Mobilization of the *incQ* Plasmid R300B with a Chromosomal Conjugation System in *Salmonella enterica* Serovar Typhi[▽]

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***Salmonella* pathogenicity island 7 (SPI-7) in *Salmonella enterica* serovar Typhi appears to be related to other genomic islands. Evidence suggests that SPI-7 is susceptible to spontaneous circularization, loss, and transposition. Here, we demonstrate that a region within SPI-7 has the ability to mobilize the small *incQ* plasmid R300B.**

Examples of conjugation systems encoded on the chromosomes of enteric bacteria by conjugative transposons are limited to only a few examples and include CTnR391 from *Proteus rettgeri* and CTnscr94 from *Salmonella enterica* serovar Senftenberg (5, 10). *Salmonella* pathogenicity island 7 (SPI-7) is a 134-kbp genomic island that is situated between two partially copied tRNA^{pheU} genes on the chromosome of *Salmonella enterica* serovar Typhi (11). The original annotation of *S. enterica* serovar Typhi CT18 predicted that SPI-7 contained mul-

tiples regions that constituted a classical pathogenicity island (9). These regions include the locus for the production and exportation of the Vi capsule and the genes responsible for the expression of a type IVB pilus, which aids attachment to human epithelial cells (13).

Modular in structure, SPI-7 appears to have evolved via a series of several independent horizontal gene transfer events. This theory is supported by the truncated nature of corresponding islands in *Salmonella enterica* serovars Paratyphi C

FIG. 1. Alignment of SPI-7 from *S. enterica* serovar Typhi with HAI2 from *Erwinia carotovora* subsp. *atroseptica* SCRI1043. Schematic representation of a pairwise alignment of SPI-7 from *S. enterica* serovar Typhi CT18 with HAI2 from *Erwinia carotovora* subsp. *atroseptica* SCRI1043. Regions of significant amino acid homology are represented by gray bars. The arrows represent the coding sequences, and the colors of the arrows correspond to the predicted functions: blue, *mob*; yellow, *pil*; and purple, *tra* (Table 1). The gene names correspond to the gene names in the respective genome sequences. The numbers at the top and bottom of the figure correspond to the coordinates within the respective genomes (left and right) and the approximate sizes of the compared fragments that have been calculated (center). Also highlighted are the locations of the constructed mutations in SPI-7, $\Delta pilS$, $\Delta pilV$, $\Delta 1$, $\Delta 2$, and $\Delta 3$.

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TABLE 1. PSI-BLAST results of hypothetical regions of SPI-7 in *S. enterica* serovar Typhi CT18^a

Gene ID no.	Annotation ^b	Strand	Size (no. of aa)	Predicted product	Predicted function
STY4521	Polysaccharide synthesis	+	344	Plasmid partitioning (<i>soj</i>)	Plasmid replication
STY4522	DNA helicase	+	454	DNA helicase	Plasmid replication
STY4523	Hypothetical	+	599	DNA binding protein	Plasmid replication
STY4524	Doubtful CDS	+	101		
STY4525	Hypothetical	+	193		
STY4526	Hypothetical	+	185		
STY4528	Hypothetical	+	447		
Miscellaneous	Low G-C	+		<i>oriV</i>	Plasmid replication
STY4529	Hypothetical	+	259		
STY4530	Topoisomerase	+	664	Topoisomerase	Plasmid replication
STY4534	Hypothetical	+	149	Periplasmic protein	Unknown
STY4535	Doubtful CDS	+	72		
STY4536	Single-stranded binding	+	178	Single-stranded binding	Plasmid replication
STY4537	Pseudogene-transposase	+	316		
STY4539–STY4553	<i>pil</i> locus				
STY4554	Transferase	+	291	<i>traE</i>	Conjugation
STY4557	Hypothetical	+	304	Plasmid transfer gene	Conjugation
STY4558	Hypothetical	+	246	<i>traG</i>	Conjugation
STY4559	Hypothetical	+	183	<i>traL</i>	Conjugation
STY4560	Hypothetical	+	166	<i>traW</i>	Conjugation
STY4561	Hypothetical	+	189	Gene from R46 plasmid	Unknown
STY4562	<i>traG</i>	+	696	<i>traD</i>	Conjugation
STY4563	Hypothetical	+	252	Maturase	Secretion
STY4564	Hypothetical	+	90	Effector protein	Secretion
STY4565	Hypothetical	+	79		
STY4566	Hypothetical	+	120		
STY4568	Hypothetical	+	217	DnaA chromosomal replication	Plasmid replication
STY4569	Hypothetical	+	299		
STY4570	Hypothetical	+	492	<i>traB</i>	Conjugation
STY4571	Hypothetical	+	147	Secreted peptidase	Secretion
STY4572	Hypothetical	+	139	<i>traC</i>	Conjugation
STY4573	<i>virB4</i>	+	807	<i>traC</i>	Conjugation
STY4574	Hypothetical	+	128		
STY4575	Outer membrane	+	130	ATP binding protein	Conjugation
STY4576	Hypothetical	+	322	<i>traU</i>	Conjugation
STY4577	Hypothetical	+	476	ATP binding protein	Conjugation
STY4578	Doubtful CDS	+	110		
STY4579	Hypothetical	+	501	<i>traG</i>	Conjugation
STY4580	Doubtful CDS		129		
STY4582	Hypothetical		119		
STY4583	<i>ybdN</i>	+	415	<i>ybdN</i>	Plasmid replication
STY4584	<i>ybdN</i>	+	204	Plasmid replication	Plasmid replication
STY4585	Hypothetical		180		
STY4586	Integrase	+	306	DNA binding protein	Plasmid replication
STY4587	Hypothetical	+	169	Topoisomerase	Plasmid replication
STY4588	Hypothetical	+	126	DNA binding protein	Plasmid replication
STY4589	Hypothetical	+	194		
STY4590	Hypothetical	+	98		
STY4591	Hypothetical	+	152	ATPase	Conjugation
STY4592	<i>traC</i>	+	649	<i>traC</i>	Conjugation
STY4593	Hypothetical	+	302		
STY4594	Hypothetical	+	299	ABC transporter	Conjugation
STY4595	Hypothetical	+	119		
STY4596	Hypothetical	+	89		
STY4597	<i>samB</i>	+	424	Plasmid protein <i>samA</i>	

^a The table displays the gene ID number from the *Salmonella* serovar Typhi CT18 sequence, the original annotation, the strand on which the gene is encoded, the size of the hypothetical protein (in amino acids [aa]), the predicted gene product of the best BLAST result, and the function of the predicted gene product.

^b CDS, coding sequence.

and Dublin and the plethora of genes on SPI-7 from *S. enterica* serovar Typhi that appear to be involved in recombination (11). On closer examination, SPI-7 contains several modules containing hypothetical genes that are not involved in pathogenicity but that may be involved in the transfer of the element. Evidence demonstrates that not only is SPI-7 capable of in-

serting into a different tRNA^{phe} site, but in some *S. enterica* serovar Typhi strains, SPI-7 is susceptible to spontaneous excision and deletion (3, 8). Furthermore, it has been shown that SPI-7 belongs to a family of genomic islands, including pKLC102 from *Pseudomonas aeruginosa* and HAI2 from *Erwinia carotovora* (2, 6) (Fig. 1). Therefore, SPI-7 has many of

FIG. 2. Graph showing the number of transconjugants obtained after mating *S. enterica* serovar Typhi BRD948, $\Delta pilS$, $\Delta pilV$, $\Delta 1$, $\Delta 2$, and $\Delta 3$ (all containing R300B) with *S. enterica* serovar Typhimurium. Transconjugants for each experiment were selected on medium containing tetracycline and streptomycin, where *S. enterica* serovar Typhimurium alone acted as a negative control for each experiment. The data presented are background-corrected mean averages of the results from three experiments, each containing three replicates (i.e., nine replicates in total). Significant *P* values ($P < 0.05$) determined by Student's *t* test calculated by comparing data from *S. enterica* serovar Typhi BRD948 with the corresponding experimental strain are demonstrated.

the attributes generally associated with conjugative transposons. However, the mobilization of the element or smaller plasmids by conjugative transfer has not been demonstrated.

We hypothesized that SPI-7 could behave like other conjugative transposons and had the ability to transfer to a suitable recipient. To add greater insight into the functions of some of the hypothetical genes and regions within SPI-7, we reannotated by PSI-BLAST the coding sequences between STY4521 and STY4597, encompassing approximately 62 kbp of DNA sequence (Fig. 1). First, it was apparent that there was bias toward the coding sequences on the leading strand (Table 1 and Fig. 1), which is a common feature of plasmids. Second, many of the hypothetical genes encoded on SPI-7 demonstrated similarity to genes from plasmids and other genomic islands, many of which appeared to have a role in plasmid replication or conjugative transfer.

Preliminary conjugative transfer experiments demonstrated that SPI-7 containing a kanamycin determinant could not be transferred from *S. enterica* serovar Typhi into a *Salmonella enterica* serovar Typhimurium recipient or an SPI-7-negative *S. enterica* serovar Dublin strain. However, we were able to transfer the 8,684-bp *incQ* plasmid, R300B, from *S. enterica* serovar Typhi BRD948 into a tetracycline-resistant *S. enterica* serovar Typhimurium recipient, albeit at a low transfer rate (~ 3 in 10^8 recipient cells).

Using the lambda red recombinase system described by Datsenko and Wanner (4), we constructed five individual *S. en-*

terica serovar Typhi BRD948 mutations by the insertion of a chloramphenicol resistance determinant (Fig. 1). Two of the mutations were gene specific and located within the *pil* region. The interrupted genes included the major pilin gene (*pilS*) and a gene adjacent to a *shufflon* which mediates bacterial self-association (*pilV*) (7); these mutations were named $\Delta pilS$ and $\Delta pilV$, respectively. The remaining three mutations encompassed several genes each and were in the region of SPI-7, containing the majority of the genes that we hypothesized to be involved in the conjugative transfer. This region included homologues to the conjugative transfer genes *traC* and *traG*. The mutations were named $\Delta 1$, $\Delta 2$, and $\Delta 3$, and the fragments removed by each deletion were 4,694 bp, 6,698 bp, and 6,999 bp, respectively (Fig. 1). The chloramphenicol resistance determinant was removed using pCP20, thus making the mutations nonpolar. All mutations were checked by PCR for the correct deletions, and all strains were transformed with R300B. R300B has an active *oriT* and the relaxosomal genes required for conjugation; however, it is non-self-transmissible and requires a donor mating pair formation for mobilization (12).

Conjugation experiments were performed by mixing mid-log-phase cultures of the various donors with an *S. enterica* serovar Typhimurium recipient harboring a tetracycline resistance determinant. The *S. enterica* serovar Typhimurium recipient alone acted as the negative control. The bacterial cultures were washed in 10 mM $MgSO_4$ and injected through a 0.45- μm nitrocellulose filter. The filters were placed on mini-

mal medium plates and incubated at 37°C overnight. Filters were removed and grown in Luria-Bertani medium without selection for 2 h. The bacterial cultures were pelleted via centrifugation, and transconjugants were selected on Luria-Bertani medium supplemented with 150 µg/ml of streptomycin and 100 µg/ml of tetracycline. Conjugation was initially confirmed by antibody agglutination and plasmid extraction on the transconjugants. Experiments were conducted on three separate occasions, each consisting of three replicates.

The results of the conjugative transfer of R300B into the various strains are shown in Fig. 2. The *pilS* and *pilV* mutations did not significantly reduce the mobilization rate of R300B from the *S. enterica* serovar Typhi donor to the *S. enterica* serovar Typhimurium recipient. However, $\Delta 1$, $\Delta 2$, and $\Delta 3$ all diminished the detectable level of conjugative transfer of R300B by over 10-fold. The conjugation rates of $\Delta 1$, $\Delta 2$, and $\Delta 3$ were significant ($P < 0.05$) by Student's *t* test with respect to *S. enterica* serovar Typhi BRD948.

The acquisition and maintenance of elements like SPI-7 may present a dramatic change in the phenotype of the bacteria, which can aid pathogenesis, transmission, or survival. Indeed, the gain of large fragments of DNA which aid the lifestyle of *S. enterica* serovar Typhi has been well documented (1). However, the mechanisms of acquisition and dissemination of such genomic islands are poorly understood and difficult to examine under laboratory conditions. Here we have demonstrated that SPI-7 contains a functional conjugation system that permits the transfer of a small *incQ* plasmid. We were, however, unable to detect the conjugation of the whole island into suitable recipients under these conditions, which suggests that this ability may have been lost since the original acquisition of SPI-7. These data add insight into the mechanics of how such elements are acquired and how related genomic islands may be able to be mobilized between strains or act as donor systems for the movement of other small pieces of horizontally transferred DNA.

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