

NOTES

Precise Excision and Self-Integration of a Composite Transposon as a Model for Spontaneous Large-Scale Chromosome Inversion/Deletion of the *Staphylococcus haemolyticus* Clinical Strain JCSC1435[∇]

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Large-scale chromosomal inversions (455 to 535 kbp) or deletions (266 to 320 kbp) were found to accompany spontaneous loss of beta-lactam resistance during drug-free passage of the multiresistant *Staphylococcus haemolyticus* clinical strain JCSC1435. Identification and sequencing of the rearranged chromosomal loci revealed that ISSha1 of *S. haemolyticus* is responsible for the chromosome rearrangements.

After the first report concerning the *Haemophilus influenzae* genome, a large number of microbial genomes were sequenced, and these genomes are available for comparison (2). A comparison of a pair of closely related chromosomes of members of the same species usually reveals that homologous genes, or orthologs, are arranged in the same order in the chromosome. However, noncolinear gene order is also found in certain strains of the same species, which is thought to be caused by chromosome rearrangement between the homologous sequences scattered along the chromosome (19). With this mechanism, it is presumed that genome rearrangements occur more frequently in the strains having many insertion sequences (IS) or prophages with closely related structures in their chromosomes. In fact, a comparison of three *Bordetella* species revealed that the rearrangements occurred more frequently in *Bordetella pertussis* (carrying 261 IS copies) than in *Bordetella parapertussis* and *Bordetella bronchiseptica* (112 and no IS copies, respectively) (12).

Of 11 staphylococcal strains belonging to four species whose entire genomes have been determined, *Staphylococcus haemolyticus* strain JCSC1435 has by far the greatest number of ISs in its chromosome (10, 18). This strain has as many as 82 copies of ISs in its chromosome, and notably 60 of these copies have been judged to be intact based on their nucleotide sequences. *S. haemolyticus* is notorious for its multidrug resistance phenotype and historical early acquisition of antimicrobial resistance to methicillin and glycopeptide antibiotics (3, 5). It has been reported that it is difficult to identify clinical strains as *S. haemolyticus* strains by traditional biochemical properties (17). These characteristics of *S. haemolyticus* may result from frequent structural alteration of the chromosome due to the re-

combination caused by the presence of abundant IS copies in the chromosome.

We have reported previously that large DNA segments that were up to 420 kbp long were deleted in ceftizoxime- or teicoplanin-susceptible variant strains of JCSC1435, presumably due to the IS-encoded transposase activity (18). We report here a different type of genome rearrangement, which also seems to contribute to the phenotypic instability or flexibility of *S. haemolyticus*.

In a previous study, all five of the IS-mediated genome deletions analyzed were localized in the first one-sixth of the chromosome, which, starting from *oriC*, roughly corresponded to the “*oriC* environ” (18). Following the previous study, we obtained 11 additional ceftizoxime-susceptible JCSC1435 derivatives by serial 11-day drug-free passages. Pulsed-field gel electrophoresis (PFGE) analysis showed that eight derivative strains had deletions in the *oriC* environ. However, the other three strains, UP01, UP03, and UP04, turned out to have more complicated rearrangements. They had the same pulsotype, which indicated that they had the same clonal origin. A detailed PFGE analysis of strain UP03 with three enzymes, SacII, SmaI, and BglII, revealed the presence of two chromosome rearrangements. One was an approximately 305-kbp deletion located in the *oriC* environ, and the other was a rearrangement localized on the opposite side of *oriC*. Furthermore, the deletion in the *oriC* environ was not a simple deletion, because we could not amplify a DNA fragment using a PCR primer set designed in a colinear manner on the chromosome map of JCSC1435, the parent strain. We then analyzed 10 and 18 other ceftizoxime-susceptible derivative strains obtained from two additional independent passage experiments. Each culture was started with an inoculum consisting of 100 μ l of a JCSC1435 overnight culture, and the ceftizoxime-susceptible derivatives were obtained by replica plating after 9-day serial passages. All derivatives had altered SacII-restricted PFGE patterns, and we found that strains B5EC1 and C4EC2, obtained from the two independent experiments, had banding patterns that were sim-

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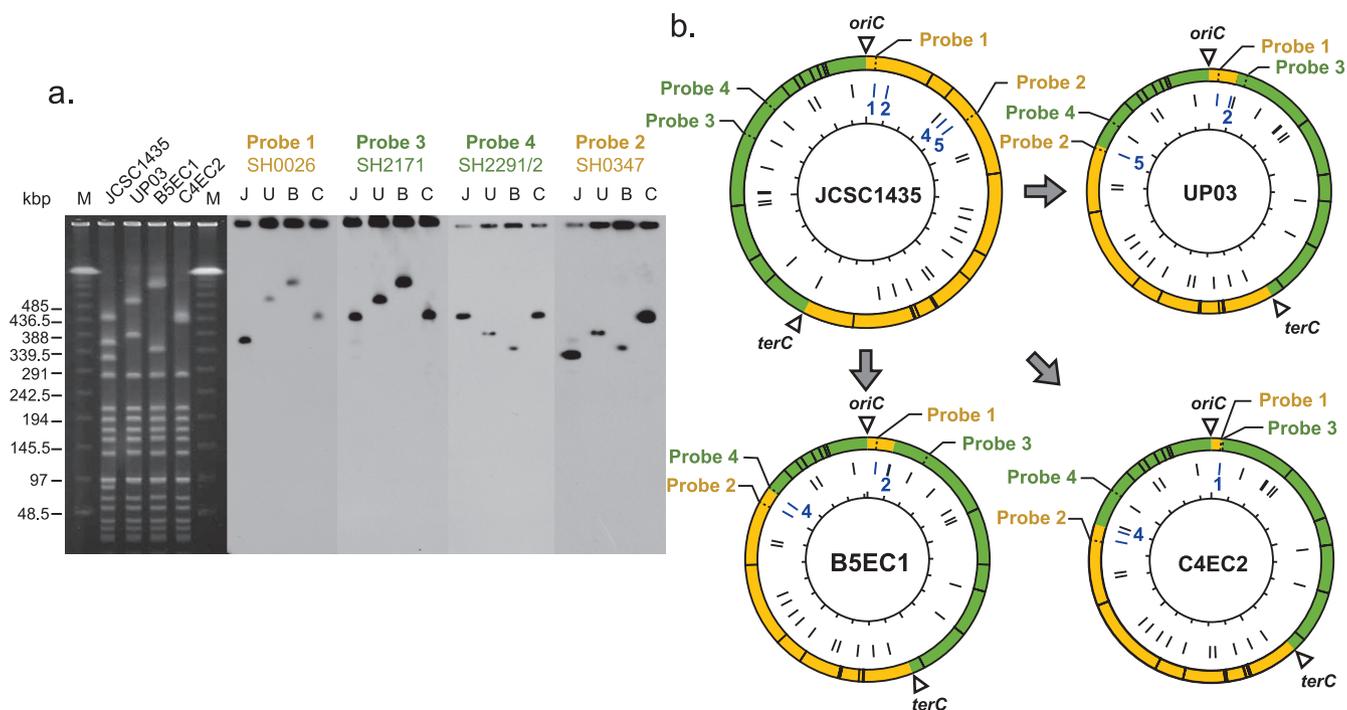


FIG. 1. PFGE and Southern blot hybridization analysis of the three ceftizoxime-susceptible derivatives (UP03, B5EC1, and C4EC2). (a) SacII-restricted PFGE and Southern blot hybridization of *S. haemolyticus* JCSC1435 and three ceftizoxime-sensitive derivatives. Southern blot hybridization was carried out using a SacII-restricted PFGE gel with PCR products obtained with SH0026, SH2171, SH2291/2292, and SH0347 as probes. Lanes M, DNA size standard (lambda ladder); lanes J, JCSC1435; lanes U, UP03; lanes B, B5EC1; lanes C, C4EC2. (b) SacII restriction sites and locations of the open reading frames used as probes in the chromosomes of JCSC1435 and ceftizoxime-susceptible derivatives. The outer circle indicates the SacII restriction sites and the locations of the open reading frames used as probes (the clockwise and counterclockwise replication arms of JCSC1435 are yellow and green, respectively). The middle circle indicates the locations of ISShaI, and the inner circle indicates the nucleotide positions (in 100-kbp increments), starting from *oriC* and moving clockwise.

ilar, but not identical, to that of UP03 (Fig. 1a). SmaI- and BglI-restricted PFGE analysis further demonstrated that similar chromosome rearrangements had occurred in the three independent derivative strains (data not shown). We then used PCR scanning and Southern blot hybridization to determine the detailed chromosome structures of the three strains (Fig. 1).

First, PCR scanning was performed in the chromosomal regions corresponding to the altered PFGE bands. With UP03, PCR produced no band in the region flanked by the two copies of the insertion sequence ISShaI-2 (from bp 88353 to 86865) and ISShaI-5 (bp 393322 to 394810) (the suffix for each ISShaI copy was assigned in the clockwise direction, starting from *oriC*). This indicated that the 310-kbp region between the two ISShaI copies was deleted. A similar strategy revealed that the regions between ISShaI-2 and ISShaI-4 (bp 356056 to 354570) of B5EC1 and between ISShaI-1 (bp 34466 to 32978) and ISShaI-4 of C4EC2 were deleted. In all the three cases, the *mecA* gene that encodes methicillin resistance was deleted along with the other regions. This was reasonable since all the strains were obtained with loss of beta-lactam resistance as the selective phenotype.

Next, DNA bands of PFGE gels were transferred to a nylon membrane, and Southern blot hybridization was carried out to identify the rearranged chromosome structures of the strains. PCR products that were about 1 kbp long (SH0026, SH0347, SH2171, and SH2291/SH2292) were amplified from the JCSC1435 chromosome and used as the hybridization probes

(Fig. 1). Probes SH2171 and SH2291/SH2292 hybridized with the 430-kbp band of parent strain JCSC1435. SH0026 hybridized with the 350-kbp band and SH0347 hybridized with the 310-kbp band of JCSC1435. On the other hand, probes SH0026 and SH2171 hybridized to aberrant bands at 490 kbp (UP03), 570 kbp (B5EC1), and 405 kbp (C4EC2), and probes SH2291/2292 and SH0347 hybridized to other aberrant bands at 375 kbp (UP03), 335 kbp (B5EC1), and 445 kbp (C4EC2). Taken together, these observations indicated that large-scale inversions had occurred in these strains in the *oriC* region.

Finally, we determined exact genome rearrangement sites by nucleotide sequencing (Fig. 2). The large-scale inversion involved two ISShaI copies located in *oriC* environ and an AT-rich octamer present on the other side of *oriC*. In addition, the regions between the two copies of ISShaI were deleted. For example, in UP03, ISShaI-2 stayed at the same position relative to *oriC*, whereas ISShaI-5 moved to the other side of *oriC* from bp 393321 to bp 2238852 (Fig. 2a). A total of 304,968 bp between the two copies of ISShaI was deleted, leaving two identical octamer sequences at nucleotide positions 2238852 and 2238859, which were located next to the IS copies ISShaI-2 and ISShaI-5, respectively. Similar inversions/deletions were found in the B5EC1 and C4EC2 chromosomes (Fig. 2b and c). The octamer sequences next to ISShaI corresponded to one of the "direct repeats" (DRs) that are known to be generated as a result of target duplication in the integration of the insertion sequence ISShaI. The characteristic 26-bp

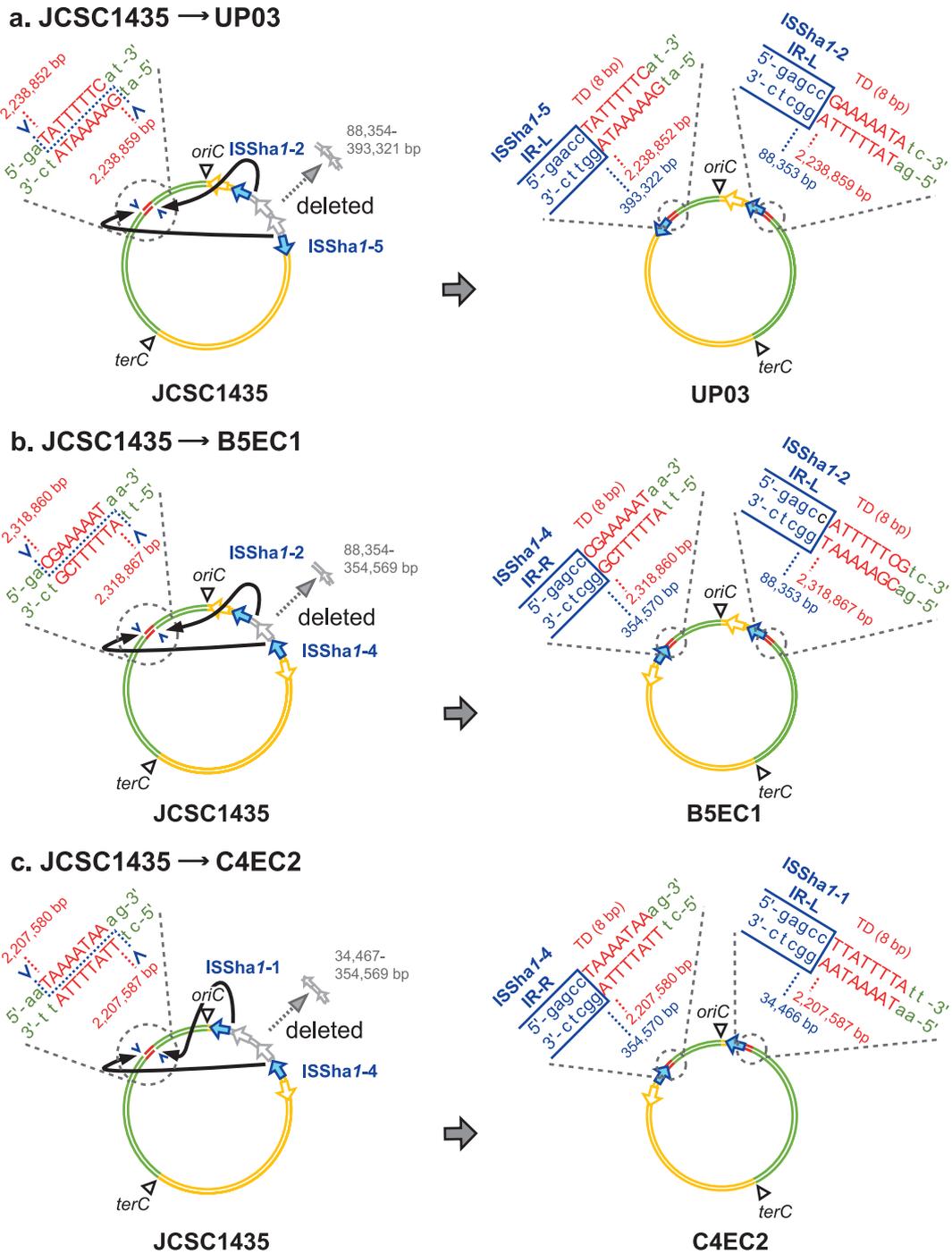


FIG. 2. Genome rearrangements observed in three derivatives of *S. haemolyticus* JCSC1435. In addition to deletion between two copies of *ISShaI*, inversion has occurred between the two ISs and the 8-bp site in opposite replication arms. The blue arrows indicate *ISShaI* involved in the genome rearrangements. The octamer sequences are indicated by uppercase red letters. They are considered to be target duplications (TD) accompanying the integration of *ISShaI*. The nucleotide positions of the derivatives are based on the nucleotide sequence of JCSC1435 (accession no. AP006716).

inverted repeats (IRs) of *ISShaI* were found to abut the newly generated octamers, as is the case with all the *ISShaI* copies in JCSC1435. *ISShaI* belongs to the ISL3 family, and each member of this family has a unique transposase (*tnp*) and a 15- to 39-bp IR and is known to produce 8-bp DRs during the integration event (11). Thus, our data clearly indicated that the

rearrangements with a newly generated octamer sequence were caused by the integration of *ISShaI*. The deletion associated with this rearrangement, therefore, seemed to be coupled with the precise excision and integration of the *ISShaI* copies. More specifically, it can be explained by the excision and self-integration of a composite transposon. Figure 3 illus-

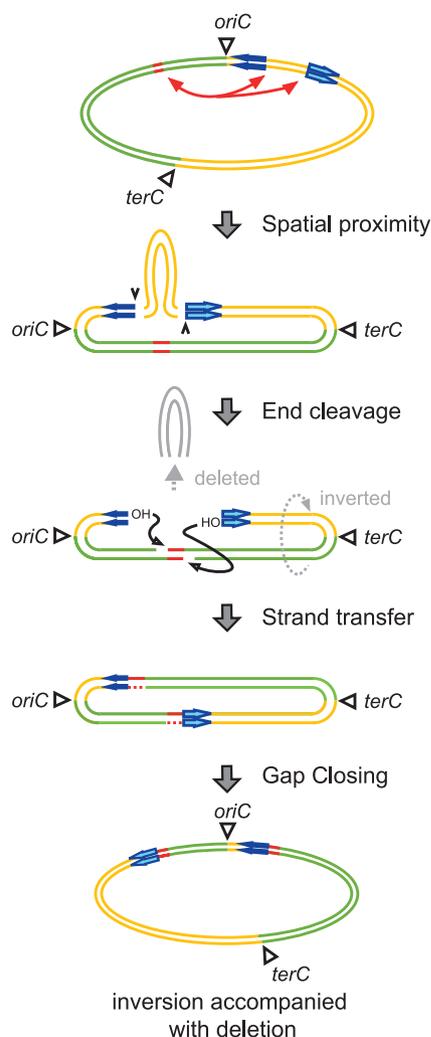


FIG. 3. Model for the ISShaI-mediated deletion/inversion and simple deletion. The diagram shows the model for ISShaI-mediated deletion/inversion. The blue and purple arrows indicate ISShaI. The deletion (designated End cleavage) is thought to occur as a result of precise excision of a huge composite transposon made up of a large *S. haemolyticus* chromosome flanked by two copies of ISShaI at both extremities. Subsequent to the excision, the transposon is integrated into itself at a preferred site (8-bp AT-rich sequence). The resultant genome is characteristic in its big part of the chromosome inverted relative to the *oriC* region. The integration makes the target site duplication that is identifiable (as "inverted" repeats) in the chromosomes of derivative strains adjacent to the two ISShaI copies involved in the integration.

trates this hypothesis. A composite transposon with a large chromosomal region flanked by two ISShaI copies excises itself (with cleavage sites at the outside edges of the IRs) and subsequently integrates itself into the self DNA, generating the octamer DR at the target site of integration. As a result, the chromosome is left with a big inversion and deletion.

ISs are known to cause inversion or deletion through a homologous recombination mechanism (4). However, the members of many IS families are reported to form chromosomal deletions mediated by their transposases, where recombination takes place precisely at the end of the IS element (1, 7, 8, 13). In staphylococci, we previously described IS431 trans-

posase-mediated large-scale deletions in a methicillin-resistant *Staphylococcus aureus* chromosome (20). All deletions start precisely at the edge of a unique IS431 copy but end at various chromosomal loci in the chromosome. *S. haemolyticus* possesses IS431 copies. ISs also generate deletions in *S. haemolyticus* strains (6) but were found only occasionally in JCSC1435 (18).

IS10/Tn10 also causes deletions with a similar mechanism (15). Tn10 is a composite transposon demarcated by two IS10 copies at both ends. It has been reported recently that an experimentally inserted Tn10 copy in the bacteriophage lambda genome or the *Salmonella enterica* serovar Typhimurium chromosome causes two types of genome rearrangements similar to those observed in this study (7–9, 14, 16). The first type is a transposase-promoted deletion that removes a single contiguous DNA segment starting precisely at one internal terminus of IS10 of the transposon. The second type is a more complex rearrangement involving both an inversion and a specific deletion of Tn10. The observed DNA rearrangements occurred almost always at the internal termini of IS10 copies. The ISShaI-mediated simple deletion and inversions/deletions described here appear to be caused by a mechanism similar to the IS10/Tn10 mechanism (Fig. 3).

ISShaI may be the most frequent cause of genome rearrangements in *S. haemolyticus* (it is by far the most abundant IS in JCSC1435). In order to investigate the distribution of ISShaI among staphylococci, PCR detection of ISShaI was carried out using 74 *S. haemolyticus* clinical strains, 75 clinical strains of coagulase-negative staphylococci belonging to other species, and 28 *Staphylococcus* type strains, including *S. haemolyticus* type strain ATCC 29970. ISShaI was harbored by all 74 clinical isolates of *S. haemolyticus* tested. No staphylococcal type strain except *S. haemolyticus* ATCC 29970 carried ISShaI. When clinically isolated coagulase-negative staphylococcal strains were examined, only three species other than *S. haemolyticus* carried the IS element; these species were *Staphylococcus capitis* (1/7 strains), *Staphylococcus epidermidis* (4/42 strains), and *Staphylococcus warneri* (1/1 strain). Strains of six other species did not carry ISShaI; these species were *Staphylococcus auricularis* (four strains), *Staphylococcus caprae* (six strains), *Staphylococcus hominis* (five strains), *Staphylococcus hyicus* (one strain), *Staphylococcus lungdunensis* (one strain), and *Staphylococcus sciuri* (eight strains). This strongly suggests that carriage of ISShaI is one of the significant features of *S. haemolyticus*.

JCSC1435 is highly resistant to teicoplanin (MIC, 64 mg/liter). The extreme flexibility of the *S. haemolyticus* genome demonstrated in this study may also be associated with the acquisition of resistance. Studies of JCSC1435 taking advantage of its frequent chromosome rearrangement are under way.

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