens of this genus, *Staphylococcus aureus* and *Staphylococcus epidermidis*, colonize a sizable portion of the human population. The predominant species, *S. epidermidis*, is fairly widespread throughout the cutaneous ecosystem, whereas *S. aureus* is carried primarily on mucosal surfaces. Within this context, staphylococci generally have a benign symbiotic relationship with their host. However, breach of the cutaneous organ system by trauma, inoculation needles, or direct implantation of medical devices enables the staphylococci to gain entry into the host and acquire the role of a pathogen. *S. epidermidis* is primarily associated with infections of implanted medical devices, such as prosthetic heart valves and joint prostheses (49). On the other hand, *S. aureus* is a more aggressive pathogen, causing a range of acute and pyogenic infections, including abscesses, bacteremia, central nervous system infections, endocarditis, osteomyelitis, pneumonia, urinary tract infections, chronic lung infections associated with cystic fibrosis, and several syndromes caused by exotoxins and enterotoxins, including food poisoning and scalded skin and toxic shock syndromes (32, 41).

Successive acquisition of resistance to most classes of antimicrobial agents, such as penicillins, macrolides, aminoglycosides, chloramphenicol, and tetracycline has made treatment and control of staphylococcal infections increasingly difficult. The widespread use of methicillin and other semisynthetic penicillins in the late 1960s led to the emergence of methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE), which continue to persist in both the health care and community environments (45). Currently, greater than 60% of *S. aureus* isolates are resistant to methicillin and some strains have de-

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† Supplemental material for this article may be found at http://jb.asm.org/.

veloped resistance to more than 20 different antimicrobial agents (40). The remaining effective therapy against most strains of multidrug-resistant staphylococci, including MRSA and MRSE, is the glycopeptide antibiotic vancomycin (51). However, the emergence in 1997 (6) of *S. aureus* with intermediate levels of resistance to vancomycin (vancomycin-intermediate *S. aureus*) and the most recent emergence of *S. aureus* with high levels of resistance to vancomycin (vancomycin-resistant *S. aureus*) (7) has limited its effectiveness. Finally, the increasing incidence of hypervirulent community-acquired *S. aureus* (45, 48) has become a major concern to the global health community and reinforced the critical need for new methods of control and treatment.

We have determined the complete genome sequences of *S. aureus* COL, an early MRSA isolate, and *S. epidermidis* RP62a, an MRSE biofilm-producing clinical isolate. Comparison of these genomes with other sequenced staphylococcal genomes provides insights into genome features that contribute to increasing pathogenicity in *S. aureus* and has led to the identification of novel genome islands in *S. epidermidis* that may contribute to the evolution of this species from a commensal pathogen to a more aggressive pathogen.

MATERIALS AND METHODS

Bacterial sources. *S. aureus* COL was obtained from Brian Wilkinson (Illinois State University), who has maintained the culture as a frozen stock since 1976. The COL strain was reportedly isolated as a penicillinase-negative strain in the early 1960s from the operating theatre in a hospital in Colindale, England (17, 43). COL was one of the first MRSA isolates to be identified and has been used extensively in biochemical investigations of methicillin and vancomycin resistance (16).

S. epidermidis RP62a (ATCC 35984) is a slime-producing strain isolated during the 1979 to 1980 Memphis, Tennessee, outbreak of intravascular catheter-associated sepsis (9, 10). RP62a is capable of accumulated growth and subsequent biofilm formation, which contribute to its pathogenicity in foreign-body infections (22).

Other strains used for comparative genomic analyses are *S. aureus* Mu50 (28), N315 (28), and MW2 (3) and *S. epidermidis* ATCC 12228 (54). Mu50 is a clinical MRSA strain isolated in 1996 from a Japanese patient with infection of a surgical incision site which was resistance to vancomycin therapy (19, 20). N315 is a Japanese clinical MRSA isolate identified in 1982 (28, 37). MW2 is a highly virulent community MRSA strain isolated in 1998 from a 16-month-old girl in North Dakota and initially associated with four pediatric deaths in Minnesota and North Dakota (3, 5). *S. epidermidis* ATCC 12228 is a non-biofilm-forming reference strain also isolated in the United States (2, 54).

Genome sequencing, assembly, and ORF prediction. *S. aureus* strain COL and *S. epidermidis* strain RP62a were sequenced to closure by the random shotgun method, with cloning, sequencing, and assembly completed as described previously for genomes sequenced at The Institute for Genomic Research (TIGR) (39). One small-insert plasmid library (2.0 to 3.0 kb) and one medium-insert plasmid library (10 to 12 kb) was constructed for each strain by random mechanical shearing and cloning of genomic DNA. In the initial random-sequencing phase, eightfold sequence coverage was achieved from the two libraries (one sequenced to fivefold coverage and the other sequenced to threefold coverage). The sequences from the respective strains were assembled separately with TIGR Assembler or Celera Assembler (www.tigr.org). All sequence and physical gaps were closed by editing the ends of sequence traces, primer walking on plasmid clones, and combinatorial PCR, followed by the sequencing of the PCR product.

An initial set of open reading frames (ORFs) that likely encode proteins was identified with GLIMMER (14), and those shorter than 90 bp as well as some of those with overlaps were eliminated. A region containing the likely origin of replication was identified, and bp 1 was designated adjacent to the *dnaA* gene, located in this region. All ORFs were searched against a nonredundant protein database as previously described (39). Frameshifts and point mutations were detected and corrected where appropriate. The remaining frameshifts and point mutations are considered authentic, and the corresponding regions were annotated as authentic frameshift or authentic point mutation, respectively. The ORF prediction and gene family identifications were completed by methodology described previously (39). Two sets of hidden Markov models (HMMs) were used to determine ORF membership in families and superfamilies. These included 721 HMMs from Pfam, version 2.0, and 631 HMMs from the TIGR ortholog resource. TMHMM (27) was used to identify membrane-spanning domains in proteins.

Comparative genomics. For the identification of species-specific and strain-specific genes, all predicted ORFs from the TIGR-sequenced staphylococcal genomes (*S. aureus* COL and *S. epidermidis* RP62a) and published staphylococcal genomes (*S. aureus* N315 and Mu50 (28), *S. aureus* MW2 (3), and *S. epidermidis* ATCC 12228 (54) were searched against an in-house database composed of 195 prokaryotic, 8 eukaryotic, 175 phage, 63 virus, and 46 plasmid genomes with WU-BLASTP (http://BLAST.wustl.edu). Those genes that matched a non-self genomic sequence at a P value of $\leq 10^{-5}$, an identity of $\geq 35\%$, and match lengths of at least 75% of the length of both query and subject sequences were considered nonunique. These comparisons were used to generate match tables (see Supplemental Table 6 [http://www.tigr.org/tdb/staphylococcus; all supplemental tables are at this website]). Single nucleotide polymorphisms (SNPs) were identified by comparing the genome of *S. aureus* COL to those of *S. aureus* N315, Mu50, and MW2 and by comparing the genome of *S. epidermidis* RP62a to that of *S. epidermidis* ATCC 12228 with MUMer (15). Because we did not have access to underlying sequence data for published staphylococcal genomes, identification of SNPs was based on the final draft sequence. By mapping the position of the SNP to the annotation in the *S. aureus* COL and *S. epidermidis* RP62a genomes, it was possible to determine the location of the SNP (intergenic versus intragenic) and its effect on the deduced polypeptide (synonymous versus nonsynonymous). For each deduced polypeptide, the degree of relatedness across strains was calculated by using a BLAST score ratio. The BLASTP raw score was obtained for the alignment against itself (REF_SCORE) and the most similar protein in the query strains (QUE_SCORE). Scores were normalized by dividing the QUE_SCORE for each query genome by REF_SCORE. Normalized scores were plotted as *xy* coordinates.

A comparative database of all staphylococcal ORFs was generated for position effect determination by identifying all matches among the six sequenced genomes by a BLAST-Extend-Repraze (BER) search ($P < 0.1$; bit score > 50). These BER matches were then run through Position Effect software (TIGR) to determine conservation of gene order. The query and hit genes from each match were defined as anchor points in gene sets composed of adjacent genes, with up to 10 genes upstream and downstream from each anchor gene used in creating the gene sets. An optimal alignment between the ordered gene sets was calculated by using percent similarity from BER and applying a linear gap penalty of 100. Positive-scoring optimal alignments containing gene sets of four or more matching genes were stored in the database.

Nucleotide sequence accession numbers. Nucleotide sequences for *S. aureus* COL (accession numbers CP000046 for the chromosome sequence and CP000045 for the plasmid sequence) and *S. epidermidis* (accession numbers CP000029 for the chromosome sequence and CP000028 for the plasmid sequence) have been deposited at GenBank. The genome sequences and the annotation of the TIGR-sequenced strains are available in the TIGR Comprehensive Microbial Resource at www.tigr.org. The *S. aureus* COL SAXXXX and *S. epidermidis* RP62a SEXXXX locus numbers are listed as SACOLXXXX and SERPXXXX, respectively, in GenBank.

RESULTS AND DISCUSSION

S. aureus is one of the leading causes of infectious disease in hospital settings and, recently, is an increasing cause of disease in the community (45, 48). Since the emergence of MRSA in the 1970s, *S. aureus* has continued to acquire additional antimicrobial resistance factors to the point where some isolates are resistant to more than 20 different antimicrobial agents (40). Development of antimicrobial resistance factors along with additional virulence factors and their movement through this species have likely occurred through gene transfer mediated by mobile genome islands, bacteriophage, plasmids, transposons, and insertion sequences (IS). The most recent example of such gene movement is the acquisition of the *Enterococcus faecalis* Tn*1546* vancomycin resistance element by plasmid-mediated transfer into *S. aureus* (52). *S. epidermidis*, the less-virulent member of this genus frequently associated with hospital-acquired and biomedical device infections, has also

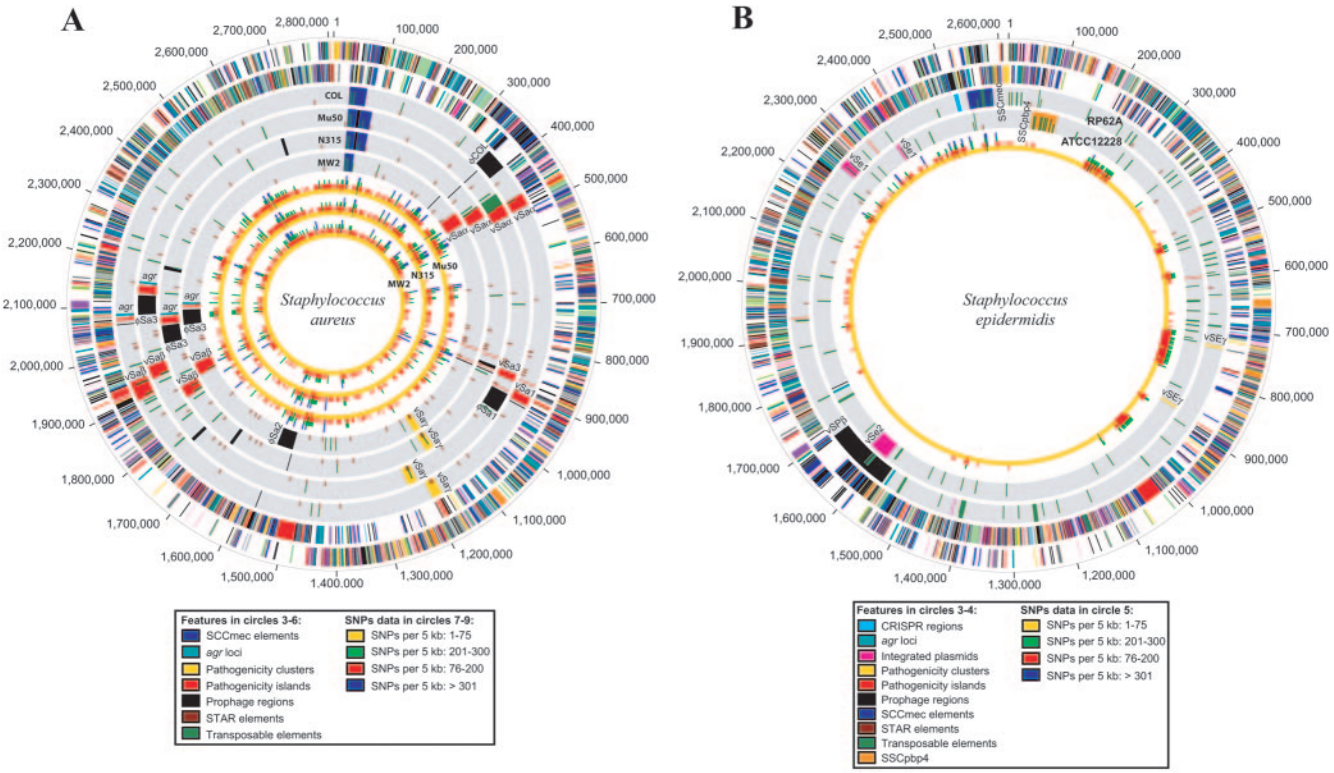

FIG. 1. Circular representation of the sequenced *S. aureus* and *S. epidermidis* genomes. Each concentric circle represents genomic data for *S. aureus* (A) and *S. epidermidis* (B) and is numbered from the outermost circle to the innermost circle. The outermost circles indicate the genome coordinates in base pairs. The second and third circles represent the predicted *S. aureus* COL and *S. epidermidis* RP62a ORFs on the plus and minus strands, respectively, colored by role categories: salmon, amino acid biosynthesis; light blue, biosynthesis of cofactors and prosthetic groups and carriers; light green, cell envelope; red, cellular processes; brown, central intermediary metabolism; yellow, DNA metabolism; green, energy metabolism; purple, fatty acid and phospholipid metabolism; pink, protein fate and synthesis; orange, purines, pyrimidines, nucleosides, and nucleotides; blue, regulatory functions; grey, transcription; teal, transport and binding proteins; black, hypothetical and conserved hypothetical proteins. The fourth (strain COL), fifth (strain Mu50), sixth (strain N315), and seventh (strain MW2) circles in *S. aureus* and the fourth (strain RP62a) and fifth (strain ATCC 12228) circles in *S. epidermidis* indicate genome islands involved in virulence (red or yellow), regulatory loci (*agr*) (blue-green), prophage (black), SSC*mec* (blue), SSC*pbp4* (orange), integrated plasmids (pink), STAR elements (brown), transposable elements (dark green), and CRISPR regions (light blue). The 8th (strain Mu50), 9th (strain N315), and 10th (strain MW2) circles in *S. aureus* and the sixth (strain ATCC 12228) circle in *S. epidermidis* represent the number of SNPs per 5 kb compared to *S. aureus* strain COL and *S. epidermidis* strain RP62a. Gold ticks, 1 to 75 SNPs; red ticks, 76 to 200 SNPs; dark green ticks, 201 to 300 SNPs; blue ticks, more than 301 SNPs. Complete DNA sequence and annotation for *S. aureus* MW2, N315, and Mu50 and *S. epidermidis* ATCC 12228 were obtained from GenBank accession numbers BA000033, BA000018, BA000017, and AE015929, respectively.

acquired multiple resistance factors through similar processes. It is likely that gene transfer among multiple members of the staphylococcal species is a frequent event, allowing for adaptation to shifting host environments.

Genome features and islands. General genome features of *S. aureus* COL and *S. epidermidis* RP62a, along with those of *S. aureus* N315 (28), Mu50 (28), and MW2 (3) and *S. epidermidis* ATCC 12228 (54) are presented in Supplemental Table 1 (http://www.tigr.org/tdb/staphylococcus). The genome sequences of two clinical *S. aureus* isolates, MSSA476 and MRSA252, were published (21) just prior to submission of the manuscript and were not included in our whole-genome comparisons. Significant aspects of MSSA476 and MRSA252 are, however, included within the following results and discussion. Whole-genome analysis indicated that the genomes of *S. aureus* and *S. epidermidis* are syntenic throughout a well-conserved core region (data not shown), with differences the result of genomic elements including genome islands (νSa, νSe, SSC*mec*, and staphylococcus cassette chromosome [SSC]-like elements), integrated prophage, IS elements, composite transposons, and integrated plasmids (Table 1) which are associated with disease and virulence. These genomic elements make up approximately 7% of the *S. aureus* COL genome and 9% of the *S. epidermidis* RP62a genome, percentages that are similar to those for other gram-positive pathogens, such as group A streptococcus (~10%) (4) but lower than that for *Enterococcus faecalis* (25%) (39).

Seven pathogenicity genomic islands (νSa), in positions conserved across all sequenced genomes, have been identified in *S. aureus* (Table 1 and Fig. 1). These islands carry approximately one-half of the *S. aureus* toxins or virulence factors, and allelic variation of these genes, along with presence or absence of individual νSa, contributes to the pathogenic potential of this species (Table 2). For example, island νSa3 is unique to *S. aureus* MW2 and carries allelic forms of enterotoxin genes *sel2* and *sec4*, which may contribute to its increased virulence. On the other hand, *S. aureus* MRSA252 (21) has a novel island, SaPI4, that contains homologs of pathogenicity proteins found

TABLE 1. Genomic islands in six sequenced staphylococcal genomes

Inland	Genes found on island[b]	Type, location of island (predicted integration sequence[a]) for:						Allele family member(s)
		S. aureus				*S. epidermidis*		
		COL	Mu50	N315	MW2	RP62A	ATCC 12228	
vSa1	Enterotoxin genes (*seb*, *tsst*, *ear*)	903332–919283 (A)	NP	NP	NP	NP	NP	SaPI1, SaPI3
vSa2	*sec*, *tsst*	NP	NP	NP	NP	NP	NP	SaPIbov
vSa3	Type I, *fhuD*; type II, *sel2*, *sec4*, *ear*	NP	I, 868373–882872 (B)	NP	II, 839358–853808 (B)	NP	NP	SaPI3
vSa4	Type I, *sel*, *sec3*, *tsst*; type II, four unknown ORFs	II, 2072899–2076041 (C)	I, 2148912–2133235 (D)	I, 2056679–2072358 (D)	II, 2097809–2100950 (C)	NP	NP	SaPI2
vSaα	Type I, *set6–15*, *lpl1–9*; type II, *set16–26*, *lpl10–14*, type III, *set1–5*, *lpl2,7,8,11,13*	III, 465424–489723	I, 461919–491326	I, 436162–466813	II, 416307–452099	NP	NP	
vSaβ	Type I, *splA–F*, *lukDE*, *ear*, epidermin gene cluster; type II, *splA–F*, *lukDE*, *ear*, *seg*, *sen*, *sei*, *sem*, *seo*, epidermin gene cluster	I, 1902466–1938731	II, 1932523–1961464	I, 1854608–1881615	I, 1890800–1922552	NP	NP	
vSaγ	*set*, *eta*, *psm*β	1173206–1193358	1208629–1230219	1132235–1153775	1133469–1153549	NP	NP	
vSeγ	*psm*β					734975–737635	840908–843572	
SCC*mec*	*mecA*, *ermA*, *bleO*, *addD*	I, 68085–34173 (E)	II, 87085–34158 (E)	II, 87119–34153 (E)	IV, 58278–34150 (E)	II, 2536574–2584194 (F)	NP	Types I, II, III, IVa, IVb
SSC*pbp4*	*pbp4*, *merT*, *merB*, *merA*, *tagF*	NP	NP	NP	NP	NP	32031–99960 (G)	
ϕSa1	72 ORFs	NP	917453–962005 (H)	NP	NP	NP	NP	ϕ11, ϕETA
ϕSa2	*lukS-PV*, *lukF-PV*	NP	NP	NP	1575042–1529123 (I)	NP	NP	ϕSLT
ϕSa3	*sak*, *sea*, *sep*, *seg2*, *sek2*	NP	2126304–2083238 (J)	2049591–2005321 (J)	2088820–2046205 (J)	NP	NP	ϕ42
ϕCOL	Integrated in *geh*	354674–398267 (K)	NP	NP	NP	NP	NP	ϕL-54
ϕSPβ	Integrated in *yeeE*, LPXTG surface protein gene	NP	NP	NP	NP	1567637–1694334 (L)	NP	
Tn*5801*	*tetM*, two unknown ORFs	NP	I, 436002–461791 (M)	NP	II, 415784–417798 (M)	NP	NP	
vSe1	Type I, *cadCD*; type II, one unknown ORF	NP	NP	NP	NP	I, 2250348–2267496 (N)	II, 2255537–2259992 (N)	
vSe2	*srtA*, two LPXTG surface protein genes	NP	NP	NP	NP	NP	1558081–1519667 (O)	

[a] A, TTATTCCTGCTAAATAA; B, TCCCGCCGTCTCCAT; C, GTTTTACATCATTCC[CT]GGCAT; D, GTTTTACATCATTCCCGGCAT; E, TTATGATA[CA]GC[CT]TCT; F, TTATGATA[TA]GCTTCT; G, TCATTTATGATATGCTTCT; H, TCGAAATGGAAGGTAGTATTGGATAGCTTTAAAC[CA][CG]CGTTGTTAAGCCATTCTTGACTTCCGGAAATGG[CG]TAT; I, AAATAAACATATC[GA]TCATAATGTGATGGT; J, TGTATCCAAACTGG; K, ATCATACAAGGATGGGAT; L, CAATGCATAAAA; M, GAGTGGGAATA; N, CTAAATATTTTC; O, CGGAGAGTGAGGGAT. NP, not present.

[b] Gene symbols: sortase, *srtA*; cadmium efflux, *cadCD*; lipase, *geh*; staphylococcal kinase, *sak*; β-lactamase, *ear*; ferric hydroxamate uptake, *fhuD*; leukotoxin, *lukDE*; tandem lipoprotein, *lpl*; PSM beta subunit, *psm* β; staphylococcal entertoxins, *sec3*, *sec4*, *seg*, *seg2*, *sea*, *sek2*, *sel*, *sel2*, *sem*, *sen*, *seo*, *sep*; serine protease, *splA–F*; toxic shock syndrome toxin, *tsst*; panton-valentine leukocidin components S and F, *lukS-PV*, *lukF-PV*; methicillin resistance, *mecA*; penicillin binding protein *4*, *pbp4*; *ermA*, *bleO*, *addD*, *tetM*, *yeeE*; alkytmercury lyase, *merB*; mercury transport protein, *merT*; mercuric reductase, *merA*; teichoic acid biosynthesis protein F, *tagF*.

TABLE 2. Virulence factors and genomic islands in six sequenced staphylococcal genomes

Virulence factor	*S. aureus*								*S. epidermidis*			
	COL		Mu50		N315		MW2		RP62a		ATCC 12228	
	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island
Enterotoxin	SA1657 *sea*		SAV2009 *sec3*	vSa4	SA1430 *sea*[d]		MW1889 *sea*	ϕSa3				
	SA0907 *seb*	vSa1	SAV1824 *seg*	vSaβ	SA1817 *sec3*	vSa4	MW0759 *sec2*	vSa3				
	SA0887 *sei*	vSal	SAV1828 *sei*	vSaβ	SA1642 *seg*	vSaβ	MW1937 *seg*	ϕSa3				
	SA0886 *sek*	vSal	SAV2008 *sel*	vSa4	SA1646 *sei*	vSaβ	MW0051 *seh*					
			SAV1829 *sem*	vSaβ	SA1816 *sel*	vSa4	MW0760 *sel*	vSa3				
			SAV1825 *sen*	vSaβ	SA1647 *sem*	vSaβ	MW1938 *sek*	ϕSa3				
			SAV1830 *seo*	vSaβ	SA1643 *sen*	vSaβ	MW0052 *seo*[d]					
			SAV1948 *sep*	ϕSa3	SA1648 *seo*	vSaβ						
			SAV1601 *sep*		SA1761 *sep*	ϕSa3						
			SAV1827 *seu*	vSaβ	SA1645 *yent1*	vSaβ						
					SA1644 *yent2*	vSaβ						
Exotoxin	SA1178 *set1*	vSaγ	SAV0422 *set6*	vSaα	SA1009 *set1*	vSaγ	MW1047 *set1*	vSaγ				
	SA0469 *set1*	vSaα	SAV0423 *set7*	vSaα	SA0357 *set2*		MW1048 *set4*	vSaγ				
	SA0468 *set3*	vSaα	SAV0424 *set8*	vSaα	SA1011 *set3*	vSaγ	MW0382 *set16*	vSaα				
	SA0478 *set3*	vSaα	SAV0425 *set10*	vSaα	SA1010 *set4*	vSaγ	MW0383 *set17*	vSaα				
	SA1180 *set3*	vSaγ	SAV0426 *set11*	vSaα	SA0382 *set6*	vSaα	MW0384 *set18*	vSaα				
	SA0474 *set4*	vSaα	SAV0427 *set12*	vSaα	SA0383 *set7*	vSaα	MW0385 *set19*	vSaα				
	SA1179 *set4*	vSaγ	SAV1168 *set12*	vSaγ	SA0384 *set8*	vSaα	MW0386 *set20*	vSaα				
	SA0473 *set5*	vSaα	SAV0428 *set13*	vSaα	SA0385 *set9*	vSaα	MW0387 *set21*	vSaα				
	SA0470 *set*[d]	vSaα	SAV0429 *set14*	vSaα	SA0386 *set10*	vSaα	MW0388 *set22*	vSaα				
	SA0472 *set*[d]	vSaα	SAV0433 *set15*	vSaα	SA0387 *set11*	vSaα	MW0389 *set23*	vSaα				
					SA0388 *set12*	vSaα	MW0390 *set24*	vSaα				
					SA0389 *set13*	vSaα	MW0391 *set25*	vSaα				
					SA0390 *set14*	vSaα	MW0394 *set26*	vSaα				
					SA0393 *set15*	vSaα	MW0345 *set*[d]					
Exfoliative toxin	SA1184 *et*[d]	vSaγ	SAV1173 *eta*	vSaγ	SA1016 *eta*	vSaγ	MW1054 *eta*	vSaγ				
Toxic shock syndrome toxin			SAV2011 *tsst*	vSa4	SA1819 *tsst*	vSa4						
Esterase	SA2345		SAV2350[d]		SA2140[d]		MW2271[d]		SE1941		SE1929	
	SA2549[d]		SAV2535[d]		SA2323[d]		MW2456[d]		SE2109[d]		SE2095	
Serine protease	SA1869 *splA*	vSaβ	SAV1813 *splA*	vSaβ	SA1631 *splA*	vSaβ	MW1755 *splA*	vSaβ				
	SA1868 *splB*	vSaβ	SAV1812 *splB*	vSaβ	SA1630 *splB*	vSaβ	MW1754 *splB*	vSaβ				
	SA1867 *splC*	vSaβ	SAV1811 *splC*	vSaβ	SA1629 *splC*	vSaβ	MW1753 *splC*	vSaβ				
	SA1866 *splD*	vSaβ	SAV1810 *splD*	vSaβ	SA1628 *splD*	vSaβ	MW1752 *splF*	vSaβ				
	SA1865 *splE*	vSaβ	SAV1809 *splF*	vSaβ	SA1627 *splF*	vSaβ	MW0903 *htrA*	vSaβ				
	SA1864 *splF*	vSaβ	SAV1023 *htrA*		SA0879 *htrA*		MW1670 *htrA*					
	SA1028 *htrA*								SE2390 *htrA*		SE0722 *htrA*	
	SA1777 *htr*[d]								SE2401		SE0723	
Staphylokinase			SAV1944	ϕSa3	SA1758	ϕSa3	MW1885	ϕSa3				
Serine V8 protease	SA1057 *sspA*		SAV1048 *sspA*		SA0901 *sspA*		MW0932 *sspA*		SE1397 *sspA*		SE1543 *sspA*	
Cysteine protease	SA1056 *sspB*		SAV1047 *sspB*		SA0900 *sspB*		MW0931 *sspB*		SE2390 *sspB*		SE0184 *sspB*	
	SA1970 *sspB*		SAV1046 *sspC*		SA0899 *sspC*		MW0930 *sspC*		SE2391 *sspC*		SE0183 *sspC*	
	SA1055 *sspC*											
Lipase	SA2694 *lip*		SAV2671 *lip*		SA2463 *lip*		MW2590 *lip*		SE2336 *lip*		SE0245 *lip*	
	SA0317 *geh* (amino)[a]		SAV0320 *geh*		SA0309 *geh*		MW0297 *geh*		SE0018 *geh*		SE2403 *geh*	
	SA0390 *geh* (carboxy)[a]								SE2297 *geh1*, *gehC*		SE0281 *geh1*, *gehC*	
									SE2388 *geh2*, *gehD*		SE0185 *geh2*, *gehD*	
Lipase/esterase	SA0712 *lipA*[d]		SAV0655 *lipA*[d]		SA0610 *lipA*[d]		MW0617 *lipA*[d]		SE0309 *lipA*		SE0424 *lipA*	
Extracellular elastasc precursor									SE2252 *sepA*		SE2219 *sepA*	
Leukotoxin D	SA1880 *lukD*	vSaβ	SAV1819 *lukD*	vSaβ	SA1637 *lukD*	vSaβ	MW1767 *lukD*	vSaβ				
Leukotoxin E	SA1881 *lukE*	vSaβ	SAV1820 *lukE*	vSaβ	SA1638 *lukE*	vSaβ	MW1768 *lukE*	vSaβ				
Synergohymenotropic toxin							MW1379 *lukSPV*	ϕSa2				
Leukocidin F					SA1812 *lukF*		MW1378 *lukFPV*	ϕSa2				
Leukocidin M	SA2006 *lukM*				SA1813 *lukM*		MW1942 *lukM*					
Alpha hemolysin	SA1173 *hly*	vSaγ	SAV1163 *hly*	vSaγ	SA1007 *hly*	vSaγ	MW0955 *hly*	vSaγ				
Beta hemolysin	SA2003 *hlb*		SAV2003 *hlb*[e]		SA1752 *hlb*[e]		MW1940 *hlb*[e]		SE2544 *hlb*		SE0008 *hlb*	
Delta hemolysin	SA2022 *hld*		SAV2035 *hld*		SAS065 *hld*		MW1959 *hld*		SE1489 *hld*		SE1634 *hld*	
Gamma hemolysin, component A	SA2419 *hlgA*		SAV2419 *hlgA*		SA2207 *hlgA*		MW2342 *hlgA*					
Gamma hemolysin, component C	SA2421 *hlgC*		SAV2420 *hlgC*		SA2208 *hlgC*		MW2343 *hlgC*					
Gamma hemolysin, component B	SA2422 *hlgB*		SAV2421 *hlgB*		SA2209 *hlgB*		MW2344 *hlgB*					
Hemolysin III	SA2160[d]		SAV2170		SA1973[d]		MW2096[d]		SE1769[d]		SE1760[d]	
Hemolysin	SA0762[d]		SAV0919		SA0780[d]		MW0664[d]		SE2258[d]		SE2226[d]	
Hyaluronate lyase	SA2194 *hysA*		SAV2202 *hysA*		SA2003 *hysA*		MW2129 *hysA*					
Thermonuclease nuclease	SA1357 *nuc*		SAV1324 *nuc*		SA1160 *nuc*		MW1211 *nuc*		SE0891 *nuc*		SE1004 *nuc*	
	SA0860		SAV0815		SA0746		MW0769		SE1570	ϕSPβ		
Cell wall hydrolase	SA1264 *lytN*		SAV1247 *lytN*		SA1090 *lytN*		MW1130 *lytN*					
Zinc metalloprotense	SA1281		SAV1262		SA1105		MW1145		SE0829[d]		SE0938[d]	
Clp protease, procolytic subunit	SA0833 *clpP*		SAV0768 *clpP*		SA0723 *clpP*		MW0730 *clpP*		SE0436 *clpP*		SE0551 *clpP*	

Continued on facing page

TABLE 2—*Continued*

Virulence factor	*S. aureus*								*S. epidermidis*			
	COL		Mu50		N315		MW2		RP62a		ATCC 12228	
	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island	Locus and gene	Island
Clp protease, ATP binding subunit	SA0979 *clpB*		SAV0975 *clpB*		SA0835 *clpB*		MW0857 *clpB*		SE0564 *clpB*		SE0674 *clpB*	
Clp protease, ATP binding subunit	SA1721 *clpX*		SAV1674 *clpX*		SA1498 *clpX*		MW1618 *clpX*		SE1238 *clpX*		SE1349 *clpX*	
Clp protease, ATP binding subunit	SA0570 *clpC*		SAV0523 *clpC*		SA2336 *clpC*		MW2469 *clpC*		SE0165 *clpC*		SE0287 *clpC*	
Staphylococcal protein A Spa	SA0095 *spa*		SAV0111 *spa*		SA0107 *spa*		MW0084 *spa*					
Phenol-soluble modulin	SA1186 *beta*	vSaγ	NT02SA1161 *beta*[b]	vSaγ	NT01SA1111 *beta*[b]	vSaγ	MW1056 *beta*	vSaγ	SE0736 *beta1*	vSeγ	SE0846 *beta1*	vSeγ
	SA1187 *beta*	vSaγ	NT02SA1162 *beta*[b]	vSaγ	NT01SA1112 *beta*[b]	vSaγ	MW1057 *beta*	vSaγ	SE0737 *beta1*	vSeγ	SE0847 *beta1*	vSeγ
	SA2022 *hld*[c]		SAV2035 *hld*[c]		SAS065 *hld*[c]		MW2121 *hld*[c]		SE0738 *beta1*	vSeγ	SE0848 *beta1*	vSeγ
									SE0739 *beta2*	vSeγ	SE0849 *beta2*	vSeγ
									SE2397 *beta1*		SE0177 *beta*	
									SE2400 *beta1*		SE0174 *beta1*	
									SE0083 *alpha*			
									SE1489 *delta*[c]		SE1634 *delta*[c]	

[a] geh in COL is disrupted by insertion of ϕCOL near the carboxy terminus of the protein.
[b] Not in original annotation. NT02SA1161, NT025A1162, NT01SA1111, and NT01SA1112 were identified by TIGR annotation.
[c] Also delta hemolysin.
[d] Putative.
[e] Truncated.

in previously characterized νSa1 and νSa2 islands (Table 1) but does not carry known virulence genes. Our analysis identified a novel genomic island, νSaγ (νSeγ), that is found in all *S. aureus* and *S. epidermidis* genomes (Table 1 and Fig. 2). The *S. epidermidis* νSeγ allele contains genes for a cluster of four members of the phenol-soluble modulin (PSM) family, a potential virulence factor of *S. epidermidis* (38, 50). The *S. aureus* νSaγ allele contains a cluster of two PSM genes and a small secondary cluster of exotoxin genes similar to those in νSaα. Our analysis of *S. epidermidis* RP62a and ATCC 12228 also identified two integrated plasmids, νSe1 and νSe2 (Table 1 and Fig. 2; Supplemental Table 2), which contain prophage integrase genes in a structure similar to that for *S. aureus* genome islands. While neither νSe1 or νSe2 carries virulence factors found in *S. aureus*, the νSe1 island in RP62a contains genes for cadmium resistance and the νSe2 island in ATCC 12228 encodes a second strain-specific sortase (encoded by *srtC*) not found in other staphylococci and two strain-specific LPXTG cell surface attachment proteins with likely roles in adhesion to host tissue (Supplemental Table 2).

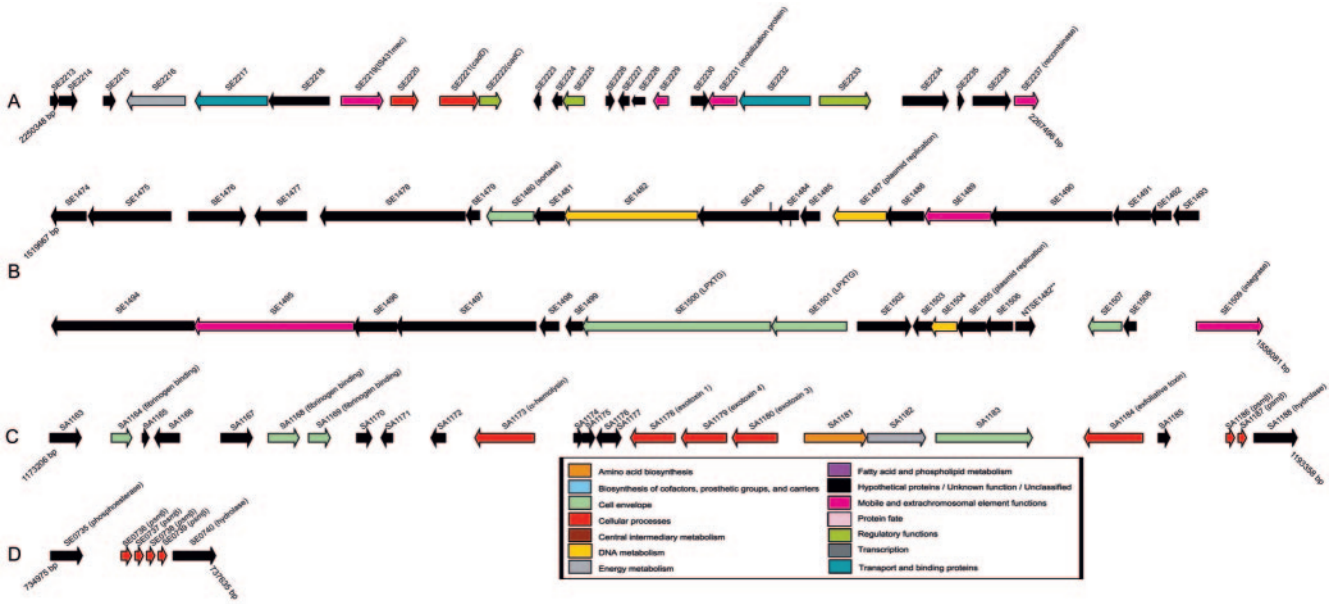

FIG. 2. Novel integrated plasmids and genome islands in *S. aureus* and *S. epidermidis*. Shown are the schematic diagrams of integrated plasmids of νSe1 in *S. epidermidis* RP62a (A), νSe2 in *S. epidermidis* ATCC 12228 (B), νSaγ in *S. aureus* COL (C), and νSeγ in *S. epidermidis* RP62a (D). ORFs are marked in the direction of transcription as arrows and are colored according to functional categories as indicated. Positions within the respective genomes are indicated as genome coordinates on the ends of each schematic. Putative functions of selected ORFs accompany the gene locus number. Putative functions of all ORFs and their locus numbers are presented in Supplementary Table 2.

Five types of integrated prophage were identified, with at least one phage in every genome except that of *S. epidermidis* ATCC 12228 (Table 1 and Fig. 1). In *S. aureus* COL, a L54-like phage (30), which we have named ϕCOL, was integrated near the 3′ end of the lipase gene (*geh*). The ϕSa3 phage, which is integrated into the beta-hemolysin gene (*hlb*) of *S. aureus* N315, Mu50, MW2, MRSA252, and MSSA476, was not found in *S. aureus* COL. A single *Bacillus subtilis* ϕSPβ-like phage (29) was identified in *S. epidermidis* RP62a (Table 1; Supplemental Table 3; see Fig. S1 in the supplemental material), where it is inserted in *att* sites within *yeeE*. Comparative genome hybridization of multiple *S. epidermidis* clinical isolates (S. Gill, unpublished data) shows that acquisition of the ϕSPβ-like phage is unique to RP62a and likely a recent event. The ϕSPβ-like phage is a mosaic structure carrying multiple staphylococcal IS elements and genes encoding a staphylococcal nuclease and an RP62a-specific LPXTG surface protein, indicating that multiple recombination events have likely occurred following entry of the phage into RP62a.

Three types of SCC*mec* islands (types I, II, and IVa) (23, 24) were previously identified among the *S. aureus* COL, N315, Mu50, and MW2 genomes (Table 1; Supplemental Table 4; see Fig. S2 in the supplemental material). The SSC*mec* islands are characterized by a set of site-specific recombinase genes (*ccrA* and *ccrB*) which promote site-specific integration into an *att* site within *orfX* and a *mecA* gene which encodes resistance to methicillin (1, 23). Our analysis of *S. epidermidis* RP62a identified a type II SSC*mec* which is 98% identical at the nucleotide level and identical in gene organization (with the exception of the region from pUB110 flanked by IS*431mec)* to that of the *S. aureus* type II SSC*mec*. Acquisition of additional transposon and IS elements, such as Tn*554*, by SSC*mec* types II and III corresponds with the need for *S. aureus* to survive the increased use of antibiotics in clinical environments. Previous structural analysis of identified SSC*mec* elements suggests that the *ccrAB* genes may form an independent mobile SSC element that mediates staphylococcal interspecies transfer of antimicrobial or virulence genes (25, 26). Our analysis of *S. epidermidis* ATCC 12228 has identified such an SSC element, named SSC*pbp4* (also identified by Mongkolrattanothai et al. [34]), which lacks *mec* but which contains two pairs *of ccrA1* and *ccrB* genes along with multiple IS elements, a restriction-modification system (*hsdS* and *hsdM*), and genes encoding penicillin binding protein 4 (*pbp4*) and resistance to mercury and cadmium (see Fig. S1 in the supplemental material). The presence of two *ccrAB* pairs and multiple putative *att* sites in addition to *orfX* suggests that SSC*pbp4* is the result of two independent insertion events. The existence of SSC*pbp4* in *S. epidermidis* and a novel SSC*mec*-like element (SSC*far)* in the genome of MSSA476 (21) suggests that similar SSC elements capable of transferring virulence factors between *S. aureus* and *S. epidermidis* may already exist within these species.

The seven types of IS elements identified in *S. aureus* and *S. epidermidis* are randomly distributed throughout their genomes (Supplemental Table 1; Fig. 1). A new staphylococcal IS element, IS*Sep1*, was identified in both *S. epidermidis* genomes. Composite transposons Tn*554* and Tn*4001* were identified in *S. aureus* N315 and Mu50 and *S. epidermidis* RP62a, respectively, but not in *S. epidermidis* ATCC 12228 or *S. aureus* COL.

Multiple copies of the GC-rich STAR (*S. aureus* repeat element) signature sequence (11) were found dispersed throughout intergenic regions of the *S. aureus* and *S. epidermidis* genomes (Fig. 1). STAR elements are more abundant in *S. aureus*, but in neither species are they associated with regions of atypical genome composition or with predicted mobile genes. A single copy of the extragenic CRISPR (clustered regularly interspaced palindromic repeats) DNA repeat element (20, 21) was identified near the *dnaA* gene at the replication origin of the *S. epidermidis* RP62a genome (Supplemental Table 5; Fig. 1). CRISPRs were not identified in *S. epidermidis* ATCC 12228 or in the *S. aureus* genomes.

Comparative genomics and evolution of virulence. A comparison of the six staphylococcal genomes against each other revealed (i) a total of 454 species-specific genes that are common to the *S. aureus* COL, N315, Mu50, and MW2 genomes but not found in *S. epidermidis* RP62a or ATCC 12228, (ii) a total of 286 species-specific genes that are common to the *S. epidermidis* RP62a and ATCC 12228 genomes but not found in the *S. aureus* genomes, (iii) 332 strain-specific genes that are found in *S. epidermidis* ATCC 12228 but not in *S. epidermidis* RP62a, and (iv) 346 strain-specific genes that are found in *S. epidermidis* RP62a but not in *S. epidermidis* ATCC 12228 (Supplemental Table 6). A core set of 1,681 genes common among all strains and both species was also identified (Supplemental Table 6). The majority of the unique genes can be accounted for by the presence or absence of prophage and genomic islands. For example, the 127-kb ϕSPβ-like prophage in *S. epidermidis* RP62a (Table 1; Fig. 1; see Fig. S1 in the supplemental material) represents approximately 5% of the RP62a genome. Similarly, νSa1 in *S. aureus* COL represents 0.5% of the genome and carries the staphylococcal enterotoxin B gene (*seb*), a major virulence factor.

Comparative analysis of the *S. aureus* isolates suggested variations in the evolutionary history of the pathogenicity islands, some of which appear to have been created as a result of integration and subsequent mobilization of resident prophage into other members of this species (31, 42). Movement of these islands, such as mobilization of νSa1 (SaP1) by phage 80α (31, 42), into multiple *S. aureus* isolates may enable them to evolve and grow through the acquisition of additional virulence genes. For example, of the seven identified pathogenicity islands (Table 1), vSa1 and vSa2 share conservation in gene order in strains COL and MW2, respectively. In *S. aureus* COL, however, SEB (*seb*) is encoded in the same position as the toxic shock syndrome toxin (encoded by *tsst*) in MW2, suggesting either gene displacement or independent acquisition events through phages. In νSa4, COL and MW2 share an *att* site into which four ORFs, including a phage integrase gene and remnants of phage have inserted. The same *att* site in N315 and Mu50 is occupied by a νSa4 which contains additional genes, including the enterotoxin K (*sel*), enterotoxin C (*sec3*), and *tsst* genes. The similarity of the phage integrases suggests that the same phage integrated in all strains but that the *sek*, *entC*, and *tsst* genes have been lost from COL and MW2.

Genome islands as vectors of virulence determinants in the staphylococci contrasts with what is observed in the other low-GC-content gram-positive pathogens for which we have complete genomes available. For example, the *Listeria monocytogenes* genomes demonstrate a high degree of synteny, with variations in the genomes due to extensive SNPs (35). This sug-

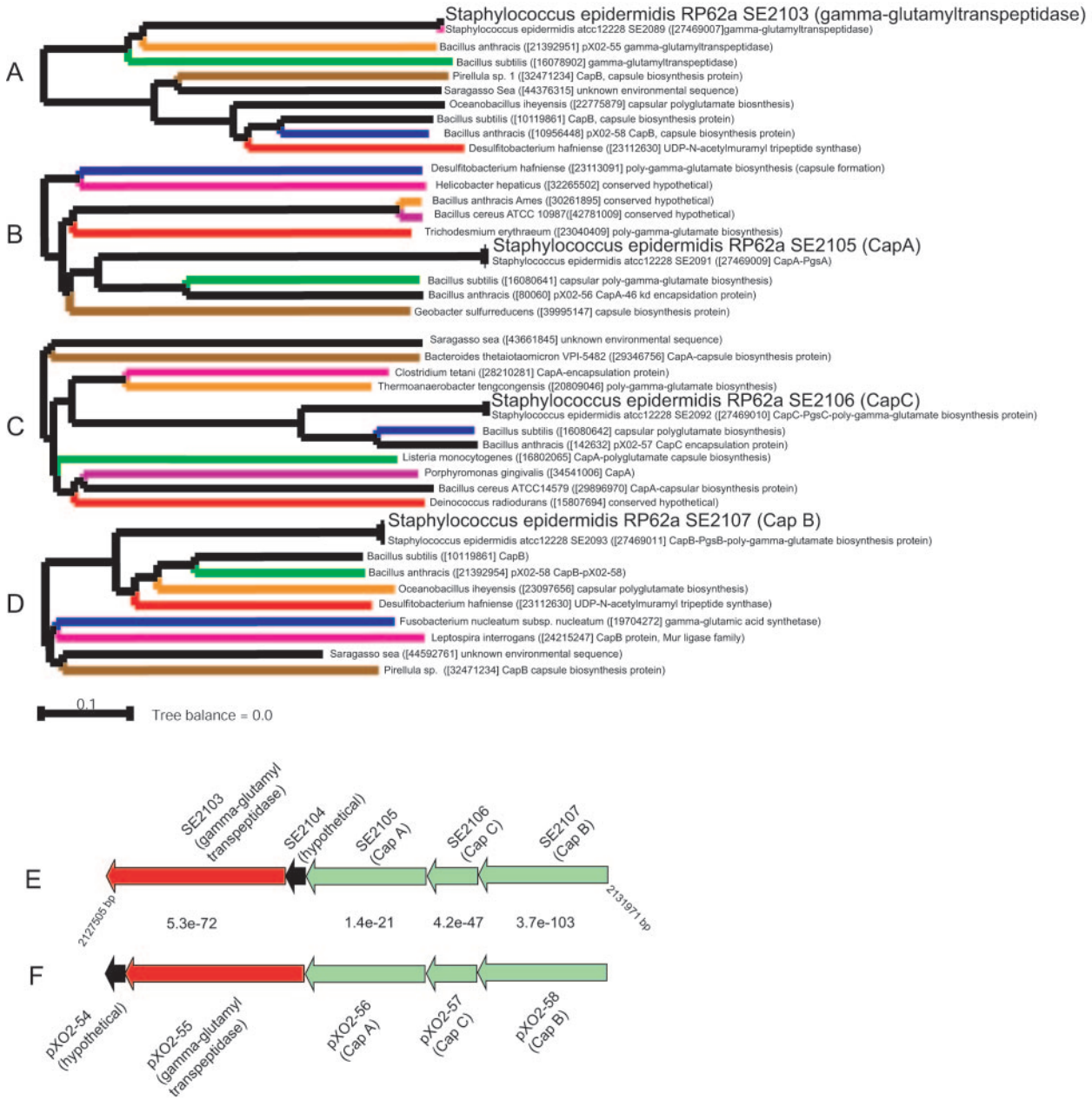

FIG. 3. Phylogenetic analysis and organization of the Cap operon in *S. epidermidis* RP62a. Homologs of *S. epidermidis* RP62a Cap operon (E) were identified by BLASTP of the WU-BLAST formatted database of all complete bacterial genomes. Each gene in the Cap operon was aligned against respective homologs with ClustalW, and phylogenetic trees were generated with Belvu for gamma-glutamyltranspeptidase (A), CapA (B), CapC (C), and CapB (D). Organization of the *S. epidermidis* RP62a and *B. anthracis* Cap operons is shown in schematics E and F, respectively. Positions within the respective genomes are indicted as genome coordinates on the ends of each schematic. BLASTP e values determined from homolog search results are shown between each ORF in schematics E and F. GenBank accession numbers are in parentheses and accompany all matches on the phylogenetic tree.

gests that the adaptation to infectious diseases in this species relies on small but specific genomic differences. In *Bacillus anthracis*, the majority of differences across strains are also in SNPs (but to a smaller degree than in *Listeria*'s) and in the presence or absence of the anthrax toxin genes carried on plasmid pX02. By comparison, *S. aureus* seems to demonstrate variable capabilities of virulence, depending on a combination of both genome islands in the form of phage and pathogenicity islands, as well as the presence of SNPs (see below). These differences may be the most significant factor contributing to the successive acquisition of resistance, as well as virulence factors.

Acquisition of virulence factors also appears to occur as a result of plasmid-mediated gene transfer between staphylococci and other low-GC-content gram-positive pathogens. For example, our analysis of the *S. epidermidis* RP62a and ATCC 12228 genomes revealed the presence of a *cap* operon (*capABC*) and gamma-glutamyl transpeptidase gene (Fig. 3)

TABLE 3. SNPs in *S. aureus*[b] and *S. epidermidis*[c]

Type of SNP	No.[d] in			
	S. aureus			*S. epidermidis* ATCC 12228
	Mu50	N315	MW2	
Total	22,888	22,160	19,599	10,297
In-gene	17,966	17,352	15,358	7,938
Intergenic	4,922	4,808	4,241	2,359
Synonymous	11,519	11,264	9,968	5,359
Codon position 1	531	518	490	239
Codon position 2	2[a]	2[a]	4[a]	1[a]
Codon position 3	10,986	10,744	9,474	5,119
Nonsynonymous	6,447	6,088	5,390	2,579
Codon position 1	2,850	2,710	2,436	1,281
Codon position 2	2,072	1,927	1,739	826
Codon position 3	1,525	1,451	1,215	472

[a] Synonymous SNPs at the second position of a stop codon TGA → TAA or synonymous SNPs at both the first and second position of a serine codon.
[b] Compared to *S. aureus* COL.
[c] Compared to *S. epidermidis* RP62a.
[d] Transition rates were 62.21, 62.49, 63.15, and 66.17 for *S. aureus* Mu50, N315, and MW2 and *S. epidermidis* ATCC 12228, respectively, transversion rates were 37.79, 37.51, 36.85, and 33.83, respectively.

similar to that found on the *B. anthracis* pX02 plasmid, where it encodes the polyglutamate capsule, which is essential for *B. anthracis* virulence. Experimental verification of a functional polyglutamate capsule in *S. epidermidis* remains to be done, but polyglutamate may play a role in the formation of *S. epidermidis* biofilms. Phylogenetic analysis of *cap* genes in the operon (Fig. 3) indicates that the acquisition of this locus may have been the result of a plasmid-mediated transfer event from an ancestor of the bacilli to *S. epidermidis*. However, note that a number of species-specific metabolic functions, such as acetoin dehydrogenase and polyphosphate synthesis, that are encoded by complete operons in *S. epidermidis* could also be the result of gene loss by a common ancestor.

A total of 22,888, 22,160, and 19,599 SNPs were found in the genomes of *S. aureus* Mu50, N315, and MW2, respectively, compared to that of strain COL. Of these SNPs, 6,447, 6,088, and 5,390 resulted in a nonsynonymous (NS) change in amino acid sequence in strains Mu50, N315 and MW2, respectively (Table 3 and Fig. 1). A total of 10,297 SNPs were found in the genome of *S. epidermidis* ATCC 12228 compared to that of strain RP62a. Of these SNPs, 2,579 resulted in an NS change in amino acid sequence. In *S. aureus*, SNPs are clustered in genome islands and, when these are grouped by function, it is found that there are a higher number of SNPs making up the cell envelope than performing other functions (~20% of total SNPs for Mu50, N315, and MW2) (Fig. 1; see Fig. S3 in the supplemental material). In *S. epidermidis*, although the majority of SNPs are found in genes for hypothetical proteins, a significant number (12.5% of the total SNPs for ATCC 12228) are in genes encoding proteins with cell envelope functions (Fig. 1; see Fig. S4 in the supplemental material). Variations in cell envelope or surface proteins, such as the LPXTG/NPQTN proteins and fibronectin binding proteins (encoded by *fnbA* and *-B*) likely reflect their immunogenicity and the high level of protective antibodies against these proteins which are present in human sera (18). Changes in amino acid sequence within highly immunogenic domains of these proteins may enable the bacteria to evade attack by the immune system.

Although much is known about staphylococcal virulence, very little is known about the metabolism of staphylococci. Previous studies on the metabolism and physiology of these organisms have been limited, but the complete genome sequence has allowed for an increased understanding of the basic biology of these species. In addition to the previously identified pathways for the synthesis of various amino acids, we have identified pathways (Fig. 4) for the synthesis of the amino acids leucine, valine, aspartate, isoleucine, glycine, and methionine. Pathways that would enable growth on a range of simple and complex sugars via the glycolytic pathway, the phosphate pathway, and the tricarboxylic acid cycle were also identified. The mevalonate pathway, required for the synthesis of isopentenyl-PP, essential for cell wall biosynthesis, as well as menaquinones and ubiquinones, needed for electron transport, were also identified.

S. aureus is primarily an inhabitant of mucous membranes, and *S. epidermidis* is primarily an inhabitant of the skin surface; in both environments the organisms are likely to encounter osmotic stress. With respect to transport, *S. aureus* and *S. epidermidis* possess seven and eight predicted sodium ion/proton exchangers, respectively. Both organisms are well adapted for osmotic stress, with six transport systems for proline, glycine betaine, or other probable osmoprotectants (Fig. 4). Other transporters related to osmoregulation include the MscL and MscS mechanosensitive ion channels and two Trk potassium ion channels.

Probably the major difference between *S. aureus* and *S. epidermidis* in terms of transport is the absence of three PTS sugar transporters, for mannitol, sorbitol, and pentitols, and an ABC family maltose transporter from *S. epidermidis*. Both species have a variety of transporters for inorganic cations and anions (Fig. 4), but it appears that iron acquisition is a serious priority. Six complete or partial ferric iron ABC uptake systems, four additional orphan ferric iron binding proteins, and two ferrous iron FeoB uptake systems were identified in *S. aureus*. In comparison, there are three complete or partial ferric iron ABC uptake systems, two additional orphan ferric iron binding proteins, and two ferrous iron FeoB uptake systems in *S. epidermidis*. Both staphylococcal species include a large number of predicted drug efflux systems, including the previously described NorA fluoroquinolone transporter and a probable ortholog of the *Lactococcus lactis* LmrP multidrug efflux protein.

Pathogenicity and *S. epidermidis* as an emerging pathogen. Genome-wide analysis of the six staphylococcal genomes revealed that approximately 11 and 7% of the total ORFs are predicted to encode cell surface proteins (Table 4) and secreted virulence factors (Table 2), respectively. Many surface proteins with essential roles in host colonization, biofilm formation, and evasion of host defense mechanisms have a common C-terminal LPXTG/NPQTN cell wall attachment motif and companion sortase processing enzymes (encoded by *srtA* and *-B*), which are conserved in all gram-positive bacterial pathogens (33, 44, 47). Our analysis of the LPXTG/NPQTN surface proteins revealed that only the accumulation-associated protein (encoded by *aap*) and most members of the Sdr gene family (*sdrCDEFG*) are functional homologs in both species. Those unique to each species likely reflect key differences in host tissue specificity and multifactorial adherence mechanisms used by *S. aureus* and *S. epidermidis*. *S. epider-*

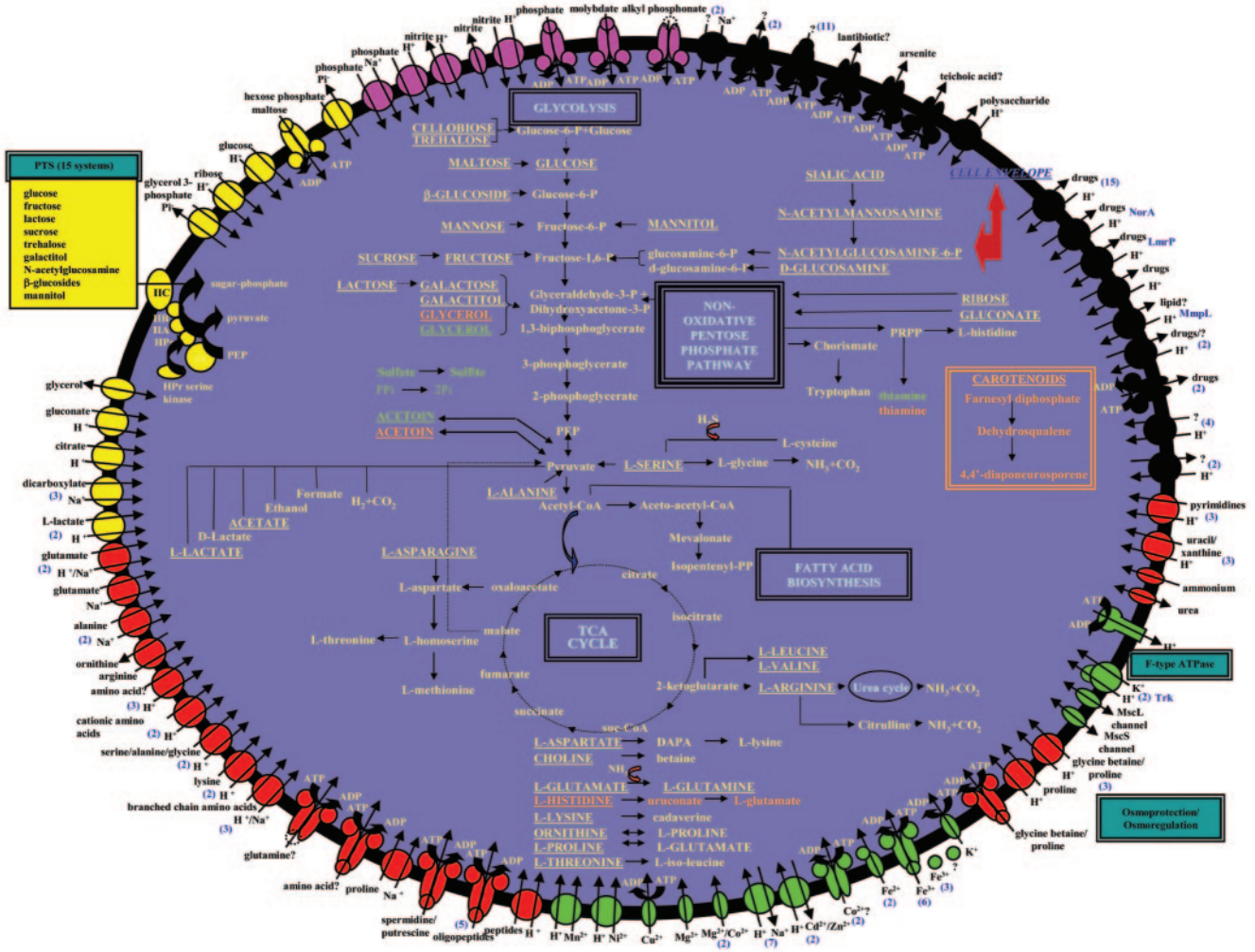

FIG. 4. Overview of metabolism and transport in *S. aureus* and *S. epidermidis*. Pathways for energy production, metabolism of organic compounds, and synthesis of carotenoids are shown. Orange text, processes unique to *S. aureus*; green text, processes unique to *S. epidermidis*. Transporters are grouped by substrate specificity as follows: inorganic cations (green); inorganic anions (pink); carbohydrates and carboxylates (yellow); amino acids, peptides, amines, and purines and pyrimidines (red); and drug efflux and other (black). Question marks indicate uncertainty about the substrate transported. Export or import of solutes is designated by the direction of the arrow through the transporter. The energy-coupling mechanisms of the transporters are also shown: double-headed arrow, solutes transported by channel proteins; two arrows, secondary transporters, indicating both the solute and the coupling ion; single arrow, transporters with an unknown energy coupling mechanism. ATP-driven transporters are indicated by the ATP hydrolysis reactions. Components of transporter systems that function as multisubunit complexes that were not identified are outlined with dotted lines. Where multiple homologous transporters with similar substrate predictions exist, the number of that type of transporter is indicated in parentheses.

midis ATCC 12228 encodes a novel third sortase (encoded by *srtC*) not found in other staphylococci and most closely related to sortases of *L. lactis* and *Streptococcus suis*. Many of the secreted proteins (Table 2) have roles in multiple mechanisms for invasion of host tissue and evasion of host defense systems. The relative abundance of virulence factors in *S. aureus* compared to *S. epidermidis* reflects the propensity of *S. aureus* to cause fulminant and sometimes life-threatening infections, as opposed to the more subacute or chronic infections caused by *S. epidermidis*. For example, members of the enterotoxin and exotoxin (53) families (Tables 1 and 2) which function as superantigens and inducers of a proinflammatory cytokine response are unique to *S. aureus* and have not been identified in characterized isolates of *S. epidermidis*.

The most likely candidate for a bona fide virulence factor in *S. epidermidis* is the family of small cytokine-stimulating peptides (22 to 44 amino acids in length) previously identified as PSM (38) (Table 2). Members of the PSM family are present in other staphylococci, including *S. aureus*, but our analysis has revealed that they are more numerous in *S. epidermidis*, where they appear to have expanded as a result of gene duplication within the νSeγ genome island (Fig. 2).

Expression of staphylococcal virulence factors and cell surface adhesion proteins is regulated by two previously identified regulatory loci, the accessory gene regulator locus (*agrABCD*) (36) and the staphylococcal accessory regulator family (*sarA*, etc.) (8), which respond to environmental or host stimuli through a quorum-sensing mechanism to coordinate adherence, tissue breakdown, and further invasion. Our analysis has identified 15 additional two-component regulatory systems

TABLE 4. Surface proteins in *S. aureus* and *S. epidermidis*

Locus	Function[a]	Functional name	Gene(s)	SD[b] present	LPXTG[c]	YSIRK[d] present
S. aureus surface proteins[e]						
SA0856	a	Clumping factor A	*clfA*	+	LPDTG	+
SA2652	a	Clumping factor B	*clfB*	+	LPETG	+
SA2511	a	Fibronectin binding protein A	*fnbA*		LPETG	+
SA2509	a	Fibronectin binding protein B	*fnbB*		LPETG	+
MW2612[g]	a	Collagen adhesion	*cna*		LPKTG	
SA0608	a	SdrC	*sdrC*	+	LPETG	+
SA0609	a	SdrD	*sdrD*	+	LPETG	+
SA0610	a	SdrE	*sdrE*	+	LPETG	+
SA0095	a,b	Protein A	*spa*		LPETG	+
SA0050	e	Methicillin resistance surface protein	*pls*	+	LPDTG	+
SA2676	e	Cell wall surface anchor protein	*sasA*		LPDTG	
SA2150	e	Phosphoglucomutase (FmtB/Mrp)	*sasB*		LPDTG	+
SA1806	e	Cell wall surface anchor protein	*sasC*		LPNTG	+
SA0119	e	Cell wall surface anchor protein	*sasD*		LPAAG	
SA1140	d	Cell wall surface anchor protein	*sasE* (*isdA*)		LPKTG	+
SA2668	e	Cell wall surface anchor protein	*sasF*		LPKAG	
SA2505	e	Cell wall surface anchor protein	*sasG* (*aap*)		LPKTG	+
SA0024	e	5′ nucleotidase family protein	*sasH*		LPKTG	
SA1781	g	Cell wall surface anchor protein	*sasI* (*harA*)		LPKTG	+
SA1138	d	Cell wall surface anchor protein	*sasJ* (*isdB*)		LPKTG	+
SA2381[h]	d	Cell wall surface anchor protein	*sasK*		LPKTG	
SA1141	e	Cell wall surface anchor protein (NPQTN)	*isdC*		NPQTN	
SA2002	a	Extracellular adherence protein	*eap, map*			
SA0858	a	Extracellular matrix and plasma binding protein	*empbp*			
SA1472	a	Cell wall-associated fibronectin binding protein	*ebh*			+
SA1164	a	Fibrinogen binding-related protein	*fib*			
SA1168	a	Fibrinogen binding protein	*efb*			
SA1522	a	Elastin binding protein	*ebp*			
SA1062	a	Biofunctional autolysin	*atl*			
S. epidermidis surface proteins[f]						
SE0026	a	SdrF (truncated) (not all strains have SdrF)	*sdrF*			+
SE0207	a	SdrG	*sdrG*	+	LPDTG	
SE1487	a	SdrH	*sdrH*	+		
SE1316	e	Cell wall surface anchor protein	*sesA*		LPLAG	+
SE2162	e	Cell wall surface anchor protein	*sesB*		LPNTG	
SE2264	e	Cell wall surface anchor protein	*sesC*		LPATG	
SE2392[i]	c	Cell wall surface anchor protein	*sesD* (*bhp*)		LPQTG	+
SE0719	e	Cell wall surface anchor protein	*sesE*		LPETG	+
SE2398	e	Cell wall surface anchor protein	*sesF* (*aap*)		LPDTG	+
SE1482[i]	e	Cell wall surface anchor protein	*sesG*		LPDTG	+
SE1483	e	Cell wall surface anchor protein	*sesH*		LPETG	
SE1654[i]	e	Cell wall surface anchor protein	*sesI*		LPETG	
SE1500[j]	e	Cell wall surface anchor protein	*sesJ*		LPKTG	
SE1501[j]	e	Cell wall surface anchor protein	*sesK*		LPNTG	
SE1011	a	Cell wall associated fibronectin binding protein	*ebh*			+
SE1048	a	Elastin binding protein	*ebp*			
SE0636	a,f	Bifunctional autolysin	*altE*			
SE0775	a	Fibrinogen binding protein	*fbe*			

[a] a, adherence to host tissue (extracellular matrix, fibrinogen, fibronectin, collagen, elastin, endothelial and epithelial cells); b, evasion of host defense; c, biofilm formation; d, binding to heme-iron; e, unknown; f, adherence to synthetic material (implanted medical devices); g, haptoglobin receptor A.
[b] SD, Serine-aspartate dipeptide repeats found in staphylococcal cell wall-attached proteins.
[c] LPXTG, attachment sequence for gram-positive cell wall-attached proteins.
[d] YSIRK, signal peptide YSIRK for gram-positive cell wall-attached proteins.
[e] *S. aureus* COL loci unless indicated otherwise.
[f] *S. epidermidis* RP62a loci unless indicated otherwise.
[g] *S. aureus* MW2.
[h] *S. aureus* N315.
[i] Only in *S. epidermidis* RP62a.
[j] Only in *S. epidermidis* ATCC 12228.

(Supplemental Table 7) that are similar to *agr* and conserved in both *S. aureus* and *S. epidermidis*, which is surprising considering the differences in adhesins and virulence factors expressed by these species. Identification of possible functional homologs of the *agr* locus in the genomes of *Clostridium acetobutylicum*, *Enterococcus faecium*, *Enterococcus faecalis*, *Lactobacillus plantarum*, and *Listeria monocytogenes* suggests a conservation of regulatory and quorum-sensing mechanisms among the low-GC-content gram-positive pathogens.

Our comparison of the *S. epidermidis* genomes revealed that a key difference between the biofilm-nonproducing ATCC 12228 type strain and the biofilm-producing RP62a is the presence of the intercellular adhesion locus (*icaABCD*) and the cell wall associated biofilm protein (Bap) or Bap homologous pro-

tein (Bhp). The *ica* locus, which encodes the polysaccharide intercellular adhesin protein with a key role in biofilm formation and bacterial accumulation on host surfaces, is present in biofilm-associated isolates, such as *S. epidermidis* RP62a, but is frequently absent in commensal isolates, such as ATCC 12228 (54). Bap was previously identified in bovine mastitis *S. aureus* isolates, where it has key roles in adherence to polystyrene surfaces, intercellular adhesion, and biofilm formation (12, 13, 49), but has not been found in human clinical *S. aureus* isolates. However, Bhp was identified in *S. epidermidis* RP62a, where it may have a function similar to that of the Bap homolog. Homologs of Bap and Bhp were identified in other bacteria, including the enterococcal surface protein or Esp in *Enterococcus faecalis*, where it plays a similar essential role in biofilm formation (46). The Esp, Bap, and Bhp surface proteins may play functional roles in biofilm formation among mixed populations of these bacteria, as documented by the recent transfer of the vancomycin conjugative transposon, Tn*1546*, from clinical isolates of *Enterococcus faecalis* to *S. aureus* (52).

Conclusion. The most significant observation from our study was evidence for gene transfer between the staphylococci and bacilli. The *cap* operon, encoding the polyglutamate capsule, a major virulence factor in *B. anthracis*, has integrated in the genomes of both *S. epidermidis* RP62a and ATCC 12228, likely as a result of plasmid-mediated gene transfer between the two genera. Evidence of active gene transfer and movement of mobile elements between the staphylococci and other low-GC-content gram-positive bacteria is suggestive not only of continued evolution of virulence and resistance in *S. aureus* but also the transition of *S. epidermidis* from a commensal pathogen to a more aggressive opportunistic pathogen through the acquisition of additional virulence factors. Evidence of *S. epidermidis* strains producing enterotoxin C (49) indicates that this gene movement has already occurred and leads us to propose the genome sequencing of additional *S. epidermidis* clinical isolates to examine the role of gene transfer in the evolution of staphylococcal virulence.

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