ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2008, p. 340–344 Vol. 52, No. 1
0066-4804/08/$08.00+0 doi:10.1128/AAC.00902-07

Salmonella Genomic Island 1 (SGI1), Variant SGI1-I, and New Variant SGI1-O in *Proteus mirabilis* Clinical and Food Isolates from China▽

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Received 10 July 2007/Returned for modification 22 August 2007/Accepted 25 October 2007

***Salmonella* genomic island 1 (SGI1) and variants (SGI1-I and the new variant SGI1-O) were mapped in five strains of *Proteus mirabilis* isolated from humans and food in China. Sequencing showed that SGI1 and variants were integrated at the 3′ end of the chromosomal *thdF* gene as previously described for *Salmonella* strains.**

Salmonella genomic island 1 (SGI1) is a 43-kb integrative mobilizable element initially characterized in *Salmonella enterica* serovar Typhimurium phage type DT104 strains (3, 7). SGI1 contains a multidrug resistance region (MDR), which is a complex class 1 integron containing all the genes necessary for resistance located between a resolvase-like gene (*res*) and a gene of unknown function (S044) (Fig. 1). SGI1 and variants of it named SGI1-A to SGI1-N have been described for other *Salmonella enterica* serovars (5, 11, 12, 14, 17, 18). In *Salmonella* SGI1 is integrated in the last 18 bp of the *thdF* gene (specific *attB* site) (7, 14). SGI1 has been shown to be mobilized using a conjugative helper plasmid and thus transferred to *Salmonella* or *Escherichia coli* recipient strains (7). Typically SGI1 integration can be confirmed by PCR targeting the left junction region with primers U7-L12 (*thdF* specific) and LJ-R1 (SGI1 *int* specific) and/or the right junction region with primer 104RJ (SGI1 S044 specific) and either primer C9-L2 (serovar Typhimurium retron *int* specific) or primer 104D (*yidY* specific) (Fig. 1) (2, 3). Recently the presence of an SGI1 variant (SGI1-L) was described in clinical strain *Proteus mirabilis* 18306, the first bacterium other than *Salmonella* shown to harbor this element (1). In *P. mirabilis* 18306 the left junction region could not be detected by PCR, and it was concluded that SGI1 may be integrated elsewhere in the genome (1). In a comment on that report it was pointed out that the *thdF* gene of *P. mirabilis* HI430 is only 70% identical to the serovar Typhimurium gene and that in fact the primer U7-L12 binding site contains seven nucleotide differences and, thus, this may explain the failure of the left junction PCR (Fig. 1) (8).

Strains characterized in this study are listed in Table 1. Primers used in this study are listed in Table 2. PCR was carried out with Amplitaq Gold (Applied Biosystems, Foster City, CA) or the FailSafe PCR System (Epicentre Biotechnologies, Madison, WI). Sequencing was by automated dideoxy cycle sequencing.

We recently screened 30 clinical *P. mirabilis* strains isolated from the environment, food, and stool of diarrheic patients in China and *P. mirabilis* ATCC 29906 and ATCC 29245 for the cassettes of class 1 integrons (5′-CS/3′-CS primers) and detected amplicons in seven strains. (Table 1). All seven strains were multidrug resistant, with the number of drugs ranging from four up to nine (Table 1). PCR mapping and/or DNA sequencing of the cassettes revealed that one strain harbored *aadA2* and bla_{PSE-1}, one strain harbored *aadA2* and *dfrA1-orfC*, three strains harbored *dfrA1-orfC*, and two strains harbored *dfrA7* (Table 1; also see below). We used the *thdF* gene of serovar Typhimurium as a query sequence in a BLAST search of the completely sequenced genome of *P. mirabilis* HI4320 (http://www.sanger.ac.uk/Projects/P_mirabilis) to find the *thdF* homologue and flanking sequence and designed primers PmLJ1 and PmRJ1 (Table 2; Fig. 1). We note that the *P. mirabilis* HI4320 *thdF* gene is located 145 bp upstream from the start of *hipB*/*hipA* toxin/antitoxin homologues (Fig. 2). We conducted PCR with primers PmLJ1 and LJ-R1 (left junction) on all 30 *P. mirabilis* clinical strains and the two ATCC strains and obtained an amplicon of the predicted size (500 bp if SGI1 was present) only with DNA from the five strains that harbored 1- and/or 1.2-kb cassettes. The seven strains that harbored cassettes were then analyzed by PCR with primers 104RJ and PmRJ1 (right junction), and only the five strains positive for the left junction region produced an amplicon of the expected size (410 bp). The two strains harboring the *dfrA7* gene (accession number X58425; 0.75-kb cassette) were not further studied, as it was concluded that although they contained integrons and were multidrug resistant, they were not associated with SGI1 (Table 1). Sequence analysis of both the left and right junction amplicons from *P. mirabilis* B02012 confirmed that the last 18 bp of the *thdF* gene corresponding to the left direct repeat (DR-L) is 100% identical to the right 18-bp direct repeat (DR-R) (Fig. 1). This result is in accordance with the sequenced genome of *P. mirabilis* HI4320, in which the last 18 bp of the putative *thdF* gene (the *attB* site) is identical to the previously described *attP* site of SGI1 (7, 8). Thus, five *P. mirabilis* strains appeared to have SGI1 or a variant integrated at the end of the *thdF* gene as in *Salmonella*. Further, all five

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▽ Published ahead of print on 19 November 2007.

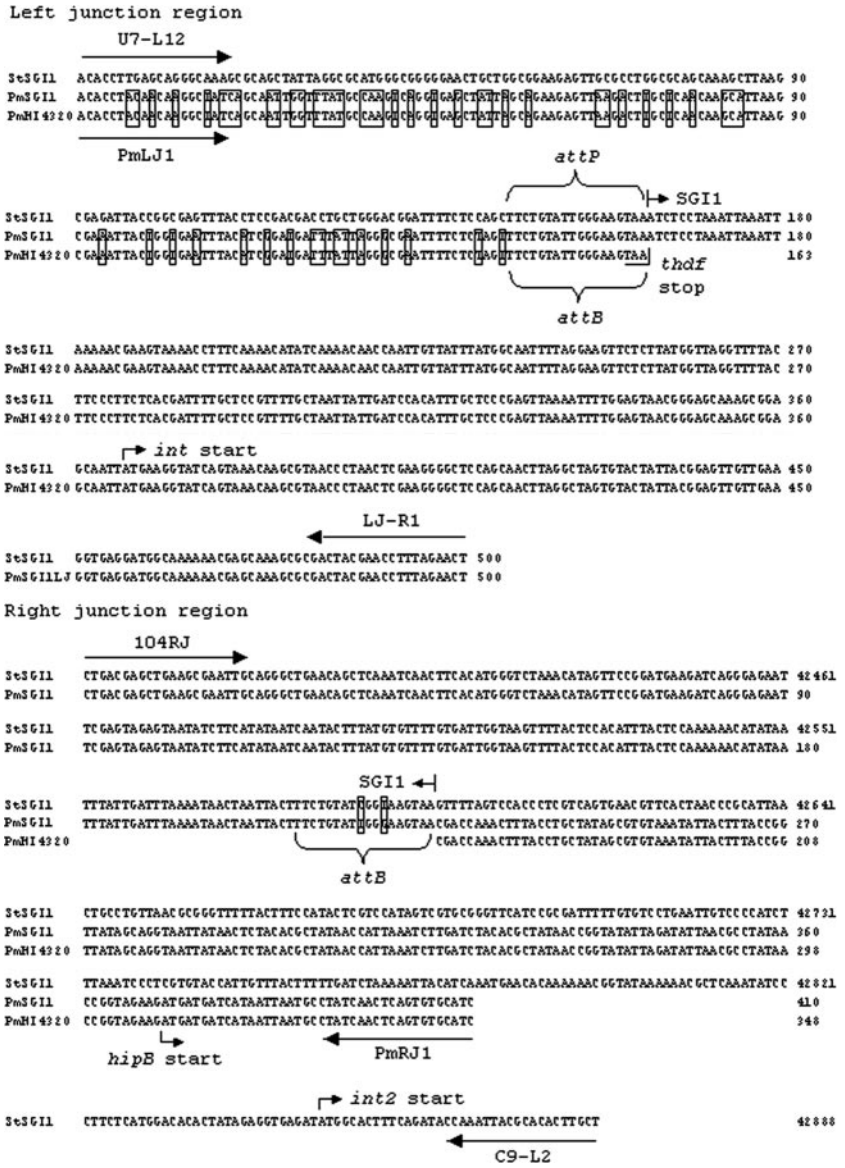

FIG. 1. Sequence alignment of the left (top) and right (bottom) junction regions of SGI1 from *S. enterica* serovar Typhimurium DT104 (StSGI1) and *P. mirabilis* B02012 (PmSGI1) and the genomic region from *P. mirabilis* HI4320 containing *thdF* and the downstream region. The nucleotide differences between the *Proteus* and *Salmonella thdF* genes are boxed (top) as are the ones in the *attB* site (bottom). The nucleotide differences between the *Salmonella* and *Proteus* sequences after the right junction (bottom) are not indicated, as these sequences are not homologous. Base pair coordinates for StSGI1 are from the sequence with accession no. AF261825. Primers used to detect the junction regions are indicated by arrows.

TABLE 1. *P. mirabilis* strains characterized in this study

Strain	Integron cassette(s)		SGI1 type	Antibiogram[c]	Isolate source
	Gene(s)[a]	Size(s) (kb)[b]			
B02012	*aadA2*, bla_{PSE-1}	1, 1.2	SGI1	AmChCiKaNaStSuTeTm	Patient
C02035	*aadA2*, *dfrA1-orfC*	1, 1.2	SGI1-I	ChStSuTeTm[d]	Food
C04014	*dfrA1-orfC*, bla_{PSE-1}-*aadA2*	1.2, 2.0	SGI1-O	AmChCiKaNaStSuTeTm[e]	Food
C05022	*dfrA1-orfC*	1.2	SGI1-O	AmChKaStSuTeTm	Patient
C05023	*dfrA1-orfC*	1.2	SGI1-O	AmChKaStSuTeTm	Patient
P06020	*dfrA7*	0.75	None	(Am)ChKaStSuTeTm	Patient
P06021	*dfrA7*	0.75	None	AmChKaStSuTeTm	Patient

[a] As determined by PCR mapping and/or DNA sequencing.
[b] Size of amplicon produced with primers 5′-CS and 3′-CS.
[c] Resistant or intermediate resistance (parentheses) to drugs shown as determined by broth microdilution. Drugs tested: amikacin, ampicillin (Am), amoxicillin-clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol (Ch), ciprofloxacin (Ci), gentamicin, kanamycin (Ka), nalidixic acid (Na), streptomycin (St), sulfizoxazole (Su), tetracycline (Te), and trimethoprim-sulfamethoxazole (Tm).
[d] By streptomycin Etest exhibited a MIC of 8 μg/ml with colonies in the zone. A single colony from the zone exhibited a MIC of 96 μg/ml.
[e] By streptomycin Etest exhibited a MIC of 32 μg/ml with colonies in the zone. A single colony from the zone exhibited a MIC of 96 μg/ml.

TABLE 2. Primers used in this study

Primer[a]	Sequence (5′–3′)	Target region[b]	PCR		Reference
			No.[c]	Size (bp)	
U9-L1 (+)	TACTACAAGCAGATAACGCC	2771–3679	St1	909	13
P1-R1 (−)	TAGAAACGACAAAGCGCGTG				13
P134-L2 (+)	TGACCCAATTCCAAAGCCAC	16784–17839	St4	1,490	13
P134-R1 (−)	GTGTTTGGGCAAGATCCCAG				13
StGP21 (+)	ATAACGGCAGGTTCCGGTTC	20173–21108	St5	936	13
StGP6 (−)	CGATGAAGCGCACAAATTTG				13
StGP24 (+)	TCAAGATTCCTATCTGCAGG	24363–25201	St6	838	13
StGP28 (−)	AGAGTTACTAGACCAAGCGC				13
LJ-R1 (−)	AGTTCTAAAGGTTCGTAGTCG	*int*	1	500	3
PmLJ1 (+)	ACACCTACAACAAGGCTATC	*thdF* of *P. mirabilis*	1	500	This study
			23	348	
104RJ (+)	CTGACGAGCTGAAGCGAATTG	S044	2	476	3
PmRJ1 (−)	GATGCACACTGAGTTGATAG	*hipB* of *P. mirabilis*	2	476	This study
			23	348	
5′-CS (+)	GGCATCCAAGCAGCAAGC	5′-CS region	3	Variable[d]	10
3′-CS (−)	AAGCAGACTTGACCTGAT	3′-CS region	3	Variable[d]	10
tetG-1s (+)	GCTCGGTGGTATCTCTGCTC	*tet*(G)	4	468	15
tetG-2s (−)	AGCAACAGAATCGGGAACAC	*tet*(G)	4	468	15
StCm-L (+)	CACGTTGAGCCTCTATATGG	*floR*	5	888	2
StCm-R (−)	ATGCAGAAGTAGAACGCGAC	*floR*	5	888	2
F4 (+)	TTCCTCACCTTCATCCTACC	*floR*	6	599	6
F6 (−)	TTGGAACAGACGGCATGG	*tetR*	6	599	6
MDR-13 (+)	TCCCGATTCTGTTGCTGCTTG	*tet*(G)	7	1,078	This study
MDR-B3 (−)	AAGCATGGCTGCTGACAAC	*orf1*	7	1,078	This study
floR-R1 (−)	TCAACGTGAGTTGGATCATAG	*floR*	8	1,430	This study
MDR-5 (+)	TAGGTATGGGGCTCATAATTG	*qacEΔ1*	8	1,430	This study
104-D4 (+)	ATGCCTAGCATTCACCTTCC	*sulI*	9	1,183[e]	This study
DB-B4 (−)	ATCACATCACCCTGGAAATGG	IS*6100*	9	1,183[e]	This study
			21	2,954[e]	
			22	2,642[e]	
DB-T1 (+)	TGCCACGCTCAATACCGAC	IS*6100*	10	930	2
DB-B6 (−)	CTGTGCCTTCTTGCGAGC	S044	10	930	This study
StGP-23 (+)	CCTTGGTACGTTCGCTAATC	*res*	11	1,417	This study
			16	1,848	
			20	2,262	
int-R (−)	GCCTTGCTGTTCTTCTACGG	*intI1*	11	1,417	This study
AadA2-L (+)	TGTTGGTTACTGTGGCCG	*aadA2*	12	538	15
AadA2-R2 (−)	TGCTTAGCTTCAAGTAAGACG	*aadA2*	12	538	3
			15	1,316, 2,360[f]	
MDR-20 (+)	TAGTTCAAAGTTTCAGCAAG	bla_{PSE-1}	13	765	This study
			17	1,751, 2,608[g]	
PSE-R2 (−)	ACAATCGCATCATTTCGCTC	bla_{PSE-1}	13	765	3
PSE-R3 (−)	CTGAAACTTTGAACTACTTGC	bla_{PSE-1}	14	935	This study
MDR-19 (−)	AACCGGATCAGAAATCCATGC	*groEL/int*	14	935	This study
			19	1,254	
MDR-1 (+)	TGATCGAAATCCAGATCCTG	*intI1*	15	1,316, 2,360[f]	This study
AadA2-R3 (−)	GGTTCGAAATTTCGATGGTC	*aadA2*	16	1,848	This study
QS-2 (−)	TGAGTGCATAACCACCAGCC	*sul1*	17	1,751, 2,608[g]	3
dfrA1-F (+)	CGAAGAATGGAGTTATCGG	*dfrA1*	18	919	11
orfC-R (−)	TCTCGAATCAAGCAGGAACC	*orfC*	18	919	11
dfrA1-R (−)	TTAGAGGCGAAGTCTTGG	*dfrA1*	19	1,254	11
			20	2,262	
orfC-F (+)	CATTACGAAGCGAA GCACC	*orfC*	21	2,954[e]	11
PSE-D1 (+)	AGAGCGAAATGATGCGATTG	bla_{PSE-1}	22	2,642[e]	This study
Ag5 (−)	ACAAACGACAAGCCACGT	*orf513*	Neg[h]	750	This study
Ag6 (+)	TATCGTCTATCGTACACTCTC	*orf513*	Neg[h]	750	This study

[a] (+), 5′–3′ is left to right in Fig. 2; (−), 5′–3′ is right to left in Fig. 2.
[b] Coordinates are from SGI1 (accession no. AF261825), and genes are as shown in Fig. 2.
[c] PCR numbers as shown in Fig. 2.
[d] Size varies depending on number of cassettes in this region.
[e] Size would be ~2.9 kb larger if this region contained common region 1 (CR1) and *dfrA10* as found in some SGI1 variants (2, 14).
[f] Size was 1,316 bp in SGI1 but 2,360 bp in *P. mirabilis* C04014.
[g] Size was 1,751 bp in SGI1 but 2,608 bp in *P. mirabilis* C04014.
[h] PCR with Ag5/Ag6 primers was negative for all strains tested but would be expected to give a product of 750 bp if CR1 was present.

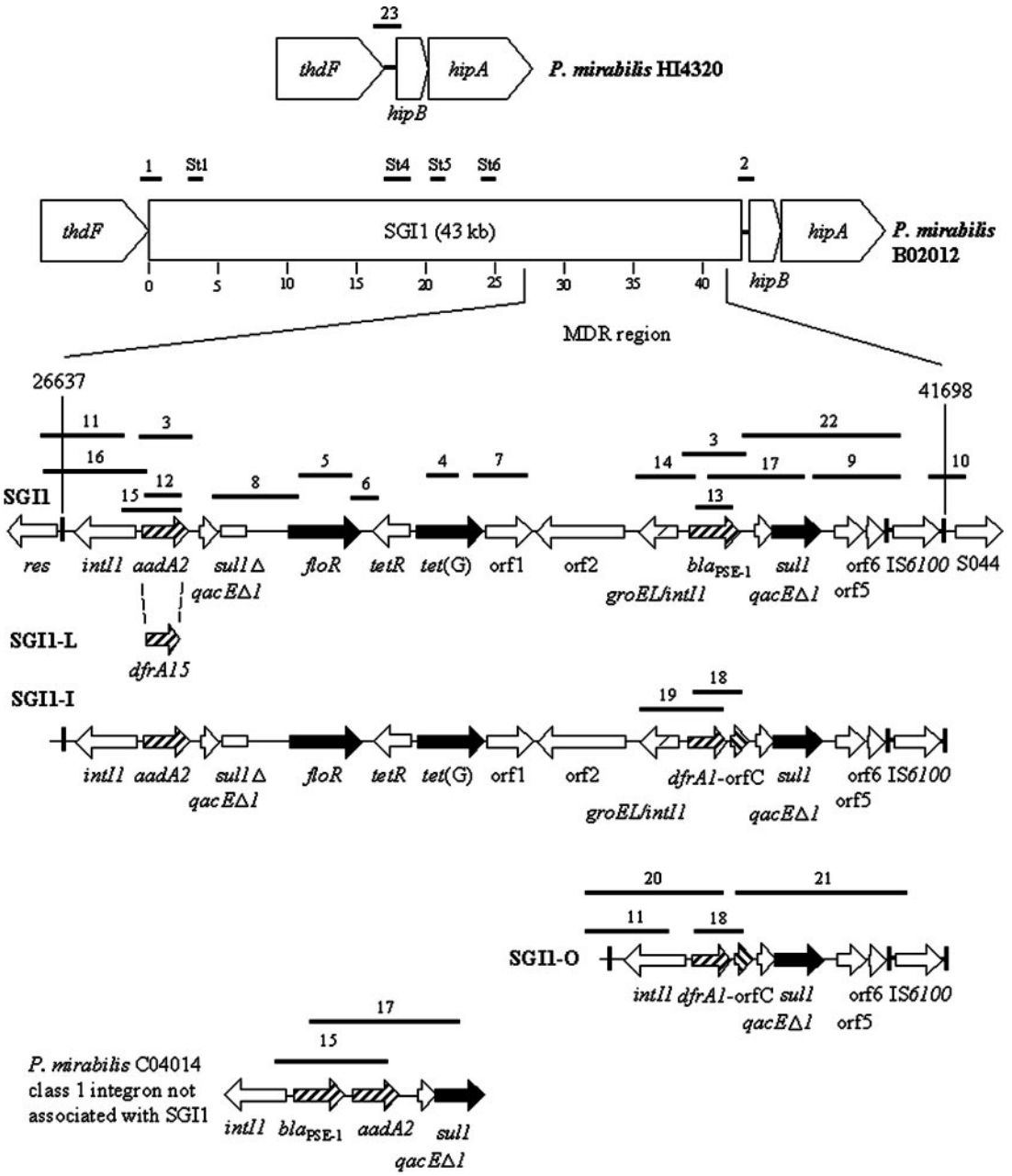

FIG. 2. Schematic view of the SGI1 and SGI1 variants as integrated in *P. mirabilis*. The *P. mirabilis* HI4320 *thdF* gene and downstream region are shown at the top, and the MDRs of SGI1, SGI1-I, SGI1-L, and SGI1-O (not to scale) are shown at the bottom. Base pair coordinates are from the sequence with accession no. AF261825. PCRs carried out to map the SGI1s are indicated by thick black bars and are numbered (Table 1). SGI1 was mapped as shown, and SGI1-I was mapped as for SGI1 and also with PCRs 18, 19, and 21. The bla_{PSE-1}/*aadA2* integron from *P. mirabilis* C04014 was negative in PCRs 9, 10, 11, and 16.

strains were positive in PCRs St1, St4, St5, and St6 designed to amplify various regions of SGI1 outside of the MDR (Fig. 2) (13). We used various combinations of the primers listed in Table 2 to map the SGI1s in the five strains (Fig. 2). One strain contained SGI1 (3), one strain contained SGI1-I (11), and three strains contained a new variant named SGI1-O, which contained only the *drfA1-orfC* cassette. The *orf513* gene from common region 1 (CR1) found in some SGI1 variants was not detected (2). Interestingly, one strain with SGI1-O, *P. mirabilis* C04014 isolated from chicken, was positive by PCR for bla_{PSE-1} and *aadA2*. However, though mapping showed these to be adjacent to one another as the only cassettes in a class 1 integron, they were not linked to regions upstream (*res*) or downstream (IS*6100*) of the MDR of SGI1 (Fig. 2).

Thus, when present SGI1 is integrated via the specific *attB* site defined by the last 18 bp of the *thdF* gene in *P. mirabilis* as predicted (Fig. 1) (8).

The identification of SGI1 and its variants in *P. mirabilis* isolated from food and human clinical isolates from China highlights the increased dissemination of the SGI1 element

both geographically and biologically. The documented potential for increased virulence in strains harboring this element in conjunction with the multidrug-resistant nature of the SGI1 element raises concerns especially since these new strains harboring SGI1 have been identified from food sources (4, 9, 16, 19).

Nucleotide sequence accession numbers. The *dfrA1-orfC* cassette region from SGI1-O and the bla_{PSE-1}-*aadA2* cassette region from *P. mirabilis* C04014 have been assigned accession numbers EU006710 and EU006711, respectively, and the left and right junction regions of SGI1 in *P. mirabilis* B02012 have been assigned accession numbers EU180707 and EU180708, respectively, in the GenBank database.

We gratefully acknowledge the expert technical assistance of Dennis Le and Romeo Hizon. The sequence data for *P. mirabilis* were produced by the *Proteus mirabilis* Sequencing Group at the Sanger Institute and can be obtained from ftp://ftp.sanger.ac.uk /pub/pathogens/pm.

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