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New Structure of Phage-Related Islands Carrying *fusB* and a Virulence Gene in Fusidic Acid-Resistant *Staphylococcus epidermidis*

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Nucleotide sequencing of the *fusB*-flanking regions in two fusidic acid-resistant *Staphylococcus epidermidis* isolates with the type IV *aj1*-leader peptide (LP)-*fusB* structure (lacking *aj1*) revealed that their *fusB* gene was located on novel phage-related islands inserted downstream of *smpB* and are here referred to as SeRI$_{fusB\text{-}3692}$ and SePI$_{fusB\text{-}857}$. The novel SePI$_{fusB\text{-}857}$ structure was followed by SeCI$_{857}$, forming a composite pathogenicity island which contained a putative virulence gene, *vapE*. The linkage of *fusB* and *vapE* may contribute to bacterial adaption.

The horizontally acquired determinant *fusB* is the most frequent determinant responsible for fusidic acid resistance in *Staphylococcus epidermidis* and is often found associated with genomic resistance islands (RIs) (1, 2). We have previously found at least three types of structures of RIs, discriminated by their sequences flanking *fusB* (*aj1*-leader peptide [LP]-*fusB*), and we have identified different insertion sites, including sites downstream of *groEL* and *rpsR* (2). However, the genetic support of *fusB* in some isolates remains unknown. To analyze the unidentified structure and gain more understanding of the prevalence of various *fusB*-carrying elements, a total of 141 fusidic acid-resistant (MIC $\geq$ 2 μg/ml) *S. epidermidis* isolates were tested. The isolates were collected from a 3-year (2008 to 2010) collection in the Bacteriology Laboratory of the National Taiwan University Hospital, a 2,500-bed teaching hospital in northern Taiwan. The species of *S. epidermidis* was initially identified using the Phoenix Automated System and was then further confirmed by *S. epidermidis*-specific PCR (3).

Detection of *fusB*, *fusC*, and *fusD* (4), performed by PCR, revealed that the majority of isolates (136/141, 96.5%) possessed *fusB*. Only four isolates carried *fusC*, and one contained a *fusA* point mutation (resulting in P404L). Various types of *aj1*-LP-*fusB* fragments for 136 *fusB*-positive isolates were determined by PCR as previously described (2). Of them, 14 type I (full-length *aj1*), 58 type II (partial *aj1* fragment, truncated from nucleotide position 93 to 421), 47 type III (a more truncated *aj1* that retained only the last 37 bp), and 17 type IV (lacking *aj1*) isolates were identified. The fusidic acid MICs for 136 *fusB*-positive isolates ranged from 2 to 16 μg/ml. The type II isolates displayed significantly higher-level resistance to fusidic acid (the MIC for 41/58 [71%] isolates was 16 μg/ml) than type III isolates (the MIC for only 12/47 [26%] isolates was 16 μg/ml) ($P < 0.05$) (Table 1).

The isolates with type IV *aj1*-LP-*fusB* sequences differed from other types by the absence of *aj1*, but *fusB*'s genetic environment was unknown. Two representative type IV isolates (NTUH-3692 and NTUH-857) were used for cloning and sequencing with a long accurate (LA)-PCR *in vitro* cloning kit (TaKaRa Shuzo Co. Ltd., Japan) (2) and by inverse PCR (see Table S1 in the supplemental material). The sequencing of amplification products was performed on an Applied Biosystems model 3100 DNA sequencer (Applied Biosystems, Foster City, CA) using the *Taq* BigDye-Deoxy Terminator cycle sequencing kit (Applied Biosystems).

TABLE 1 Distribution of fusidic acid resistance determinants and MICs among fusidic acid-resistant *S. epidermidis* isolates

Resistance determinant	*aj1*-LP-*fusB* type	No. of isolates	No. of isolates for which the MIC (μg/ml) was[a]:				
			2	4	8	16	32
fusB	I	14	0	1	8	5	0
	II	58	0	1	16	41	0
	III	47	1	13	21	12	0
	IV	17	1	1	9	6	0
fusC		4	0	0	0	1	3
fusA with point mutation		1	0	0	0	1	0
Total		141	2	16	54	66	3

[a] The MICs for isolates with a type II *aj1*-LP-*fusB* sequence were significantly ($P < 0.05$) higher than those for isolates with a type III *aj1*-LP-*fusB* sequence.

Sequencing results indicated that the *fusB* gene in NTUH-3692 was located on a 15,553-bp phage-related RI and is here referred to as SeRI$_{fusB\text{-}3692}$, where "Se" signifies "*S. epidermidis*." The *fusB* in NTUH-857 was located in a 21,003-bp composite island, here referred to as cSePI$_{fusB\text{-}857}$ (where "PI" signifies "pathogenicity island"), which was composed by SePI$_{fusB\text{-}857}$ (14,529 bp) and SeCI$_{857}$ (where "CI" signifies "chromosomal insertion") (6,474 bp) (Fig. 1). The sizes of SeRI$_{fusB\text{-}3692}$ and SePI$_{fusB\text{-}857}$ fit the criteria for a

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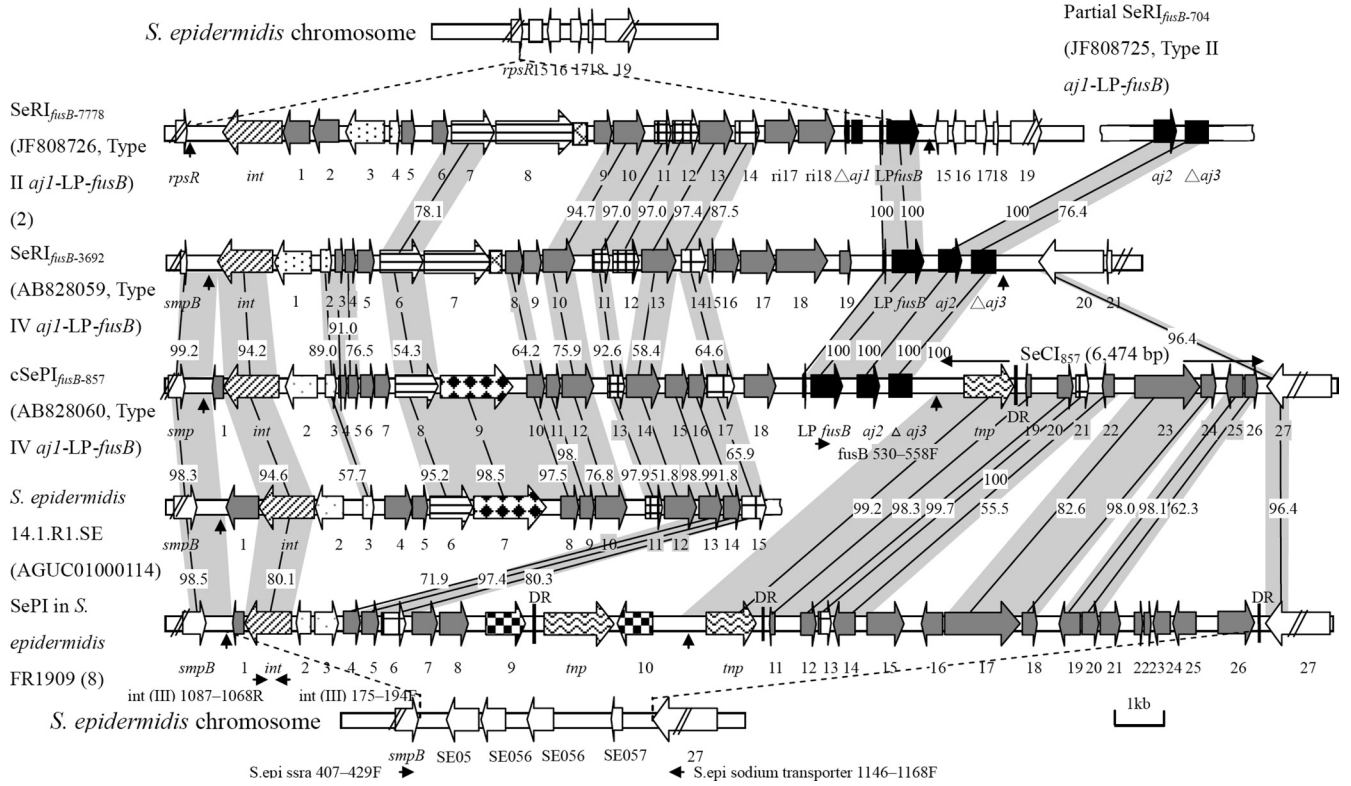

FIG 1 Genetic organization of SeRI$_{fusB\text{-}3692}$ (GenBank accession no. AB828059) and SeRI$_{fusB\text{-}857}$ (GenBank accession no. AB828060) compared with those of SeRI$_{fusB\text{-}7778}$ (GenBank accession no. JF808726), partial SeRI$_{fusB\text{-}704}$ (GenBank accession no. JF808725), *S. epidermidis* 14.1.R1.SE (GenBank accession no. AGUC01000114), and the PI in *S. epidermidis* FR1909 (GenBank accession no. AENR01000001 and AENR01000008). Genes are drawn according to their sequences and function. ▨, *int* and *xis*; ▩, transcription regulators; ▤, replication genes (including the primase gene[*pri*] and the replication initiator gene [*rep*]); ▩, the replication origin (ori); ⊞, the terminase small-subunit encapsidation gene *terS*; ▦, other encapsidation genes; ▥, *pif* (phage interference); ■, *aj1*-LP-*fusB* regions; ⊞, *vapE*; ▩, virulence factor genes; ▨, transposase genes; □, chromosome genes adjacent to SeRI$_{fusB}$; ▢, other genes coding hypothetical proteins. The predicted direct repeats are indicated by vertical arrows. The horizontal arrows represent the PCR primers used to determine the insertion sites (downstream of *smpB*). Homologous regions between resistance islands are shown with shaded connecting lines, and shaded numbers show the percentages of homology between the corresponding sequences. DR, direct repeat; S. epi, *S. epidermidis*; ssra, SsrA-binding protein encoded by *smpB*.

pathogenicity island (5). The GC contents of SeRI$_{fusB\text{-}3692}$ and cSePI$_{fusB\text{-}857}$ were 29.1% and 30.3% (SePI$_{fusB\text{-}857}$, 29.8%, and SeCI$_{857}$, 31.5%), respectively, slightly lower than that of the published whole-genome sequences of *S. epidermidis* (~32%). Both SePI$_{fusB\text{-}857}$ and SeRI$_{fusB\text{-}3692}$ were flanked by direct repeats and contained conserved phage-related core genes (6). SeRI$_{fusB\text{-}3692}$ and cSePI$_{fusB\text{-}857}$ were located downstream of *smpB*, unlike with *groEL* and *rpsR* in previously found *fusB* RIs (2). The *smpB* insertion site has been found in SaPIm4 and SaPImw2 of *Staphylococcus aureus* (7) and in a composite PI in *S. epidermidis* (8).

Sequence data confirmed the lack of the *aj1* gene in SeRI$_{fusB\text{-}3692}$ or SePI$_{fusB\text{-}857}$. Comparison of SeRI$_{fusB\text{-}3692}$ and SePI$_{fusB\text{-}857}$ to previously found SeRI$_{fusB\text{-}7778}$ (2) revealed that the genetic organizations were similar and that the sequences of LP and *fusB* were identical (Fig. 1). All three islands carried conserved phage-related core genes (Fig. 1) (6). The open reading frames (ORFs) in regions upstream of *fusB* in SeRI$_{fusB\text{-}3692}$ and SePI$_{fusB\text{-}857}$ were in general similar. Sequences of ORFs in SePI$_{fusB\text{-}857}$ showed high identity to those in an island in *S. epidermidis* 14.1.R1.SE (GenBank accession no. AGUC01000114) (Fig. 1).

Another important finding in this study is the presence of a putative virulence gene, *vapE*, in SePI$_{fusB\text{-}857}$. The *vapE* gene was originally found in virulent *Dichelobacter nodosus*, a sheep pathogen that causes severe ovine foot rot (9). The *vapE* gene has also been found in PIs of *S. aureus* (10, 11) and in *S. epidermidis* 14.1.R1.SE but is here for the first time identified in a *fusB* element. Of 136 *fusB*-positive *S. epidermidis* isolates, *vapE* was detected in nine isolates (9/136, 6.6%), including three type I, one type II, and five type IV *aj1*-LP-*fusB* isolates. However, only in the five type IV isolates were *vapE* and *fusB* linked together and located downstream of *smpB*. In one type II isolate, *vapE* was located downstream of *smpB*, but *fusB* was located downstream of *groEL*. In the three type I isolates, the location of *vapE* was unknown. Bacterial PIs usually carry either virulence genes or antibiotic resistance genes; very few PIs carry both of them (12).

Unlike in other *fusB* RIs, SePI$_{fusB\text{-}857}$ was followed by SeCI$_{857}$. SeCI$_{857}$ was similar to SeCI-1 in *S. epidermidis* FRI909 (8) (Fig. 1). Thus, cSePI$_{fusB\text{-}857}$ may arise from two independent integration events.

To compare the characteristics of isolates carrying different structures in their *fusB* element, the antimicrobial resistance profile and the presence of virulence-related genes, including the biofilm-related *icaAB* locus (13), IS*256* (14), and the resistance gene *mecA* (15), detected by PCR, were determined (Table 2). The above-named genes have been reported to be associated with nosocomial isolates but were detected in only a small subset of com-

TABLE 2 Antimicrobial susceptibilities and distribution of the virulence-related *icaAB* locus and IS*256* gene and *mecA* resistance gene among isolates with different *aj1*-LP-*fusB* types

Antibiotic(s) to which isolates were resistant or virulence-related genotype[a]	No. of isolates (%) of *aj1*-LP-*fusB* type:			
	I ($n = 14$)	II ($n = 58$)	III ($n = 47$)	IV ($n = 17$)
Clindamycin	9 (64)	45 (78)	35 (74)	4 (24)
Erythromycin	12 (86)	45 (78)	35 (74)	12 (71)
Gentamicin	13 (93)	56 (97)	42 (89)	8 (47)
Oxacillin	14 (100)	57 (98)	47 (100)	15 (88)
Trimethoprim-sulfamethoxazole	12 (86)	54 (93)	42 (89)	14 (82)
icaAB (−) IS*256* (−) *mecA* (−)	0 (0)	1 (2)	0 (0)	2 (12)
icaAB (−) IS*256* (−) *mecA* (+)	4 (29)	1 (2)	3 (6)	10 (59)
icaAB (+) IS*256* (−) *mecA* (+)	1 (7)	1 (2)	0 (0)	3 (18)
icaAB (−) IS*256* (+) *mecA* (+)	4 (29)	0 (0)	1 (2)	2 (12)
icaAB (+) IS*256* (+) *mecA* (+)	5 (36)	55 (95)	43 (91)	0 (0)

[a] (−), gene is absent; (+), gene is present.

mensal isolates (14, 16). The rates of resistance to erythromycin and trimethoprim-sulfamethoxazole (SXT) were similar among the four types. The type IV isolates exhibited a lower occurrence of resistance to oxacillin, clindamycin, and gentamicin than type I, II, or III isolates. It has been previously reported that commensal isolates are less resistant to clindamycin, SXT, and oxacillin (16), and gentamicin resistance has been recognized as a marker of nosocomially acquired staphylococci (17). Resistance to more antibiotics and a high prevalence of the *icaAB* locus, IS*256*, and *mecA* in type II or III isolates suggests their clinical significance. For type IV isolates, although they less frequently carried the *icaAB* locus, IS*256*, and *mecA* genes than isolates of the other types, the presence of *vapE* may somehow confer an advantageous attribute.

Concluding remarks. This study provides new data on the complexity and diversity of genetic elements associated with the *fusB* determinant in fusidic acid-resistant *S. epidermidis* strains. Novel SePI$_{fusB\text{-}857}$ contained both an antibiotic resistance gene (*fusB*) and a putative virulence gene (*vapE*), which may provide an advantage for bacterial survival.

Nucleotide sequence accession number. The nucleotide sequences of SeRI$_{fusB\text{-}3692}$ and cSeRI$_{fusB\text{-}857}$ have been deposited in GenBank under accession numbers AB828059 and AB828060, respectively.

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