

Florfenicol-Chloramphenicol Exporter Gene *fexA* Is Part of the Novel Transposon Tn558

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The florfenicol-chloramphenicol exporter gene *fexA* is part of the novel transposon Tn558 from *Staphylococcus lentus*. Similarities between Tn558 and Tn554 from *Staphylococcus aureus* included the arrangement of the transposase genes *tnpA* to *-C* and an *att554*-like target sequence. Circular forms of Tn558 were detected and suggest the functional activity of this transposon.

Recently the first staphylococcal florfenicol-chloramphenicol efflux gene, *fexA*, was detected on plasmid pSCFS2 of *Staphylococcus lentus* (4). To investigate whether *fexA* is part of a transposable element, the *fexA* flanking sequences in pSCFS2 were analyzed with regard to similarities to other staphylococcal transposons.

Plasmid pSCFS2 was digested with BglII, and the six resulting fragments of sizes between 1.2 and 14.0 kb were cloned separately into the BamHI-digested vector pBluescript II SK(+) (Stratagene, Amsterdam, The Netherlands). Confirmation of the *fexA* gene on the 7.1-kb BglII fragment was done by PCR and by hybridization experiments (data not shown). The PCR primers *fexA*-fw (5'-GTACTTGTAGGTGCAATTACGGCTGA-3') and *fexA*-rev (5'-CGCATCTGAGTAGGACATAGCGTC-3') (amplicon size, 1,272 bp; annealing temperature, 57°C) were used along with *Pwo* polymerase (Peqlab, Erlangen, Germany). To determine a sufficiently long sequence up- and downstream of *fexA*, sequence analysis included parts of the 7.1-kb BglII fragment and the adjacent 14.0-kb BglII fragment. The nucleotide sequence of the *fexA* flanking regions was determined by primer walking on both strands starting from the terminal parts of the *fexA* gene (MWG-Biotech, Ebersberg, Germany).

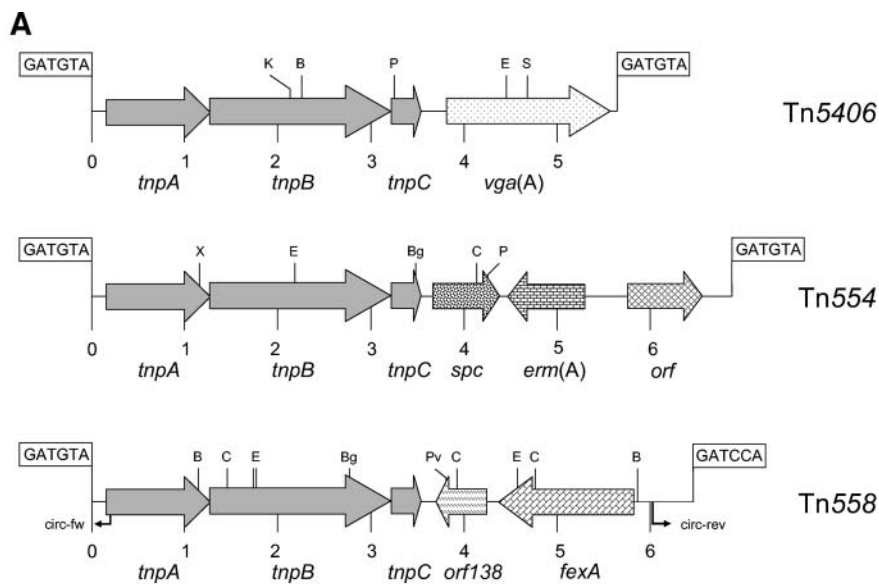
Analysis of a 7,718-bp region revealed the presence of a transposon-like element of 6,644 bp, designated Tn558. This element consisted of five reading frames of more than 120 amino acids (aa), which accounted for 78.5% of the Tn558 sequence (Fig. 1a). Three of these reading frames exhibited similarities to the genes *tnpA*, *tnpB*, and *tnpC*, whose products are involved in the transposition of the *Staphylococcus aureus* transposons Tn554 (1, 5–8) and Tn5406 (3) (Fig. 1a). It is noteworthy that despite the same arrangement and the similar sizes, the *tnpA*, *tnpB*, and *tnpC* genes of the three transposons Tn554, Tn5406, and Tn558 differed considerably in their nucleotide sequences and in the deduced amino acid sequences of their gene products (Table 1). The remaining two open reading frames of Tn558 were orientated in the opposite direction and code for the 475-aa florfenicol-chloramphenicol exporter protein FexA and a putative oxidoreductase of 138 aa,

respectively (Fig. 1a). The deduced amino acid sequence of the latter open reading frame exhibited 45 to 47% identity and 76% similarity to the NAD(P)H oxidoreductases of *Fusobacterium nucleatum* subsp. *nucleatum* (NP_604130) or *Fusobacterium nucleatum* subsp. *vincentii* (ZP_00143945).

As previously reported for Tn554 and Tn5406 (3, 9), Tn558 also did not contain inverted repeats at its ends and failed to generate a duplication of the target sequence at the integration site. Tn558 exhibited the hexanucleotide sequence 5'-GATGT A-3' at the left-end junction and a similar sequence, 5'-GATCC A-3', at the right-end junction. The sequence 5'-GATGTA-3' has previously been described as the “core” sequence of Tn554 and Tn5406 in the primary target site *att554* in the *S. aureus* chromosome (Fig. 1b) (2, 3, 11–13). Studies on serial transposition of Tn554 into primary and secondary target sites revealed that the sequences at the junctions of Tn554 varied with respect to the target sites: with each new transposition event, the sequence originally present in the target site is found at the left end of Tn554, whereas the former left-end junction is now found at the right end and the former right-end junction is lost (5, 10). A similar process is assumed to be responsible for the altered sequence found at the right-end junction of Tn558 in plasmid pSCFS2. Analysis of the regions flanking the Tn558 insertion in plasmid pSCFS2 identified a sequence similar to that of *att554*. A comparison of this pSCFS2 region, designated *att558*, with the sequences up- and downstream of insertion sites of Tn554 and Tn5406 is shown in Fig. 1b. The *att554* sites of Tn554 and Tn5406 are located within reading frames for proteins of 222 aa which show similarity to DNA repair proteins (3). The reading frame including the *att558* site codes for a putative protein of 140 aa which showed 51% identity and 68% similarity to a 147-aa DNA repair protein from *Listeria monocytogenes* (ZP_00231288).

Since transposition of Tn554 and Tn5406 includes the formation of circular forms which precede the integration of the transposon into a new target sequence (3, 5), inverse PCR assays were conducted to detect these circular intermediates. For this, the *Pwo* polymerase (Peqlab) and the two primers circ-fw (5'-CGG TGCCTAATCATTTCGTATGC-3') and circ-rev (5'-CGCTTAA CCGTTCTATCACTTCA-3') (amplicon size, 871 bp; annealing temperature, 62°C) were used; the primers positions are shown in Fig. 1a. Amplicons of the expected size were obtained in repeated experiments from several different *S. aureus* RN4220: pSCFS2 transformants (data not shown). The sequence of such

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B

<i>att554</i>	TTTAAAGGTACATTAATAAGTTCGATTGTACATCCACGTGAAATTTTTAGTATTGCGGTG - Tn554
	F K G T L N S S I V H P R E I F S I A V
<i>att155</i>	TCGATTGGATCTATTAACCAAACAGTGATTCATCCCAGAGAAATATTCAAACAGCGATA - Tn554
	S I G S I N G T V I H P R E I F K T A I
BM3252	TCGATTGGATCTATTAACCAAACAGTGATTCATCCCAGAGAAATATTCAAACAGCAATA - Tn5406
	S I G S I N G T V I H P R E I F K T A I
<i>att558</i>	CATATAGGCACGTTAGATATGTCAATCATACACCCCTAGAGATAGTTTTCAAGTTGCAATA - Tn558
	H I G T L D M S I I H P R D S F Q V A I
<i>att554</i>	AGAGAAAATGCCAATGCAATCATCGCAGTTCATAATCATCCATCCGGT GATGTA ACGCC - Tn554
	R E N A N A I I A V H N H P S G D V T P
<i>att155</i>	CTCAGTAACGCAATAGTATAATGCTCGGTATAATCATCCAAGTGG GATGTA ACACCT - Tn554
	L S N A N S I M L G H N H P S G D V T P
BM3252	CTCAGTAACGCCAACAGTATTATGCTCGGTATAATCATCCAAGTGG GATGTA ACCCCC - Tn5406
	L S N A N S I M L G H N H P S G D V T P
<i>att558</i>	ATAAATAATTGTAATCGTTATTTTTGCACATAACCACCCAAGCA GATGTA ACGCCA - Tn558
	I N N C K S V I F A H N H P S Q D V T P
<i>att554</i>	TCACAAGAAGATATCATAACAACAATGAGGTGAAGGAGTGTGGTTTATTTAGGGATA - Tn554
	S G E D I I T T M R L K E C G L I L G I
<i>att155</i>	TCTCCAGGAGATATCAAATAACTCAGAGAATAAAAGAGATTAGTGAAATGATGGGCATT - Tn554
	S P G D I K I T G R I K E I S E M M G I
BM3252	TCTCCTGAAGATATCAATGTAACCGAACGGTTAATGCTCATCAGTGAATGATGGGCATT - Tn5406
	S P E D I N V T E R L M L I S E M M G I
<i>att558</i>	TCAGTAAACGATATTATGGTTGGTAAGAGATTAAAGCTTATAGGGGAATTGCTAAGTATT - Tn558
	S V N D I M V G K R L K L I G E L L S I
<i>att554</i>	GATTTATTGGATCATATTATAATCGGTGATAATAGATTTACCAGTCTGTAGAAGCGGGT - Tn554
	D L L D H I I I G D N R F T S L V E A G
<i>att155</i>	ATATTATATGACCACATAATATTTTCAGAAAATGATCATCATTCAATTATCAAAAAAATT - Tn554
	I L Y D H I I F S E N D H H S I I K K I
BM3252	TTATTTTATGATCATATTATTTTCAGATAGTAACACCTATTCATTAGACAACATGAT - Tn5406
	L F Y D H I I F S D S N T Y S I R Q H D
<i>att558</i>	GATGTATTAGATGTTTAGTAGTAAGTGATAACAATTATACAAGTTTAGAAGAACTAGAG - Tn558
	D V L D S L V V S D N N Y T S L E E L E

TABLE 1. Comparisons between the transposase genes *tnpA*, *-B*, and *-C* and the corresponding proteins of Tn554, Tn5406, and Tn558

Transposon	Characteristic or comparison result for:											
	<i>tnpA</i>				<i>tnpB</i>				<i>tnpC</i>			
	Size		% Identity ^a		Size		% Identity ^a		Size		% Identity ^a	
	Gene (bp)	Protein (aa)	Gene	Protein	Gene (bp)	Protein (aa)	Gene	Protein	Gene (bp)	Protein (aa)	Gene	Protein
Tn554	1,086	361	100.0	100.0	1,893	630	100.0	100.0	378	125	100.0	100.0
Tn5406 ^b	1,086	361	69.8	72.5	1,899	632	71.4	73.0	384	127	65.1	62.3
Tn558 ^b	1,086	361	69.7	72.3	1,920	639	70.0	71.4	366	121	62.7	57.6

^a Identity with respect to the *tnp* gene and Tnp protein of Tn554.

^b Comparisons between Tn5406 and Tn558 revealed the following percentages of identity at the nucleotide/amino acid sequence level: for *tnpA*, 70.2/71.7; for *tnpB*, 69.5/71.2; for and *tnpC*, 60.9/56.4.

amplicons (MWG-Biotech) consisted of 229 bp of *tnpA* and its upstream region including the 6-bp core sequence (5'-GATGT A-3') at the left end of Tn558, whereas the remaining 642 bp of the amplicon represented the right end of Tn558 up to but not including the sequence 5'-GATCCA-3'. Evidence of the presence of circular Tn558 forms suggested the functional activity of this transposon in staphylococci (3, 5). Based on the aforementioned transposition model, this observation also suggested that the sequence 5'-GATGTA-3' at the left-end junction of Tn558 might be part of the *att558* insertion site.

The data presented in this study showed that the 6,644-bp transposon Tn558 is a member of the Tn554 family of staphylococcal transposons. Although the members of this transposon family share the same overall structure and mode of transposition, they differ distinctly in their resistance gene regions. The macrolide-lincosamide-streptogramin B resistance gene *erm(A)* and the spectinomycin resistance gene *spc* in Tn554 (8, 14) were replaced by a variant of the streptogramin A resistance gene *vga(A)* in Tn5406 (3) and by the florfenicol-chloramphenicol exporter gene *fexA* and a putative oxidoreductase gene in Tn558 (Fig. 1a). The identification of *fexA* as part of a functionally active transposon is an important observation with regard to the mobility of *fexA* and the spread of combined resistance to florfenicol and chloramphenicol. Although the novel transposon is nonconjugative, its location on a plasmid underlines the role of plasmids as vectors for transposon-borne resistance genes in the spread of antibiotic resistance.

Nucleotide sequence accession number. The sequence of Tn558 and its flanking regions has been deposited in the EMBL database under accession number AJ715531.

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FIG. 1. (A) Organization of the *S. lentus* transposon Tn558 in comparison to the structurally related transposons Tn554 (X03216) and Tn5406 (AF186237). A distance scale in kilobases is given below each map. The position and orientation of the genes coding for transposition functions (*tnpA*, *tnpB*, and *tnpC*), antimicrobial resistance [*vga(A)*], streptogramin A resistance; *erm(A)*], resistance to macrolides, lincosamides, and streptogramin B antibiotics; *spc*, spectinomycin resistance; *fexA*, resistance to florfenicol and chloramphenicol], or unknown functions (orf, orf138) are indicated by arrows with the direction of transcription shown by the arrowhead. The restriction endonuclease cleavage sites are abbreviated as follows: B, BclI; Bg, BglII; C, ClaI; E, EcoRI; K, KpnI; P, PstI; Pv, PvuII; X, XhoI. The positions of primers used for the detection of circular Tn554 forms are labeled circ-fw and circ-rev and are indicated by arrows. The 6-bp core nucleotide sequences at the transposon junctions are shown in boxes. (B) Nucleotide and amino acid sequence alignment of the attachment sites *att554* (in *S. aureus* N315 [3]) and *att155* (in *S. epidermidis* [13]) of Tn554, that of Tn5406 in *S. aureus* strain BM3252 (3), and *att558* of Tn558 in plasmid pSCFS2. An attachment site identical to *att554* has also been reported for Tn5406 in *S. aureus* strain BM3327 (3). Grey boxes indicate identical amino acids found in three or more of the aligned sequences. The hexanucleotide core sequences of the integration sites are framed. The black bar above the *att554* sequence indicates the minimum sequence required for transposition into this site as determined by deletion analysis (5, 6).