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-3692 and SeRI_fusB-857. The novel SePI_fusB-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 (RI) (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 ≥ 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).

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-3692, where “Se” signifies “Staphylococcus.” The fusB in NTUH-857 was located in a 21,003-bp composite island, here referred to as SePI_fusB-857 (where “PI” signifies “pathogenicity island”), which was composed by SePI_fusB-857 (14,529 bp) and SeCI_857 (where “CI” signifies “chromosomal insertion”) (6,474 bp) (Fig. 1). The sizes of SeRI_fusB-3692 and SePI_fusB-857 fit the criteria for a...
SeRI

SePI

SeCI857

FIG 1 Genetic organization of SeRI

Genetic organization of SeRI

S. epidermidis chromosome

SeRI

SePI

SeCI857

SeRI

SePI

SeCI857

FIG 1 Genetic organization of SeRI (GenBank accession no. AB828059) and SeRI (GenBank accession no. AB828060) compared with those of SeRI (GenBank accession no. IF808726), partial SeRI (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, integrase; xis, restriction enzyme; rep, replication regulator; ori, origin of replication; terS, termination small-subunit encapsidation gene; ssra, SsrA-binding protein encoded by smpB.

pathogenicity island (5). The GC contents of SeRI and cSePI were 29.1% and 30.3% (SePI, 29.8%, and SeCI857, 31.5%), respectively, slightly lower than that of the published whole-genome sequences of S. epidermidis (∼52%). Both SePI and SeRI were flanked by direct repeats and contained conserved phage-related core genes (6). SeRI and cSePI were located downstream of fusB, unlike with groEL and rpsR in previously found fusB RIs (2). The fusB insertion site has been found in SaPlm4 and SaPlmww2 of Staphylococcus aureus (7) and in a composite PI in S. epidermidis (8). DNA data confirmed the lack of the aj1 gene in SeRI or SePI. Comparison of SeRI and SePI to previously sequenced TP 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 and SePI were in general similar. Sequences of ORFs in SePI 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. 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 isolates. However, only in the five type IV isolates were vapE and fusB linked together and located downstream of fusB. In one type II isolate, vapE was located downstream of the fusB element. 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 was followed by SeCI857. SeCl was similar to SeCl- in S. epidermidis FR1909 (8) (Fig. 1). Thus, cSePI 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), IS256 (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 IS256 gene and mecA resistance gene among isolates with different ajI-LP-fusB types.

<table>
<thead>
<tr>
<th>Antibiotic(s) to which isolates were resistant or virulence-related genotypea</th>
<th>No. of isolates (%) of ajI-LP-fusB types:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>(n = 14)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>9 (64)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>12 (86)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>14 (100)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>12 (86)</td>
</tr>
<tr>
<td>icaAB (−) IS256 (−) mecA (−)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>icaAB (−) IS256 (−) mecA (+)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>icaAB (+) IS256 (−) mecA (−)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>icaAB (−) IS256 (+) mecA (+)</td>
<td>4 (29)</td>
</tr>
<tr>
<td>icaAB (+) IS256 (+) mecA (+)</td>
<td>5 (36)</td>
</tr>
</tbody>
</table>

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

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 the four types. The type IV isolates exhibited a lower occurrence of mecA type: mecA−857 contained both an antibiotic resistance gene fusB, and cSeRI-857 have been deposited in GenBank under accession numbers AB828059 and AB828060, respectively.

ACKNOWLEDGMENT

This work was supported by grant NSC 100-2320-B-002-014-MY3 from the National Science Council of Taiwan.

REFERENCES


