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Different IS*629* Transposition Frequencies Exhibited by *Escherichia coli* O157:H7 Strains in the Stepwise Evolutionary Model[▽]

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The insertion sequence IS*629*, which is highly prevalent in *Escherichia coli* O157:H7 genomes, was found to be absent in O157:H− strains, which are on a divergent pathway in the emergence of O157:H7. Although O157:H− is deficient in IS*629*, it permits IS*629* transposition, with an excision frequency higher than that of ancestral O55:H7 strains but significantly lower than that of pathogenic O157:H7 strains.

Insertion elements (IS) play an important role in the evolution and genomic diversification of *Escherichia coli* O157:H7 (somatic [O] 157 and flagellar [H] 7 antigen) lineages and have been confirmed to actively transpose in O157 genomes (8). In particular, IS*629* has been found in multiple copies in the *E. coli* O157:H7 genome and is the most prevalent IS in this serotype (8). On the other hand, the ancestral O55:H7 strain (GenBank accession no. CP001846) carries only two IS*629* copies (15). It is striking that O157:H− strains, which are on a divergent evolutionary pathway in the stepwise emergence of O157:H7 (4), are IS*629* deficient (Fig. 1). IS*629* presence/absence was determined in the strains analyzed by using IS*629*-specific primers (IS*629*-insideF, GAACGTCAGCGTCTGAAAGAGC; IS*629*-insideR, GTACTCCCTGTTGATGCCAG), targeting conserved regions of the insertion element previously described by Ooka et al. (9). The reason for the absence of IS*629* among strains in the closely related clonal complex (CC) A4 could be either that an IS*629*-carrying mobile element was excluded from infecting those strains or that CC A4 strains exhibit an IS*629* transposition inhibition mechanism, disabling IS*629* transposition. These strains possess numerous other IS that belong to the IS*3* family, as IS*629* does, and it is thereby possible that these might interfere with its transposition, as has been observed for Tn*5* transposition (10). The mechanism of IS*629* transposition is unknown. However, IS*911*, which is another member of the IS*3* family, transposes replicatively, suggesting that IS*629* could also transpose by the copy-paste mechanism (1, 2).

To investigate if IS*629* transposition is inhibited in the CC A4 strains, we constructed vector pIS*629*AB-Tc and introduced it into various strains belonging to the stepwise evolutionary model for *E. coli* O157:H7 (3). pIS*629*AB-Tc carries an actively expressed IS*629* transposase gene (ORFab), which has been shown to enhance IS*629* excision (5, 6) but which lacks the IS*629* inverted repeats (IR), rendering the transposase unable to excise. It also carries an IS*629* analogue (IS*629*-Tc) in which a tetracycline resistance gene (*tetC*) replaces the IS*629* transposase gene, embedded between both IS*629* IR, truncating the vector's ampicillin resistance (Amp^r) gene (Fig. 2). The IS*629*-Tc construct remains able to transpose if there is no inhibition of IS*629* transposition in the individual strain. In the event of precise IS*629* excision, Amp^r transformants are observed. We introduced this vector into a CC A1 strain (DEC5A), a CC A2 strain (3265-97), CC A4 *E. coli* O157:H− strains (493-89, H56929c, and H1085c), a CC A5 strain (G5101), a CC A6 strain (EDL933), and a possible evolutionary intermediary CC A3 strain (LSU-61) (Table 1). The LSU-61 strain possesses various characteristics from strains belonging to clonal complexes A4 and A5 (4). This vector allowed for determining IS*629* transposition and excision frequency in those strains.

Vector pIS*629*AB-Tc was constructed in two stages. First, ORFab was constructed by site-directed mutagenesis and ligated into vector pUC18, creating pIS*629*AB. Second, IS*629*-Tc was inserted into the *amp* gene of the vector (pIS*629*AB-Tc). In detail, IS*629* ORFab was generated by a 1-bp insertion in the overlapping region of IS*629* ORFa and ORFb by the overlap extension method (7). The *gne* gene of *E. coli* O rough:H7 MA6 containing an IS*629* element was amplified by PCR as described previously by Rump et al. (12), using Platinum *Taq* DNA polymerase high fidelity (Invitrogen, Carlsbad, CA). The ~2,700-bp amplicon was gel purified using a Qiaex II agarose gel extraction kit (Qiagen, Valencia, CA) following the manufacturer's instructions and used to generate two fragments needed to obtain the 1-bp insertion. The amplicon was derived using primers IS*629*F-1 (5′-ATATAGAGCTCATGACTAAAAATACTCGTTTTTC-3′) (restriction site SacI underlined) and IS*629*R-2 (5′-AACATCGTATCGTCGATTGTTATACCAGTCC-3′). The second PCR was conducted using primers IS*629*R-3 (5′-CGGGATCCTCAGGCTGCCAGATCA-3′) (restriction site BamHI underlined) and IS*629*alter-M (5′-TTCGACCGCCTCTGGAAAAAAATGATGCCACTGCTGGATAA-3′), which contained the 1-bp insertion (underlined). Three nanograms of each gel-purified fragment was combined with the others, followed by PCR amplification with the primers IS*629*F-1 and IS*629*R-3 using conditions described previously (7). The purified amplicon was digested accordingly and ligated into pUC18 (Stratagene, La

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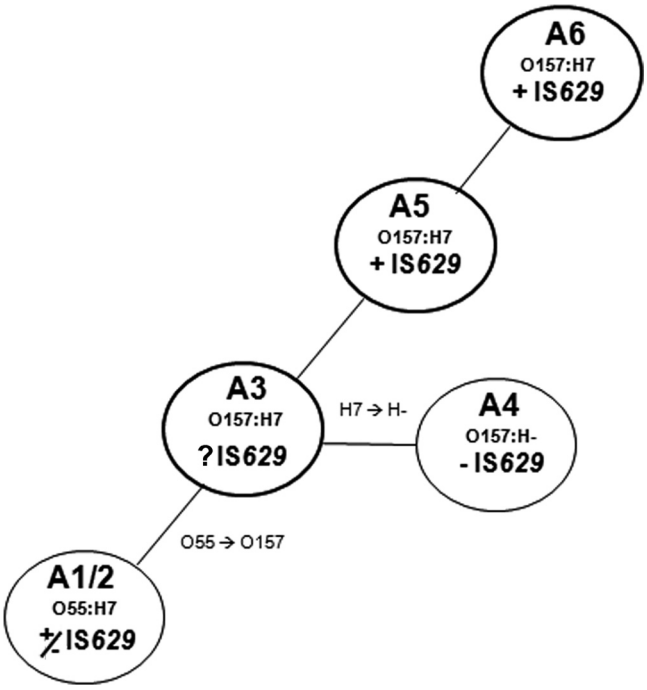

FIG. 1. IS*629* presence/absence in the stepwise evolutionary model of *E. coli* O157:H7 from ancestral O55:H7 strains (representation modified from reference 4). The circles represent the different clonal complexes. IS*629* presence and absence is indicated in bold by +IS*629* and −IS*629*, respectively. Strains belonging to hypothetical CC A3 have not yet been isolated.

Jolla, CA), and the vector construct was electroporated into *E. coli* DH5α (13). Transformants were selected on Luria-Bertani (LB) plates containing 100 μg/ml ampicillin. Amp^r colonies were PCR amplified with vector-specific primers, and those carrying the insert were sequenced in both directions by MCLAB (South San Francisco, CA) to confirm the presence of the ORFab insert in the construct (pIS*629*AB).

IS*629*-Tc was prepared by PCR using primers specific for the 5′ and 3′ ends of the *tetC* gene of the pBR322 plasmid containing both IS*629* IR (IS*629*-Tc-ScaIF [5′-ATCTGAACCGCCCCGGAAATCCTGGAGACTAAACTCCCTGAGAAAGAGGTAAACAGGATGAAATCTAACAATGCGCTCATCGTC-3′] and IS*629*-Tc-ScaIR [5′-GATTGAACCGCCCCGGGTTTCCTGGAGAGTGTTTTATCTGTGAACTCAGGTCGAGGTGGCCCGGCTCCATGC-3′]; the terminal IS*629* seq uences are underlined). The purified amplicon was ligated into the ScaI site of vector pIS*629*-AB. The new vector, pIS*629*A B-Tc, was electroporated into *E. coli* DH5α (13), and tra nsformants were selected on LB plates with 12.5 μg/ml tetrac ycline. Tetracycline-resistant (Tet^r) colonies were PCR amplified with vector-specific primers, and those carrying the insert were sequenced in both directions by MCLAB to confirm the presence of the pIS*629*-Tc insert in the construct (pIS*629*AB-Tc). For transposition frequency studies, pIS*629*A B-Tc was electroporated into the different strains, and 100 CFU of each of the transformants was grown at 37°C overnight in 100 ml of LB broth containing tetracycline (12.5 μg/ml). The transposition occurred during the overnight incubation. Each overnight culture was serially diluted and plated in triplicate on selective LB plates containing either 100 μg/ml ampicillin or 12.5 μg/ml tetracycline. The appearance of Amp^r colonies in comparison to Tet^r colonies is regarded as excision frequency.

We observed that IS*629*-Tc transposed in all strains tested, although with notably different frequencies correlating with grouping by CC, irrespective of the presence or absence of H7 antigen (Table 1). IS*629*-Tc successfully transposed in CC A4 strains, signaling that the absence of IS*629* in the CC A4 strains does not appear to be due to an IS*629* transposition inhibition mechanism. Rather, it is likely that the IS*629*s found in O55:H7 strains were lost and that the CC A4 strains were not in contact with an IS*629*-carrying mobile element after diverging from the hypothetical CC A3 (Fig. 1).

IS*629*-Tc excision frequencies in CC A5 and A6 strains were higher than those in CC A1, A2, and A4 strains. These findings agree partially with previous results from Kusumoto et al. (6), which noted that IS*629*-carrying strains have a higher IS*629* transposition frequency than IS*629*-deficient strains. However, strains from CC A1 and A2 (DEC5A and 3256-97) exhibited a low IS*629* excision frequency, regardless of the presence or absence of IS*629*. The low excision frequency determined for IS*629*-deficient strain 3256-97 (1.5×10^{-8}) was similar to that of other strains lacking IS*629* of various serotypes and genotypes (6). CC A4 strains exhibited a 100-fold-higher excision frequency than the tested CC A1 and A2 strains, although the frequency remained lower than that in CC A5 and A6 strains. This intermediate excision frequency suggested that the presence of IS*629* alone might not enhance the transposition activity of IS*629*-Tc. Kusumoto et al. (6) suggested that IS*629*-possessing strains use a "system to enhance IS*629* excision which might have been introduced by mobile genetic elements that may be linked with IS*629* or other IS elements." This mechanism was recently described by Kusumoto et al. (5) as a protein IS excision enhancer (IEE) which promotes IS*629* excision in O157:H7. Analysis of genome sequences for strains EDL933 (GenBank accession no. AE005174), G5101 (GenBank accession no. AETX01000000), and LSU-61 (GenBank accession no. AEUC01000000) showed the presence of the IEE, explaining the higher excision frequencies. Strain 3256-97 (GenBank accession no. AEUA01000000) lacks the IEE, explaining the low observed IS*629* excision frequency. On the other hand, sequence analysis of two A4 strains (493-89 [GenBank accession no. AETY01000000] and H2687 [GenBank accession no. AETZ01000000]) showed the absence of this specific gene. Hence, the elevated excision frequency in all CC A4 strains relative to that in CC A2 and A1 strains could indicate that these strains possess an upregulation mechanism different than the IEE.

Ooka et al. (8) postulated that IS-related genomic rearrangements may have significantly altered virulence and other phenotypes in O157 strains. However, the elevated IS*629* transposition frequency observed among O157:H7 strains might explain the highly diverse distribution of IS*629* in the O157:H7 genomes (14) and suggests that, in addition to impacting genomic evolution, IS*629* might increase pathogenicity in those strains. Additionally, it might contribute to the appearance of atypical pathogenic strains, like O rough:H7 (*gne*::IS*629* mutant) strains (11, 12). Consequently, should atypical O157:H7 strains become more prevalent, they will create challenges to serological detection methods and could pose a potential health risk. Regardless of the final explana-

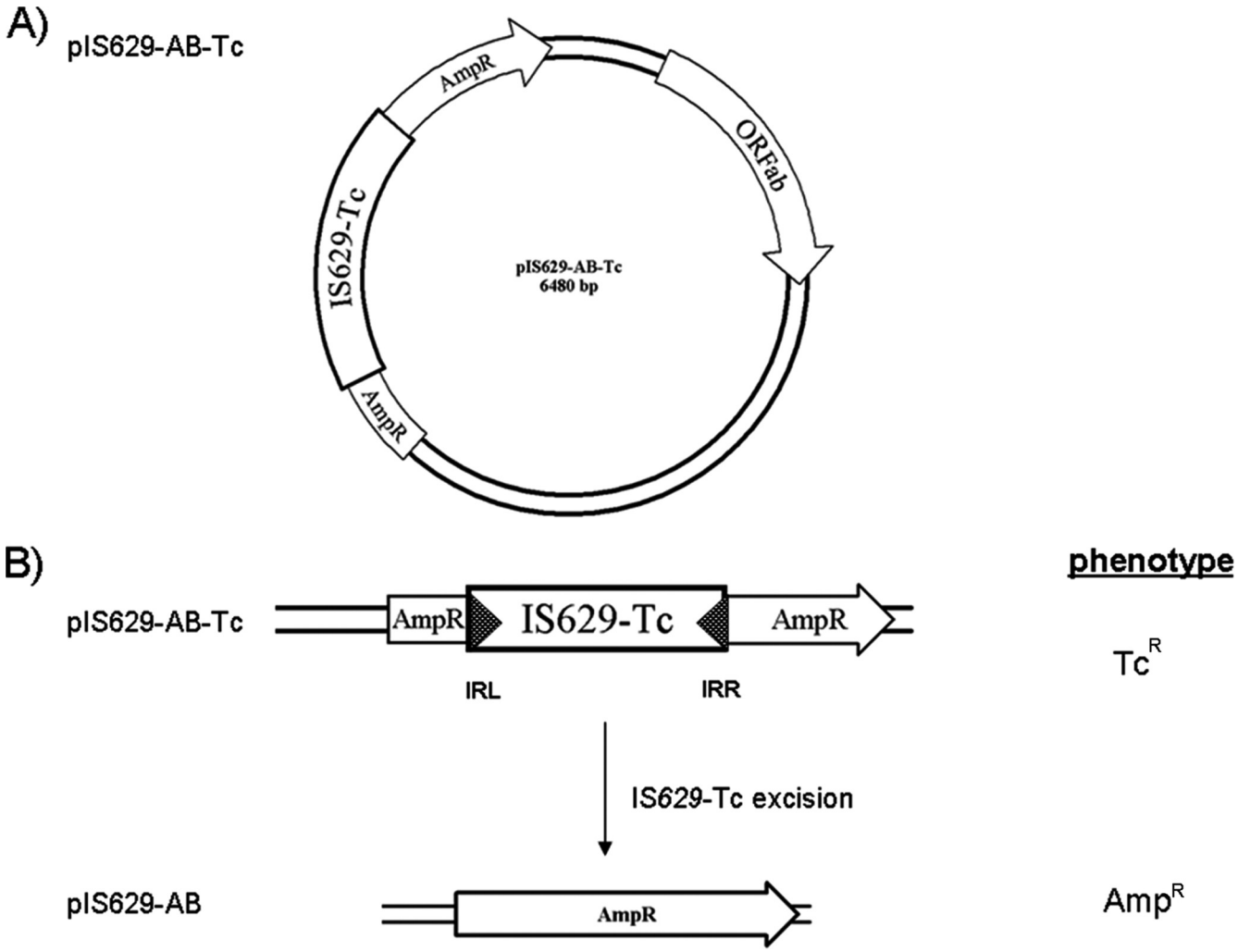

FIG. 2. Schematic representations of the plasmid construct pIS*629*AB-Tc and determination of the transposition frequency. (A) Plasmid construct pIS*629*AB-Tc, containing the IS*629* transposase gene (ORFab) and a tetracycline resistance gene (*tetC*) disrupting the ampicillin (Amp^r) gene. (B) Successful transposition results in ampicillin-resistant colonies (transposition-positive phenotype). Cells showing no transposition remain tetracycline resistant only (original phenotype). Excision frequency was calculated as follows: Amp^r/Tet^r cells.

TABLE 1. Characteristics of *E. coli* strains used in this study and IS629-Tc excision frequencies from pIS*629*AB-Tc in each strain, grouped according to CC, from recent O157 to ancestral O55 serotypes[d]

Strain name	Serotype	CC[a]	IS629[b]	Excision frequency[c]	
				Avg	SD
EDL933	O157:H7	A6	+	1.3×10^{-3}	$\pm 0.4 \times 10^{-3}$
G5101	O157:H7	A5	+	1.6×10^{-3}	$\pm 0.2 \times 10^{-3}$
LSU-61	O157:H7	A?	tr	0.6×10^{-3}	$\pm 0.2 \times 10^{-3}$
493-89	**O157:H−**	**A4**	**−**	$\mathbf{2.6 \times 10^{-6}}$	$\mathbf{\pm 0.9 \times 10^{-6}}$
H56929c	**O157:H−**	**A4**	**−**	$\mathbf{2.2 \times 10^{-6}}$	$\mathbf{\pm 0.3 \times 10^{-6}}$
H1085c	**O157:H−**	**A4**	**−**	$\mathbf{2.3 \times 10^{-6}}$	$\mathbf{\pm 0.4 \times 10^{-6}}$
3256-97	O55:H7	A2	−	1.5×10^{-8}	$\pm 0.6 \times 10^{-8}$
DEC5A	O55:H7	A1	+	2.2×10^{-7}	$\pm 0.1 \times 10^{-7}$

[a] CCs are defined in reference 4.
[b] +, presence; −, absence; tr, truncated IS629.
[c] Three independent experiments were performed for each strain. Excision frequency was calculated as follows: number of Amp^r cells/number of Tet^r cells.
[d] In bold are results for the O157:H− strains lacking IS629.

tion, it is clear that IS*629* has played an integral role in generating genetic diversity across lineages of this important and dangerous bacterial pathogen.

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