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Truncated *tni* Module Adjacent to the Complex Integron of *Salmonella* Genomic Island 1 in *Salmonella enterica* Serovar Virchow[▽]

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***Salmonella* genomic island 1 was identified for the first time in *Salmonella enterica* serovar Virchow isolated from humans in Taiwan. The complex class 1 integron conferring multidrug resistance was shown to be inserted within open reading frame (ORF) S023 and contains for the first time a partial transpositional module. The 5-bp target duplication flanking the complex integron suggests that its insertion in ORF S023 was by transposition.**

Salmonella genomic island 1 (SGI1) is a 43-kb integrative mobilizable element initially characterized in multidrug-resistant *Salmonella enterica* serovar Typhimurium phage type DT104 strains (hereafter serovar Typhimurium DT104) (1, 4). SGI1 is a site-specific integrative element, which is able to excise itself from or integrate into the last 18 bp of the chromosomal *thdF* gene (4). Recently, SGI1 was also demonstrated, during in vitro conjugation experiments, to be able to integrate into a secondary specific attachment site in the chromosome of serovar Typhimurium LT2 (6).

SGI1 contains a complex class 1 integron designated In104 responsible for the pentadrug resistance phenotype of serovar Typhimurium DT104 strains (1, 9). The complex integron In104 and variants of it named SGI1-A to SGI1-Q have been described in several other *S. enterica* serovars and also in *Proteus mirabilis* (2, 3, 7, 9, 10, 13). All In104 integrons and variants of it are found always at the same position in the SGI1 backbone, i.e., between the *res* gene (also named *tnpR*) and open reading frame (ORF) S044 of SGI1 (1, 13).

Recently, Levings et al. have described an unusual SGI1 variant in *Salmonella enterica* serovar Emek strains isolated between 1999 and 2002 (11). The complex class 1 integron initially named SGI1-J contains the *dfrA1-orfC* cassette array in the first *attI1* site and a deletion at the second *attI1* site. Flanked by the two integron-like structures are a new variant of the *floR* gene called *floR2* or *cmlA9* and the tetracycline resistance genes *tetR* and *tet*(G) (8, 11). For the first time, this complex integron, relative to In104, was found inserted within ORF S023 of the SGI1 backbone. For this major reason, Levings et al. chose to rename SGI1-J as SGI2 (9, 11). This point of nomenclature will be further discussed below.

In the present study, we examined three *Salmonella enterica* serovar Virchow strains, isolated from human blood in 1993 and 1994 in Taiwan. These strains lacked plasmids and dis-

TABLE 1. Characteristics of multidrug-resistant *S. enterica* strains

Serovar and strain(s)	Antibiotic resistance profile[a,b]	SGI1 integron-borne cassette(s)	Complex integron variant	Integron position in SGI1 (GenBank accession no. AF261825)	Chloramphenicol and florfenicol resistance gene variant	MIC (μg/ml)[c]	
						Chloramphenicol	Florfenicol
Typhimurium strain BN9181	AmpChlFloStrSptSulTet	*aadA2*, bla_{PSE-1}	In104	26636	*floR*	256	64
Virchow strains B94, B98, and B100	**ChlFloSulTetTmp**-NalFlu	*dfrA1-orfC*	InSGI1-J	19747	*floR2*	>256	32

[a] Abbreviations: Amp, ampicillin; Chl, chloramphenicol; Flo, florfenicol; Str, streptomycin; Spt, spectinomycin; Sul, sulfonamides; Tet, tetracycline; Tmp, trimethoprim; Nal, nalidixic acid; Flu, flumequine.
[b] For antibiotics indicated in boldface, resistance was conferred by SGI1.
[c] The MIC breakpoints for phenicols were defined by the Antibiogram Committee of the French Society of Microbiology, i.e., susceptible (MIC, ≤8 μg/ml) or resistant (MIC, >16 μg/ml) (12).

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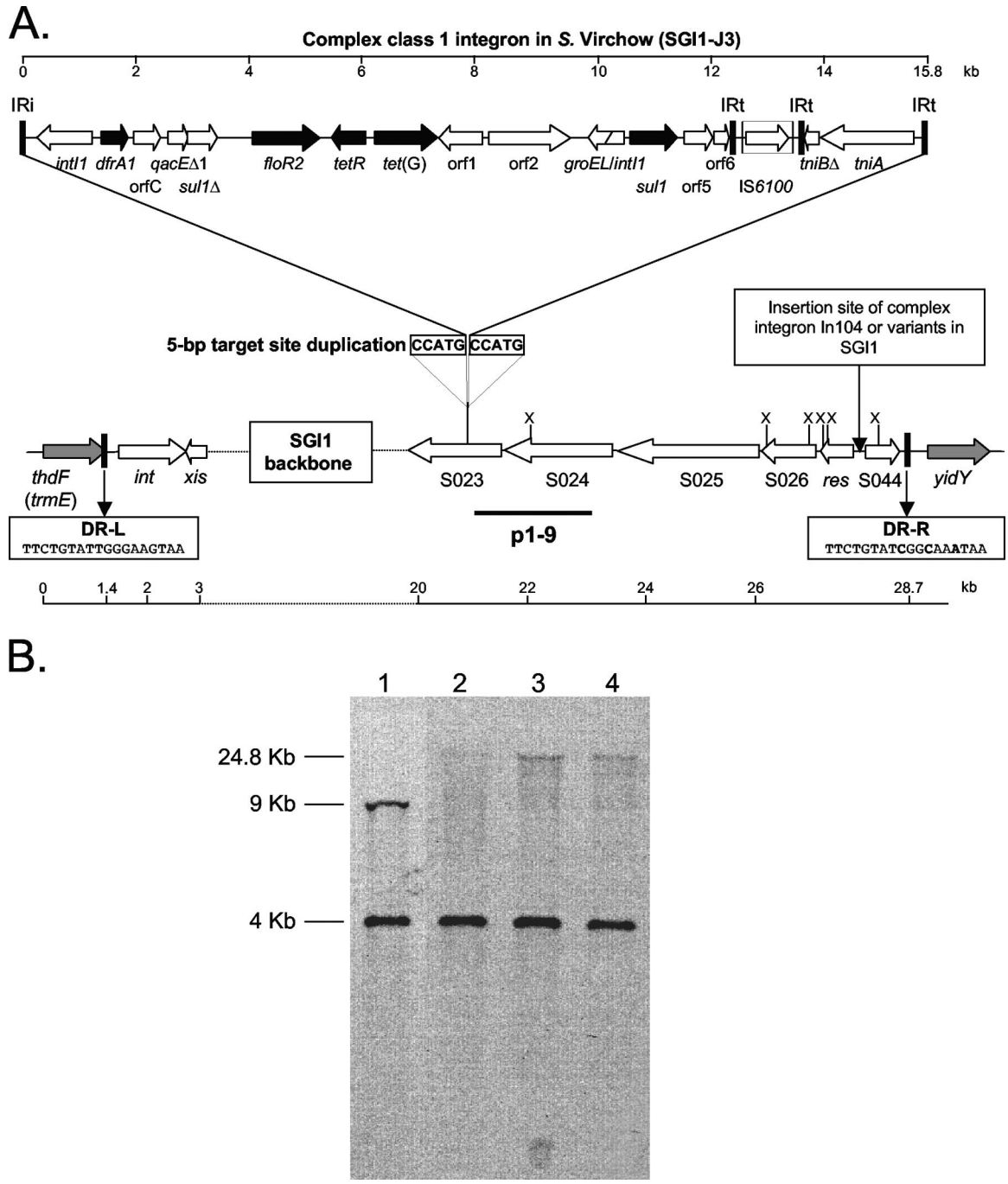

FIG. 1. (A) Genetic organization of the complex class 1 integron in SGI1-J3 from serovar Virchow isolates. Black and gray arrows correspond to SGI1 antibiotic resistance genes and chromosomal genes flanking SGI1, respectively. DR-L and DR-R are the 18-bp left and right direct repeats, respectively, bracketing SGI1. IRi and IRt are 25-bp imperfect inverted repeats defining the left and right end of complex class 1 integrons. The usual insertion point of the complex integron In104 or variants of it in SGI1 and the 5-bp target site duplication flanking the complex integron (SGI1-J3) in ORF S023 are indicated (GenBank accession number EU924797). The p1-9 probe and XbaI restriction sites (X) are indicated. (B) Southern blot hybridization with the p1-9 probe of XbaI-digested genomic DNAs of *S. enterica* serovar Typhimurium DT104 control strain BN9181 carrying SGI1 (lane 1), serovar Virchow strain B94 (lane 2), serovar Virchow strain B98 (lane 3), and serovar Virchow strain B100 (lane 4).

played a multidrug resistance profile (Table 1) suggesting the presence of SGI1.

Detection of SGI1 and its location in the chromosome were performed by PCR as previously described (5). PCR results were positive for the left and right junctions, indicating that the three serovar Virchow strains harbor SGI1 at the same chromosomal location, i.e., between the chromosomal *thdF* and *yidY* genes as in other *S. enterica* serovars (Fig. 1A). The junction PCR products were sequenced to analyze the left and right direct repeats DR-L and DR-R, respectively. The 18-bp

DR-L sequence is almost identical to the 18-bp *attP* sequence of SGI1 previously described (4). However, the 18-bp DR-R sequence showed three substitutions compared to DR-L (Fig. 1A). PCR mapping of the In104 integron of SGI1 was performed as previously described (5). This mapping revealed only the presence of the tetracycline resistance genes *tetR* and *tet*(G) (5). Thus, PCR of the integron-borne cassettes was undertaken and revealed only a 1.3-kb fragment in the three serovar Virchow strains. Sequence analysis showed the presence of the trimethoprim resistance gene cassette *dfrA1* and a gene cassette of unknown function, *orfC*. These results suggested the occurrence of the SGI1-J complex integron variant recently described in serovar Emek strains (11). Then, PCRs were carried out from the *sul1*Δ gene to the *tetR* gene and the resulting product was sequenced. Sequence analysis confirmed the occurrence of the *floR2* resistance gene (Fig. 1A). Moreover, chloramphenicol and florfenicol MICs suggested that this variant of the *floR* gene conferred a resistant phenotype on both antibiotics (Table 1) according to susceptibility breakpoints (12). Thus, the name *floR2* may be much more appropriate than *cmlA9* as proposed by Levings et al. (11), as all *cmlA* genes confer only chloramphenicol resistance and not florfenicol resistance (16). Interestingly, the region extending from IRi to the *groEL-intI1* fusion gene was also found in the antibiotic resistance gene cluster from an epidemic multidrug-resistant *Acinetobacter baumannii* strain isolated in France (8). The remaining part of the complex integron was mapped by PCR from the *tetR* gene to the IS*6100* element by using primers previously described (7).

To detect the boundaries of the complex integron with the SGI1 backbone, PCRs were applied using a forward primer in S023 with a reverse primer in the *intI1* gene and an IS*6100* forward primer with a reverse primer in S024. Sequence analysis of the left boundary of the complex integron showed that the complex integron of serovar Virchow is inserted in SGI1 exactly at the same position within ORF S023 as in serovar Emek (Table 1; Fig. 1A). However, the right boundary PCR product was 2.1 kb larger than the size expected from the SGI1-J (SGI2) sequence with the GenBank accession number AY963803 (11). Sequence analysis revealed that, adjacent to the short segment of 152 bp derived from the IRt outer end of Tn*402*, the complex integron of serovar Virchow strains harbored the right outer end of the mercury resistance transposon Tn*5058* (GenBank accession no. Y17897) (14, 15). This fragment contains a short part of the *tniB* gene (*tniB*Δ) coding for an ATPase DNA binding protein, a transposase *tniA* gene, and the IRt outer end of Tn*5058* (Fig. 1A). The 5-bp duplication target is found downstream of this third IRt copy of Tn*5058*. The location of the complex integron within S023 was confirmed by XbaI-Southern blot hybridization using the p1-9 probe (Fig. 1B) (4, 6). The p1-9 probe showed two XbaI fragments, one fragment of the expected 4-kb size as in the SGI1-carrying control strains and another less-visible fragment larger than 20 kb due to the insertion of the complex integron in S023 (Fig. 1A and B). The 5′ region of SGI1 of approximately 15 kb was mapped by PCR and did not reveal differences of genetic organization (data not shown).

The three serovar Virchow strains studied here harbored an SGI1 variant very similar to that recently described for serovar Emek (9, 11). Levings et al. sequenced 20% of the SGI1 backbone in serovar Emek and revealed more than 99.7% nucleotide identity to the sequence of SGI1 (GenBank accession no. AF261825) (11). Except the complex integron variant, the only major change from other SGI1 variants is the insertion point within the SGI1 backbone. Thus, according to the previously proposed SGI1 nomenclature, it would be preferable to keep the name SGI1 and to add a letter and a number if necessary to classify variants of SGI1 (7). We propose to maintain the name SGI1-J for the variant described in serovar Emek strains (SGI1-J corresponds to SGI2 and SGI1-J2 corresponds to SGI2-A) and to name the variant described here in serovar Virchow as SGI1-J3 according to the few differences between these two variants.

In conclusion, serovar Virchow represents the15th serovar of *S. enterica* harboring a variant of SGI1 named SGI1-J3 in which the integron transposition occurred in a different location than those in the other SGI1 variants. The presence of a large part of the *tni* module of Tn*5058* containing the transposase *tniA* gene could facilitate the transposition event of this complex integron. SGI1 seems to be a "hot spot" of acquisition of complex In4-type integrons or transposon structures as recently described in variants SGI1-K, -P, and -Q (7) and thus serves as a vehicle to transfer multidrug resistance between different bacterial genera.

Nucleotide sequence accession number. The partial sequence of the SGI1-J3 variant has been deposited in GenBank under accession number EU924797.

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