



# On the calculation of atomic displacements using damage energy

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## **SGI0, a relative of *Salmonella* genomic islands SGI1 and SGI2, lacking a class 1 integron, found in *Proteus mirabilis***

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1   **Abstract**

2   Several groups of integrative mobilizable elements (IMEs) that harbour a class 1  
3   integron carrying antibiotic resistance genes have been found at the 3'-end of the  
4   chromosomal *trmE* gene. Here, a new IME, designated SGI0, was found in *trmE* in  
5   the sequenced and assembled genome of a French clinical, multiply antibiotic  
6   resistant *Proteus mirabilis* strain, Pm1LENAR. SGI0 shares the same gene content  
7   as the backbones of SGI1 and SGI2 (overall 97.6% and 97.7% nucleotide identity,  
8   respectively) but it lacks a class 1 integron. However, SGI0 is a mosaic made up  
9   of segments with >98.5% identity to SGI1 and SGI2 interspersed with segments  
10   sharing 74-95% identity indicating that further diverged backbone types exist  
11   and that recombination between them is occurring. The structure of SGI1-V, here  
12   re-named SGI-V, which lacks two SGI1 (SGI2 and SGI0) backbone genes and  
13   includes a group of additional genes in the backbone, was re-examined. In  
14   regions shared with SGI1, the backbones shared 97.3% overall identity with the  
15   differences distributed in patches with various levels of identity. The class 1  
16   integron is also in a slightly different position with the target site duplication  
17   AAATT instead of ACTTG for SGI1 and variants, indicating that it was acquired  
18   independently. The Pm1LENAR resistance genes are in the chromosome, in Tn7  
19   and an ISEcp1-mobilised segment.

20

21   **Keywords**

22   *Salmonella* genomic island; Integrative mobilizable element; IncC plasmid

23

24   **Abbreviations**

25 AGI1: *Acinetobacter* Genomic Island 1; IME: Integrative Mobilizable Element; Orf:  
26 open reading frame; PGI1: *Proteus* Genomic Island 1; SNP: Single nucleotide  
27 polymorphism; SGI: *Salmonella* Genomic Island

28

29

## 30 1. Introduction

31 *Salmonella* genomic island 1 (SGI1) which confers ampicillin,  
32 chloramphenicol/florfenicol, streptomycin/spectinomycin, sulphonamides and  
33 tetracycline resistances, was first reported in the epidemic multidrug resistant  
34 *Salmonella enterica* subsp. *enterica* serovar Typhimurium DT104 clone (Boyd et al.,  
35 2000; Threlfall, 2000). SGI1 is an integrative mobilizable element (IME), integrated  
36 at the 3'-end of the *trmE* gene (formerly called *thdF*) (Douard et al., 2010; Doublet et  
37 al., 2005). SGI1 is made up of a 27.4 kb backbone containing 28 open reading frames  
38 (orfs) and a 15 kb complex class 1 integron, designated In104 that includes five  
39 resistance genes (Boyd et al., 2001; Levings et al., 2005). In104 is inserted upstream  
40 of the resolvase gene (*resG*, formerly S027) and flanked by a 5 bp duplication.

41 Since the first report of SGI1, many variants (SGI1-A to SGI1-Z, etc) have  
42 been reported (Hall, 2010; Lei et al., 2015; Qin et al., 2015). They have identical or  
43 nearly identical (>99.78% nucleotide identity) backbones and the variation usually  
44 results from changes in the class 1 integron that affect the resistance gene content (Bie  
45 et al., 2018; de Curraize et al., 2018; Harmer et al., 2016; Lei et al., 2015, 2014;  
46 Siebor and Neuwirth, 2013; Xiao et al., 2019). Most SGI1 variants have been found in  
47 *S. enterica* serovars or in *Proteus mirabilis* but recently SGI1 variants were also  
48 reported in *Morganella morganii* (SGI1-L) (Schultz et al., 2017), in *Providencia*  
49 *stuartii* (SGI1-W) (Soliman et al., 2019) and in *Escherichia coli* (SGI1-B) (Cummins  
50 et al., 2019).

51 However, not all IMEs found in *trmE* are directly derived from SGI1. SGI2,  
52 reported in *S. Emek* is related to SGI1 and was initially called SGI1-J (Levings et al.,  
53 2005). However, it is an independently derived genomic island (Levings et al., 2008).

54 There is 0.53% nucleotide difference between the SGI1 and SGI2 backbones and the  
55 integron in SGI2 is located in S023 instead of being upstream of *resG* (Hamidian et  
56 al., 2015a; Levings et al., 2008; Wilson and Hall, 2010). SGI1 and SGI2 are  
57 specifically mobilized by IncA and IncC (A/C) plasmids (Douard et al., 2010;  
58 Doublet et al., 2005; Harmer et al., 2016; Siebor et al., 2016). The transcriptional  
59 activator complex AcaCD of A/C plasmids promotes the excision of SGI1 and  
60 mobilization of SGI1 thanks to the presence of AcaCD binding sites on SGI1 (Carraro  
61 et al., 2014; Kiss et al., 2015).

62 Further IMEs found in *trmE* that include regions related to parts of the SGI1  
63 backbone and carry a class 1 integron, have been reported. The backbone of the  
64 element called SGI1-V has some differences in the gene content of its backbone in  
65 comparison to SGI1, though it was reported as a variant of SGI1 (Siebor and  
66 Neuwirth, 2011). More distantly related IME are *Proteus* genomic island 1 (PGI1)  
67 (Siebor and Neuwirth, 2014), PGI2 (Lei et al., 2018), and *Acinetobacter* genomic  
68 island 1 (AGI1)(Hamidian et al., 2015b). These IMEs are also not confined to the  
69 species they were found in. PGI1 has been found in *P. mirabilis* and *S. enterica*  
70 (Siebor and Neuwirth, 2014) and IME related to AGI1, first found in *Acinetobacter*  
71 *baumannii*, have also been detected in *Enterobacter cloacae*, *S. enterica* and *Vibrio*  
72 *cholerae* (Siebor et al., 2019).

73 All of the IMEs described above that target *trmE* harbour a class 1 integron  
74 that contains genes conferring resistance to various antibiotics including critical  
75 antibiotics: *bla*<sub>NDM-1</sub> (carbapenem resistance) (Girlich et al., 2015), *bla*<sub>VEB-6</sub> (Siebor  
76 and Neuwirth, 2011) and *bla*<sub>CTX-M-15</sub> (de Curraize et al., 2018) (resistance to broad  
77 spectrum cephalosporins) or *rmtC* (resistance to aminoglycosides) (Wilson and Hall,  
78 2010). Though the location of the integron is the same in all genuine SGI1 variants, it

is not the same in the other types indicating that an integron has been acquired independently by each of them.

Here, we report an IME found in *trmE* in a *P. mirabilis* isolate that consists of a backbone closely related to the SGI1 and SGI2 backbones but lacks a class 1 integron. The original classification of SGI1-V as an SGI1 variant is also re-assessed.

## 2. Materials and methods

### 2.1 Strain

A multiply antibiotic resistant *P. mirabilis*, Pm1LENAR was recovered in the course of continuous surveillance of *P. mirabilis* isolates for SGI1 or PGI1 from clinical samples in our French hospital (Siebor and Neuwirth, 2013). This isolate was identified using the ApiE 20E system (bioMérieux, Marcy l'Étoile, France) and the antibiotic susceptibility tests were performed in accordance with the guidelines of the French Committee on Antimicrobial Susceptibility Testing (Comité de l'Antibiogramme de la Société Française de Microbiologie CASFM 2013) <https://www.sfm-microbiologie.org/>.

### 2.2 Screen for IME related to SGI1 and PGI1

DNA from *P. mirabilis* isolates was extracted by heat lysis of cells and screened by PCR for the presence of the antitoxin gene, *sgiA* (S026) (Huguet et al., 2016), which is present in both SGI1 and PGI1, using primers (Table S1) and conditions previously reported (Siebor and Neuwirth, 2011).

### 2.3 Genome Sequencing and assembly

Bacterial DNA was extracted with the Gentra® Puregene® purification kit (Qiagen, Courtaboeuf, France) and was quantified using the Qubit® system (Life Technologies, Paysey, United Kingdom). The library was prepared with the

Nextera<sup>®</sup>XT kit (Illumina, Eindhoven, Netherlands) following the manufacturer's instructions. The sample was multiplexed using Illumina-supplied barcodes and paired-end sequencing was performed on the Illumina MiSeq with the 300 cycle (MiSeq<sup>®</sup> Reagent Kit v2) (Illumina, Eindhoven, Netherlands). The reads (length 300 bp) of the genome of *Pm1LENAR* were assembled using SPAdes (Bankevich et al., 2012) via the PATRIC web-based platform <https://www.patricbrc.org>. Contigs were analyzed with the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). PCR and Sanger sequencing were performed to check the single nucleotide polymorphisms (SNPs) not present in SGI1 (KU499918) (Siebor et al., 2016). This sequence was chosen for comparison because the original sequence (AF261825) (Boyd et al., 2001) differs slightly from most recent SGI1 sequences.

The short reads from Leekitcharoenphon's study (Leekitcharoenphon et al., 2016) were retrieved and assembled using SPAdes (Bankevich et al., 2012) from the PATRIC web-based platform <https://www.patricbrc.org>.

#### 2.4 Sequence analysis

BLAST, Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) and Artemis Comparison Tool (Carver et al., 2005) were used to compare nucleic acid (na) and amino acid (aa) sequences. SGI2 (AY963803) (Hamidian et al., 2015a) and SGI1-V (HQ888851) (Siebor and Neuwirth, 2011) sequences were used for alignment analysis.

#### 2.5 Plasmid transfer

The IncC plasmid, pPV835TEM24 (GenBank accession number MN148427), originally from a *Proteus vulgaris* strain, Pv835, was used to induce IME excision. It harbours a *floR* gene encoding for chloramphenicol and florfenicol resistance, an

*aacA4* gene encoding kanamycin, tobramycin, netilmicin and amikacin resistances, a *dfrA1* gene encoding trimethoprim resistance, an *aadA1* encoding streptomycin, spectinomycin resistances and a *sul1* encoding sulphonamides resistance. The *Enterobacter aerogenes* BOL (EABOL) strain harbouring the IncC plasmid pPV835TEM24 (donor) was mated with the *Proteus mirabilis* Pm1LENAR which had an IME (recipient) in a 4:1 ratio at the end of the exponential growth phase in Luria Bertani broth at 37°C as previously described (Siebor et al., 2016). After overnight incubation at 37°C without shaking of the mating broth, 10-fold serial dilutions of the bacterial cells were plated on Drigalski agar media (ThermoFisher Scientific®) (which inhibits the swarming of *P. mirabilis*), supplemented with 15 mg/L of colistin to select for *P. mirabilis* and 50 mg/L of chloramphenicol to select for pPV835TEM24 and were incubated 48h at 37°C. The transconjugants that had acquired the pPV835TEM24 plasmid were recovered on this media.

## 2.6 Loss of the IME in cells

IMEs and IncC plasmids are unstable together (Harmer et al., 2016; Huguet et al., 2016). Therefore, DNA was extracted from transconjugants grown on media selecting for the retention of the IncC plasmid (media with 15 mg/L of colistin and 50 mg/L of chloramphenicol), and PCR assays were performed to check whether they have lost the IME. Primers used are listed in Table S1. The presence or absence of the IME was screened by PCR targeting the antitoxin gene *sgiA* (S026), the left and right junctions between the IME and the *trmE* gene in the chromosome. To confirm the absence of the IME in the *trmE* gene, a PCR of the intact *trmE* (*attB*) was also performed. When loss of the IME from the chromosome was detected, colonies were subcultured on the same selective media. DNA was extracted and screened by a two-step DNA amplification of the *attP* site to ensure no extra-chromosomal circular form

of the IME remained in cells. A negative and a positive control were run for all PCR assays. The negative and positive controls were DNA extracts of *P. mirabilis* with or without an SGI1.

## 2.7 Nucleotide sequence accession numbers

The nucleotide sequences of the complete SGI0 and the pPV835TEM24 plasmid were assigned the GenBank accession numbers MH734355 and MN148427, respectively. The Whole Genome Shotgun project for the *Pm1LENAR* strain has been deposited at DDBJ/ENA/GenBank under the accession VIYO000000000. The version described in this paper is version VIYO01000000.

## 3. Results

### 3.1 Characteristics of the *P. mirabilis* isolate, *Pm1LENAR*

The multiply antibiotic resistant *P. mirabilis*, *Pm1LENAR*, was isolated in 2013 in France, from stool of a patient hospitalized in the haematology intensive care unit. This isolate had an unusual antibiotic resistance profile; it was resistant to amoxicillin (32 mg/L), amoxicillin + clavulanic acid (32 mg/L), cefotaxime (cephalosporinase producer) (32 mg/L), kanamycin (256 mg/L), streptomycin (256 mg/L), spectinomycin (256 mg/L) and trimethoprim (32 mg/L). *P. mirabilis* is intrinsically resistant to tetracyclines (256 mg/L) and colistin (>2 mg/L) (Magalhães et al., 1998; Sud and Feingold, 1970). As the SGI1/PGI1 antitoxin gene, *sgiA* (S026) was detected by PCR; the genome of *Pm1LENAR* was sequenced and assembled. An IME located, at the 3'-end of the chromosomal *trmE* gene and related to SGI1, was found in one contig (coverage 51X) (MH734355). However, an integron carrying resistance genes was not contained in this structure and for this reason; we have called it, SGI0.

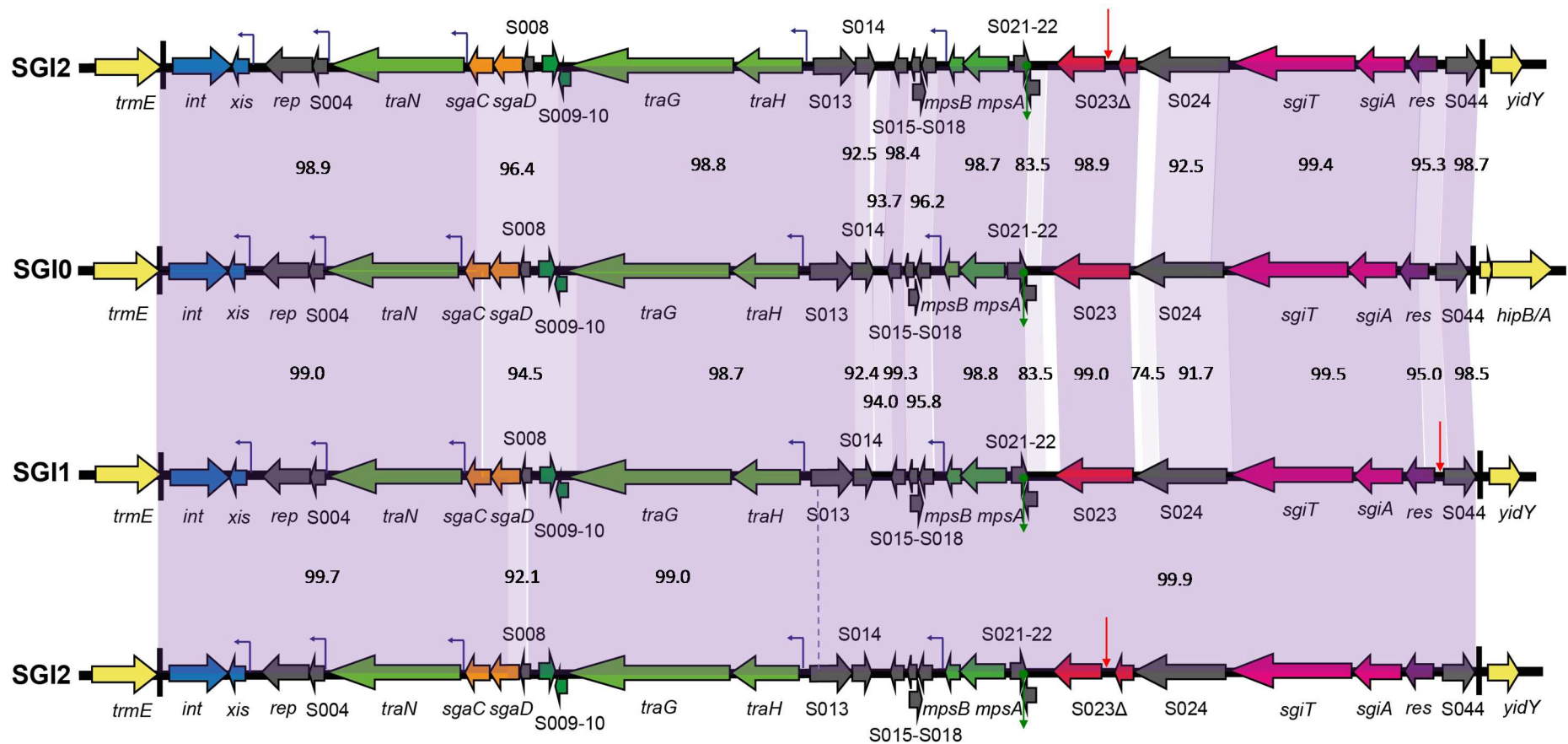
The resistance genes were found in three different contigs in the *Pm1*LENAR draft genome. The first 50.42 kb contig (number 00025) harboured a structure containing an *ISEcpI* with a *bla<sub>CMY-2</sub>* gene encoding a cephalosporinase. An 11.63 kb segment flanked by 5 bp duplication and therefore inserted by an *ISEcpI*- mediated transposition event was found in a 37.6 kb integrative element. An identical segment harbouring *ISEcpI* with a *bla<sub>CMY-2</sub>* (99.85% identity) was previously reported in a *S. Newport* plasmid (DQ164214) (Kang et al., 2006). A 44.47 kb contig (number 00028) contained a Tn7 transposon (at 7421 bp to 21488 bp) with *dfrAI* and *aadAI* genes conferring resistance to trimethoprim, and streptomycin and spectinomycin, respectively. This Tn7 is inserted downstream the *glmS* gene as is usual (Mitra et al., 2010). The third 1.07 kb contig (number 00075) harboured an *aphA1b* gene encoding resistance to kanamycin and neomycin.

ResFinder also recorded the intrinsic chromosomal gene *tet(J)* conferring tetracycline resistance as expected (Magalhães et al., 1998) and a *cat* gene encoding a chloramphenicol acetyltransferase. However, *Pm1*LENAR was susceptible to chloramphenicol. It has been reported that this *cat* gene is often present in *P. mirabilis* and can result in an intermediate level of chloramphenicol resistance (Charles et al., 1985; Okamoto et al., 1967).

### 3.2 *SGI0*, a new *IME* in *trmE*,

*SGI0* was 27.3 kb and contained 28 orfs closely related to those in the backbones of *SGI1* and *SGI2*. However, it did not harbour a class 1 integron and it was not interrupted by other elements. *SGI0* shared 97.59% (26123/26767) nucleotide identity with the *SGI1* backbone and 97.72% (26158/26767) nucleotide identity with the *SGI2* backbone. Most of the backbone shared >98.5% identity with *SGI1* and

203 SGI2 but the nucleotide identity in different segments, ranged from 74.5% to 99.5%  
204 for SGI1 and from 83.5% to 99.4% for SGI2 clearly indicating a mosaic structure  
205 (Fig. 1).  
206



**Figure 1: Comparison of SGI0 backbone with SGI1 and SGI2 backbones**

The percentages of nucleotide identity are indicated between the backbones. The following colours were used for characterizing the orfs: chromosomal genes (yellow), DNA recombination (blue), conjugal transfer (green), regulatory genes (orange), toxin/antitoxin system (pink), helicase (red), resolvase (purple), unknown function (grey). The blue hooked arrows indicate the AcaCD binding sites, the vertical green arrows indicate the *oriT* site, the vertical red arrows indicates the position of the class 1 integron. The thick black vertical bars indicate the attachment sites, *attL* and *attR* of SGI.

SgaC, MpsA and SgiT of SGI0 shared more aa identities with SGI1 than with SGI2, whereas SgaD, S008, TraG, S016, S017 and S044 shared more identity with SGI2. SNPs were distributed throughout the backbone but there were also some spots with more SNPs such as in a region between *sgaD* gene (S007) and S009 and also in S024 (Table 1) (Fig. 1). Some of SNPs result in variation in the length of the predicted polypeptide sequence. For instance, S014 and S021 of SGI0 were 21 aa and 42 aa longer respectively than for SGI1 and SGI2. S017 and S023 were 72 aa and 2 aa shorter respectively due to the presence of a point-nonsense mutation. S024 shared only 85.65% and 86.12% aa identity with S024 from SGI1 and SGI2, respectively. All of these orfs have **unknown** function. All of the essential known proteins were conserved. Hence, the SGI0 integrase shared 99.75% aa identity 99.10% nucleotide identity with SGI1 and SGI2 ones. The toxin/antitoxin system, SgiAT (Huguet et al., 2016), was also very conserved > 99.5% aa identical to SGI1 and SGI2. Moreover, the recently described mobilization proteins, MpsB/MpsA (Kiss et al., 2019), were also very conserved > 99% aa identical to SGI1 and SGI2. Interestingly, the DNA segment immediately after the *oriT* in SGI0 shared only a 83.5% percentage of nucleotide identity with SGI1 and SGI2. **The *oriT* region seems to be a hot spot for recombination events as previously reported for plasmids (Llosa et al., 1994).**

231 **Table 1: Comparison of the 28 orfs of SGI0 with those of SGI1, SGI2 and SGI-V**

<sup>a</sup> orf	gene name	<sup>b</sup> SGI0 (aa length)	<sup>c</sup> SGI1 identity (%)	<sup>b</sup> SGI1 (aa length)	<sup>b</sup> number of aa changed	<sup>c</sup> SGI2 identity (%)	<sup>b</sup> SGI2 (aa length)	<sup>b</sup> number of aa changed	<sup>c</sup> SGI-V identity (%)	<sup>b</sup> SGI-V (aa length)	<sup>b</sup> number of aa changed
S001	<i>integrase</i>	405	99.75	405	1	99.75	405	1	99.51	405	2
S002	<i>excisionase</i>	122	99.18	122	1	99.18	122	1	99.18	122	1
S003	<i>replicase</i>	317	99.37	317	2	99.37	317	2	99.05	317	3
S004		96	98.96	96	1	98.96	96	1	98.96	96	1
S005	<i>traN</i>	919	99.56	919	4	99.56	919	4	99.35	919	6
S006	<i>sgaC</i>	177	100.00	177	0	99.44	177	1	99.44	177	1
S007	<i>sgaD</i>	203	91.63	203	17	93.60	203	13	92.12	203	16
S008		73	87.67	73	9	97.26	73	2	86.30	73	10
S009		96	96.88	96	3	95.83	96	4	95.83	96	4
S010		84	98.81	84	1	98.81	84	1	97.62	84	2
S011	<i>traG</i>	1134	98.68	1134	15	99.21	1134	9	99.03	1134	11
S012	<i>traH</i>	474	98.95	474	5	98.52	474	7	98.10	474	9
S013		285	98.60	285	4	98.60	285	4	96.14	285	11
S014		152	90.84	131	12	90.84	131	12	insertion in	104	-
S015		89	98.88	89	1	98.88	89	1	98.88	89	1
S016		61	95.08	61	3	96.72	61	2	96.72	61	2
S017		41	92.68	84	6	95.12	84	4	95.12	84	4
S018		83	92.77	105	8	92.77	105	8	92.77	105	8
S019	<i>mpsB</i>	98	100.00	98	0	100.00	98	0	98.98	98	1
S020	<i>mpsA</i>	321	99.69	321	1	99.38	321	2	97.51	321	8
S021		153	99.10	111	1	99.10	111	1	98.69	153	2
S022		91	72.53	91	25	72.53	91	25	100.00	91	0
S023		544	96.14	546	21	insertion in	-	-	absent	-	-
S024		642	85.65	641	91	86.12	641	89	absent	-	-
S025	<i>sgiT</i>	847	99.76	847	2	99.65	847	3	90.20	847	83
S026	<i>sgiA</i>	329	100.00	329	0	100.00	329	0	99.39	194	1
S027	<i>resG</i>	194	98.97	194	2	98.97	194	2	100.00	209	0
S044		209	98.56	209	3	99.04	209	2	truncated	-	-

232

233 <sup>a</sup>orf: open reading frame; <sup>b</sup>aa: amino acid; <sup>c</sup> Percentages represent nucleotide identity

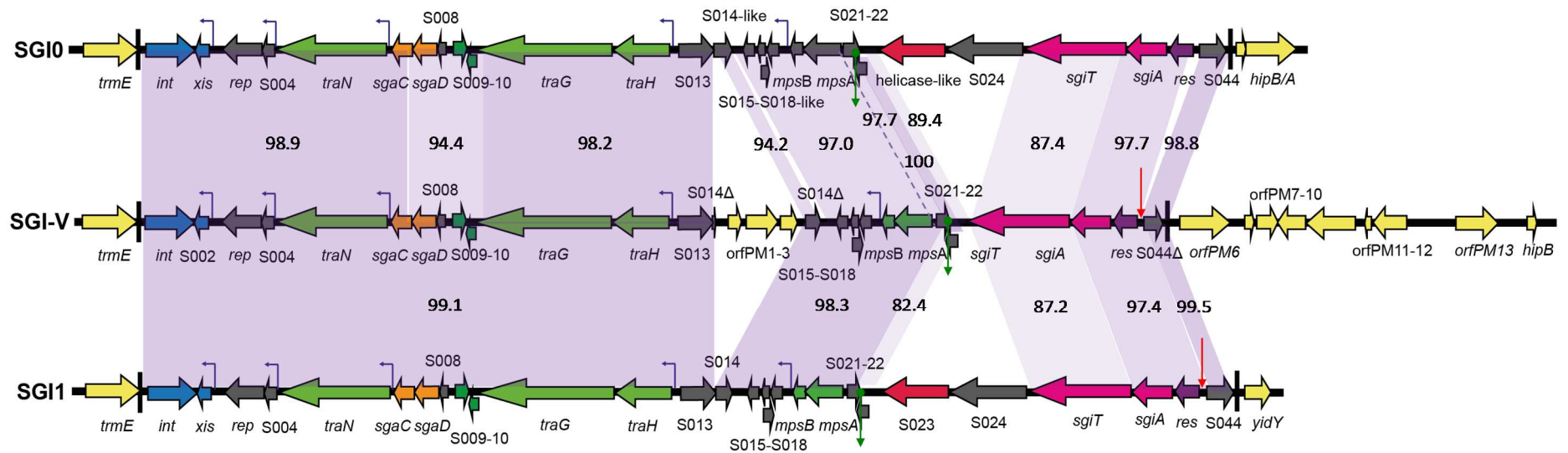
### 3.3 Loss of SGI0

As previously reported for SGI1, SGI0 also harboured the five AcaCD binding sites (Fig. 1). The sequences were same as in SGI1, except the one upstream of the *xis* gene, which had one mutation. The presence of these AcaCD binding sites and the high percentage of identity of the SGI0 integrase with SGI1, led us to test whether SGI0 could be excised when an IncC plasmid was present. Hence, the IncC plasmid pPV835TEM24 was introduced into *Pm1*LENAR harbouring SGI0. Ten colonies were detected on the selective media for the retention of the plasmid. All of them kept the IncC plasmid. PCR screening revealed that a few colonies kept both the IncC plasmid and SGI0 but most of them had lost SGI0 as the *sgiA* gene, as well as the left and right junctions of SGI1 in *trmE* were not detected. In addition, the PCR targeting the intact *trmE* gene (*attB* site) yielded a product of the correct size confirming that SGI0 was not present in the chromosome. The complete loss of SGI0 from these cells was confirmed by the fact that the extra-chromosomal form of SGI0 was not detected by PCR. As SGI0 did not harbour a resistance gene, transfer of SGI0 into another bacterium was not looked for.

### 3.4 SGI1-V is also an independently derived SGI

The backbone of SGI1-V reported previously (Siebor and Neuwirth, 2011) has some significant differences from the SGI1 backbone (Fig. 2). Therefore, SGI1-V was re-examined. In addition to the deletion and the insertion in SGI1-V relative to SGI1 (Fig. 2), in the backbone regions shared with the SGI1, the SGI1-V backbone was on average 97.25% (22671/23312 nucleotides) identical to that of SGI1. There are also polymorphisms in the SGI1-V backbone relative to SGI0 and SGI2. It shared 96.48% (22499/23320) and 97.03% (22620/23312) nucleotide identity, respectively. As for SGI2, SgaD, TraG, S016, S017, S022, ResG shared more aa identities with SGI0 than with SGI1 (Table 1).

259 In addition, the 5 bp duplication flanking the SGI1-V integron (AAATT) is different  
260 to that flanking SGI1 (ACTTG), indicating that the class 1 integron was inserted in a  
261 different position to In104 in SGI1 and hence was acquired in an independent event. The  
262 backbone differences and the different position of the class 1 integron indicate that, like SGI2  
263 (Wilson and Hall, 2010), SGI1-V is an independently derived IME. Therefore, we have re-  
264 named it SGI-V.  
265



**Figure 2: Comparison of SGI-V backbone with SGI0 and SGI1 backbones**

The percentages of nucleotide identity are indicated between the backbones. The following colours were used for characterizing the orfs: chromosomal genes (yellow), DNA recombination (blue), conjugal transfer (green), regulatory genes (orange), toxin/antitoxin system (pink), helicase (red), resolvase (purple), **unknown** function (grey). The blue **hooked** arrows indicate the AcaCD binding sites, the vertical green arrows indicate the *oriT* site, the vertical red arrows indicates the position of the class 1 integron. The thick black vertical bars indicate the attachment sites, *attL* and *attR* of SGI.

#### 4. Discussion

This study describes an IME, SGI0, in a *P. mirabilis* isolate that is related to the backbones of SGI1 and SGI2. SGI0 shared varying levels of identity with parts of the SGI1 or SGI2 backbones. It also shared regions with the SGI-V backbone. Hence, SGI0 is a mosaic that includes segments shared with the backbones of three SGIs. All of the SGI types that include a class 1 integron, namely SGI1, SGI2 and SGI-V, acquired the integron independently. SGI0 likely represents a group of integron-free IMEs that share a common ancestor and can be the forerunners of an SGI type that carries an integron.

SGI-like structures without any resistance gene have been reported in previous studies. The first was reported in 2008 in a susceptible *S. Typhimurium* DT104 that came from Health Protection Agency, in United Kingdom. In this isolate, the presence of a SGI-like structure was found using an SGI1-fingerprinting assay but the class 1 integron was not detected (Amar et al., 2008). However, no sequence is available. A later study detected the presence of an SGI-like element without a class 1 integron in a *P. mirabilis* isolate, which was collected from a dog in 2013, in France (Schultz et al., 2015). Only 3.9 kb of this structure was sequenced (some parts of S007 (*sgaD*), S008 and S011) (GenBank accession number KR296941). Interestingly, these parts are identical to the corresponding parts of SGI0, reported here and it might be identical to SGI0. The isolate harbouring this SGI, came from a different area (Pyrénées orientales) than ours (Bourgogne). However, like *Pm1LENAR*, this isolate was also resistant to amoxicillin, amoxicillin + clavulanic acid, kanamycin, spectinomycin, trimethoprim. It would be interesting to completely sequence it to determine whether it belongs to the same clone as *Pm1LENAR*.

A third study that analyzed the genomes of *S. Typhimurium* DT104 from worldwide isolates collected from 1969 to 2012, reported a few isolates harbouring SGI1 but without

any resistance genes (Leekitcharoenphon et al., 2016). However, we retrieved, assembled, and analyzed the short reads and found that all genomes harbouring an SGI1 derivative had at least part of a class 1 integron. Hence, they do not resemble SGI0.

Finally, the presence of AcaCD binding sites and the **loss of SGI0** in presence of an IncC plasmid suggest that SGI0 might be mobilized by A/C plasmids as the other IMEs in *trmE* (Doublet et al., 2005; Harmer et al., 2016; Siebor et al., 2016). SGI0 and further **related** IMEs in *trmE* without class 1 integron might be also prevalent.

## **5. Conclusion**

SGI0 is a new IME in *trmE* closely related to the backbones of SGI1, SGI2 and **parts of SGI-V** but characterized by the lack of class 1 integron. All of these SGIs backbones are likely to be derived from an original common ancestor, which has diverged overtime and the lineages have repeatedly recombined. Further **IME like SGI0** without class 1 integron might **be circulating** but have not yet been detected.

## **Conflict of interest**

The authors declare no conflict of interest.

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## **Author contributions**

CC conceptualized, performed loss assay, analysed data and wrote the manuscript. ES conceptualized and performed the sequencing and assembly of the genomes and reviewed the preparation of manuscript. CN conceptualized, supervised, revised and reviewed the

preparation of manuscript. RMH analysed data and supervised, revised and reviewed the preparation of manuscript.

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