FEMS Microbiology Letters, 368, 2021, fnab086

https://doi.org/10.1093/femsle/fnab086
Advance Access Publication Date: 15 July 2021
Research Letter

RESEARCH LETTER – Pathogens & Pathogenicity

Serratia marcescens SCH909 as reservoir and source of genetic elements related to wide dissemination of antimicrobial resistance mechanisms

Anahí S. Gambino[1,†,§], Maxime Déraspe[2,3,4,†], Verónica E. Álvarez[1], María Paula Quiroga[1], Jacques Corbeil[2,3,4], Paul H. Roy[4,5,‡] and Daniela Centrón[1,*,‡]

[1]Instituto de Investigaciones en Microbiología y Parasitología Médica, Universidad de Buenos Aires - Consejo Nacional de Investigaciones Científicas y Técnicas (IMPaM, UBA-CONICET), Facultad de Medicina, Paraguay 2155, piso 12, Ciudad Autónoma de Buenos Aires, Argentina, [2]Département de Médecine Moléculaire, Université Laval, Avenue de la Médecine 1050, Pavillon Ferdinand-Vandry, Suite 4835, Québec, Canada, [3]Centre de Recherche en Données Massives, Université Laval, Avenue de la Médecine 1050, Pavillon Adrien-Pouliot PLT-3947, Québec, Canada, [4]Centre de Recherche en Infectiologie, Université Laval, Boulevard Laurier 2705, Local RC-709, Québec, Canada and [5]Département de Biochimie, de Microbiologie et de Bio-informatique, Université Laval, Faculté des sciences et de génie, pavillon Alexandre-Vachon 1045, av. de la Médecine, local 3261, Québec, Canada

*Corresponding author: IMPaM (UBA-CONICET), Facultad de Medicina, Paraguay 2155 piso 12, Ciudad Autónoma de Buenos Aires 1121, Argentina. Tel: +54 911 50987496; E-mail: dcentron@gmail.com

One sentence summary: Genomic plasticity of *Serratia marcescens* capable of hosting multiple antibiotic resistance genes.

†Anahí S. Gambino and Maxime Déraspe contributed equally to this work.
‡Daniela Centrón and Paul H. Roy are corresponding authors of this work.

Editor: Lesley Hoyles

§Anahí S. Gambino, http://orcid.org/0000-0002-7365-8618

ABSTRACT

Serratia marcescens SCH909 is a multidrug resistant strain isolated in 1988 harboring three class 1 integrons. We wondered if these integrons were retained over time and if there were other antimicrobial resistant determinants contributing to its multidrug resistant profile. Genomic analysis showed a fourth multidrug resistance integron, a Tn*7* transposon with *dfrA1-sat2-ybeA-ybfA-ybfB-ybgA* gene cassettes in the variable region. Insertion sequences were involved in the genesis of novel composite transposons in the L4 subtype plasmid pSCH909, such as Tn*6824* carrying an arsenic regulon and two head to head class 1 integrons surrounded by two complete IS*1*. Remarkably, a novel chromosomal genomic island, SmaR, was identified, closely related to Multiple Antimicrobial Resistance Regions (MARR), usually found in AbaR0-type and AbGRI2-0 from global clones of *Acinetobacter baumannii*, and in M-type plasmids circulating in *Enterobacteriaceae*. Maintenance studies showed that the three class 1 integrons were maintained over 1 month without antimicrobial pressure. Since *S. marcescens* is considered a relevant nosocomial pathogen that can have a wide range of niches – human, plant, animal, soil and inanimate surfaces, our findings support the ability of this species to capture, maintain and spread a broad variety of antimicrobial resistance elements.

Received: 2 December 2020; **Accepted:** 9 July 2021

Keywords: *Serratia marcescens*; MARR (multiple antibiotic resistance region)

INTRODUCTION

Serratia marcescens is a nosocomial pathogen able to develop extreme and pandrug resistance, and survives in reservoirs such as drinking water, pipes and hospital disinfectants, being also able to infect plants and animals (Hejazi and Falkiner 1997; Merkier *et al.* 2013; Iguchi *et al.* 2014; Vilacoba *et al.* 2014). Multidrug resistant *S. marcescens* strains are reported to cause more invasive infections with a tendency to spread rapidly in the nosocomial environment (Almuneef *et al.* 2001; Merkier *et al.* 2013; Vilacoba *et al.* 2014; Álvarez *et al.* 2020; Bielli *et al.* 2020). Recently, the World Health Organization has identified this species as a main concern for the acquisition of antimicrobial resistance genes, and they promote the initiative to reinforce efforts focussed on development of new antibacterial agents (Davies 2017). Previous genomic studies revealed a noteworthy intraspecies genetic diversity including high genome flexibility, which may be a sign of the diversity of niches inhabited by members of this species (Iguchi *et al.* 2014).

Serratia marcescens SCH909 is a multidrug clinical strain isolated in 1988 in Greece that has been found to harbor three class 1 integrons (Centrón and Roy 2002). The mobile class C group II *Se.ma.*I2 intron that was identified within the *attC* site of the *aadB* gene cassette, has been suggested to have a role in gene cassette formation (Centrón and Roy 2002; Quiroga, Roy and Centrón 2008; Le´on and Roy 2009).

Here, we investigate the genomic content of *S. marcescens* SCH909, that may be involved in the reorganization and capture of new antimicrobial resistance elements capable of later propagation by the mechanisms of Lateral Genetic Transfer (LGT) to other clinical strains.

MATERIALS AND METHODS

Bacterial strain, conservation and growth conditions

Serratia marcescens SCH909 clinical strain was isolated in 1988 in Greece and was part of a collection of aminoglycoside multidrug resistant isolates from Schering Plough Corporation (Levesque *et al.* 1995; Centro´n and Roy 2002). Since 1988 it has been conserved at 10% glycerol in Luria–Bertani (LB) broth at −80°C. For our studies, it was aerobically grown at 37°C in LB broth with shaking or on LB agar plates.

Genomic sequencing and annotation

Serratia marcescens SCH909 was sequenced by MiSeq and PacBio, yielding 35 and two contigs, respectively, with an N_{50} contig size of 263 670 bp (Illumina, San Diego, CA USA).

The chromosome and the plasmid pSCH909 were assembled with SPAdes version 3.9.0 (Bankevich *et al.* 2012) and were 5 315 598 and 83 750 bp, respectively. The genome was annotated with Prodigal (Hyatt *et al.* 2010) and used the RefSeq database for the genome annotation which was completed with specific analysis using Blast (Altschul 1990). Further editing and manual annotation were carried out using Artemis (Carver *et al.* 2008). Further comparative analysis of the DNA sequence was done with ACT (Carver *et al.* 2008). Insertion sequences were identified and annotated manually with the aid of ISfinder (Siguier 2006) and phages with PHASTER (Arndt *et al.* 2016). Antimicrobial resistance genes were identified using RESfinder (Zankari *et al.* 2012), CARD (McArthur *et al.* 2013) and Blastn (Altschul 1990) with a cut-off e-value of e^{-10}.

Strains and plasmids compared to *S. marcescens* SCH909 and pSCH909

Serratia marcescens strain SM39 (AP013063) was isolated from a septicemic patient in Japan in 1999 (Nakamura *et al.* 2002). This strain harbored two plasmids, pSMC1 (AP013064) and pSMC2 (AP013065). *Serratia marcescens* Db11 (HG326223) was a spontaneous streptomycin-resistant derivative of strain Db10 which was isolated from a moribund *Drosophila melanogaster* in Sweden (Flyg, Kenne and Boman 1980).

Plasmid R471 (KM406489) was isolated in 1977 from *S. marcescens* in the USA and classified as IncL-type by Richards and Datta (1979), Carattoli *et al.* 2015 and Hedges and Jacob (1974). Plasmid pKP112 (LN864819) was isolated from *K. pneumoniae* from Lebanon (unpublished results) and pFDAARGOS_631_1 (CP044032) was isolated from *K. pneumoniae* from USA (unpublished results).

Nucleotide sequence accession numbers

The complete sequence of the chromosome of *S. marcescens* SCH909 has been submitted to GenBank and assigned accession number CP063238. Plasmid pSCH909 was assigned accession number CP063239.

Nucleotide comparison methods

For the comparison analysis of pSCH909, its sequence was used excluding insertions or mobile elements. In this way, a backbone was generated for comparisons with other plasmids. The tool used for the analysis was web BLAST on the NCBI database with a cutoff value of e^{-10}. We used the pMLST site to determine the incompatibility groups of the plasmids in this work (Carattoli and Hasman 2020). We used the work of Blackwell, Doughty and Moran (2021) to determine the pSCH909 plasmid subtype.

The SmaR genomic island (CP063239.1, 1 144 372–1 191 076 bp) was compared by a nucleotide search of the NCBI database using the web-based BLAST program (Camacho *et al.* 2009). A minimum cut-off of 23 340 bp in the Max score parameter was used for this study. Repeated sequences within the search were eliminated from the resulting BLASTn data set.

For the distance tree we used the results of BLASTn and NCBI Tree Viewer (the graphical display for a pairwise alignment based tree created automatically by NCBI BLAST). The NCBI distance tree of results was made using the web site with the Neighbor Joining method (Saitou and Nei 1987); the max sequence difference used was 0.75. The Evolview v3 web tool was used to visualize the tree (Subramanian *et al.* 2019).

Average nucleotide identity calculation

Average nucleotide identity was calculated using ANI calculator (Rodriguez-R and Konstantinidis 2016) for the three chromosomes used in this study. For the prediction of the incompatibility groups of the plasmids analysed in this work we used pMLST in an online program PLSDB (Carattoli *et al.* 2014; Galata *et al.* 2019).

Maintenance studies of class 1 integron

Serratia marcescens SCH909 was grown at 37°C overnight in 2 mL LB broth. Subcultures were carried out daily for 30 days (Moffatt *et al.* 2010). At first, seventh and 30th day, 30 colonies were

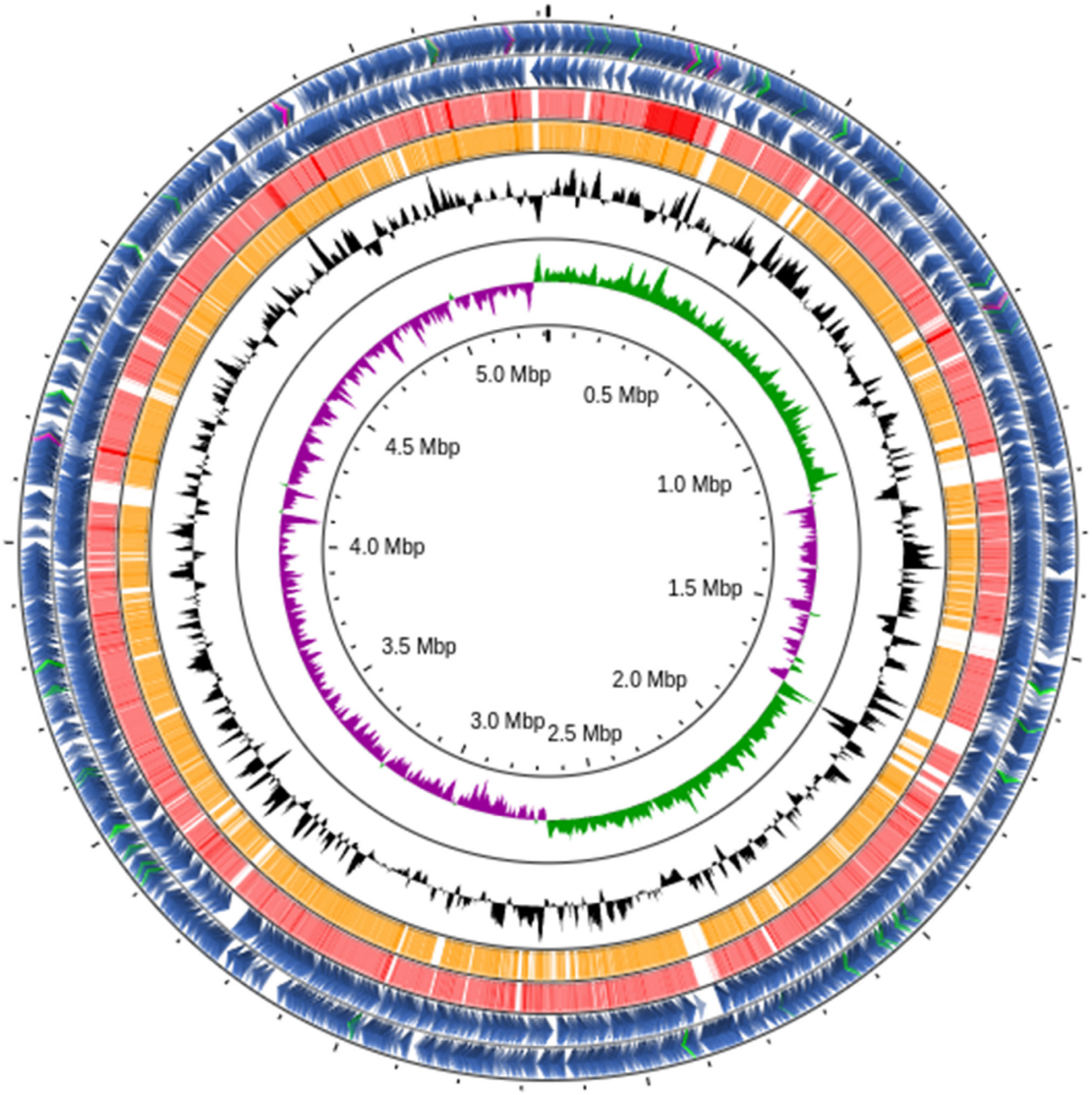

Figure 1. Map of *S. marcescens* SCH909 (CP063238). The outermost two circles indicate positions of CDSs in plus (circle 1) and minus (circle 2) strands colored by functional category: CDSs are identified with blue arrows, green arrows are tRNA, and pink arrows are rRNA. The next two circles map pairwise blastn alignments (expected threshold: $1e^{-20}$) with *S. marcescens* SM39 (AP013063.1) in red and *S. marcescens* subsp. *marcescens* Db11 (HG326223.1) in orange. The white spaces correspond to Regions of Genomic Plasticity. The fifth circle shows G + C content (deviation from average), and the sixth circle illustrates GC skew in green (+) and purple (-). The scale (in Mbp) is indicated on the innermost circle. CGview software (Petkau *et al.* 2010) was used to construct the genome map.

tested for presence of the *intI1-dfrA1*, *intI1-aadB* and *intI1-aacC1* fragments by PCR with two pairs of specific primer, that detected the *intI1* and *dfrA1*, *aadB* and *aacC1* genes, respectively (Levesque *et al.* 1995). The experiments were performed in triplicate.

RESULTS

Genomic analysis of *S. marcescens* SCH909

The chromosome of *S. marcescens* SCH909 is 5 315 598 bp (Fig. 1) with a G + C percentage of 59.9%, very similar to *S. marcescens* SM39 (59.8%), a multidrug resistant clinical isolate (AP013063) and *S. marcescens* Db11 (59.5%), a strain isolated from an insect (HG326223; Table 1). *Serratia marcescens* SCH909 harbored one plasmid, pSCH909 (83 750 bp) associated with antibiotic resistance (Table 1).

We found that the chromosome of *S. marcescens* SCH909 has 98.92% Average Nucleotide Identity (ANI) with the chromosome of *S. marcescens* SM39, isolated 11 years later, on different continents. Both *S. marcescens* SCH909 and SM39 have around 95% ANI with the *S. marcescens* Db11 chromosome (95.04% and 95.05%, respectively).

Antibiotic resistance determinant elements present in *S. marcescens* SCH909

As previously described, *S. marcescens* SCH909 was a multidrug resistant strain with decreased susceptibilities to cefoxitin, cefotaxime, ceftazidime, gentamicin, tobramycin, neomycin, streptomycin, piperacillin, trimethoprim, sulfamethoxazole and chloramphenicol (Centrón and Roy 2002). Genomic analysis revealed several antibiotic resistance determinants in the genome of *S. marcescens* SCH909 (Table 1) including intrinsic genes *aac(6′)-Ic* (M94066) and bla_{SRT-2} (AY524276) and multiple acquired determinants summarized in Table 1.

Table 1. Genomic features of *Serratia marcescens* SCH909.

***Serratia marcescens* SCH909**	
Chromosome	
Size (bp)	5 315 598
G + C content (%)	59.9
CDSs	4803 [9]
rRNA operons	7
tRNAs	88
Prophages	4
IS elements	13
ARGs	*aac(3)-Ia*, *aac(6')-Ic*, *aadA1*, *aph(3')-Ia*, bla_{SRT-2}, bla_{TEM-1}, *catA1*, *sul1*, *tet(A)*, *dfrA1*, *sat2*, *qacE*Δ1
Plasmid	**pSCH909**
Size (bp)	83 750
G + C content (%)	52.0
CDSs	103 [3]
IS elements	3
Incompatibility group	IncL4
ARGs	*aadA1*, *ant(2")-Ia*, *ant(3")-II-aac(6')-IId*, bla_{TEM-1}, *sul1*, *dfrA1*, *qacE*Δ1
Phenotype of decreased susceptibilities agents	
Antimicrobial agents	CTX, FOX, CAZ, GM, PRL, S, SMX, TOB, TP

[-] The number of pseudogenes is indicated in brackets.
Abbreviations for antimicrobials agents: cefotaxime, CTX; cefoxitin, FOX; ceftazidime, CAZ; gentamicin, GM; piperacillin, PRL; streptomycin, S; sulfamethoxazole, SMX; tobramycin, TOB and trimethoprim, TP.

A Tn*7* transposon located downstream of the *glmS* gene was found with the *intI2* gene harboring the characteristic premature stop codon, and an infrequent arrangement of *dfrA1-sat2-ybeA-ybfA-ybfB-ybgA* gene cassettes in the variable region corresponding to Tn*7*::In2-3 (In2-3 with 95% identity with INTEGRALL database, JX867127; Rami´rez, Pineiro and Centro´n 2010). A novel antimicrobial resistance genomic island named SmaR, located in the chromosome, harboring *tet(A)*, *sul1*, *catA1*, bla_{TEM-1} and *aphA1b* as well as class 1 integron In618 with *aac*C1-orfP-orfP-orfQ-*aadA1* within the variable region was found. A new plasmid pSCH909 possessing two class 1 integrons with *dfrA1-aadA1* and *aadB-Se.ma*.I2-*aadA11/aac(6')-IId*-orfO-Δbla_{OXA-10} within the respective variable regions were also identified (see below).

Features of the novel L-type L4 subtype plasmid pSCH909

We identified pSCH909 (83 750 bp) as an L-type L4 subtype plasmid (Carattoli *et al*. 2015; Blackwell, Doughty and Moran 2021) with a complete and functional module for conjugation as previously shown in experiments with *E. coli* C600 as receptor (Centro´n and Roy 2002). The pSCH909 plasmid had a total of 103 CDSs with 39.8% encoding proteins with specific plasmid functions, 33% hypothetical proteins and 22.7% encoding other functions; regulons, antimicrobial resistance determinants and mobile genetic elements were also identified (Fig. 2A). The overall G + C content of the plasmid (52.0%) differed significantly from that of the chromosome (59.9%).

The genes of the replication module (*repA, repB* and *repC*), the genes of the partition module (*parA* and *parB*), the genes involved in conjugation and mobilization (*tra* genes and *mob* genes), the gene involved in the maintenance of the plasmid (*tir*), as well as genes related to UV resistance (*mucA* and *mucB*), shared more than 98.4% identity with 95% coverage, at the nucleotide level, with the R471 plasmid (86 784 bp) (KM406489, Carattoli *et al*. 2015) the type plasmid of the IncL incompatibility group and subtyped by Blackwell Doughty and Moran 2021 within the L1 subtype plasmid (as shown in Fig. 2A). The exclusion system (*excA* and *traY*) and relaxase (*traX*) genes, that differ markedly with those of M-type plasmids, had 99.39%, 99.86% and 99.83% identity with R471, respectively.

In silico comparison of pSCH909 also revealed a high degree of nucleotide identity with L4 subtype *Klebsiella pneumoniae* plasmids; pKP112 (84 252 bp) 99.98% identity in 74% of query cover (LN864819.1, unpublished results) and pFDAARGOS_631_1 (63 589 bp) 99.99% identity in 99.99% of query cover (CP044032.1, unpublished results). The comparison of the plasmids is shown in Fig. 2A, where the query cover is 74% and 68%, respectively.

Both pSCH909 and R471 plasmids had a complete Tn*3* transposon that included the bla_{TEM-1} gene, located close to *repA* (Fig. 2A). In the case of pSCH909, the *tnpA* was invaded by a second transposon of the Tn*3* family, 5562 bp in length, possessing a complete arsenic resistance regulon *arsRDABCA* (Fig. 2B). This new complex transposable element was designated Tn*6824* (https://transposon.lstmed.ac.uk/) according to the criteria proposed by Roberts (Roberts *et al*. 2008; Tansirichaiya, *et al*. 2019). The *tnpA* of Tn*3* split by the regulon of arsenic resistance showed 100% identity to GZS04_22 800 from the plasmid p6A from *Citrobacter freundii* (CP048383). Future studies are warranted to investigate the mobility of Tn*6824*.

A second insertion relative to R471 contained two head to head class 1 integrons surrounded by two complete IS*1* forming a novel pseudo-compound transposon in pSCH909 (14 362 bp in length; Fig. 2C). One class 1 integron possessed *dfrA1-aadA1* and the second one *aadB-Se.ma*.I2-*aadA11/aac(6')-IId*-orfO-Δbla_{OXA-10} within the respective variable regions, which were separated by two hypothetical proteins, an IS*26* variant sequence IS*26*-v1 (also known with the name IS*15*Δ*1* belonging to IS*6*-family elements with three SNPs compared to IS*26*; Pong *et al*. 2019) and *tnpR* and *tnpM* genes of Tn*21* (Fig. 2C). This later class 1 integron contained the group IIC-*attC* intron, *Se.ma*.I2, inserted in the *attC* site of the *aadB* gene (Centro´n and Roy 2002).

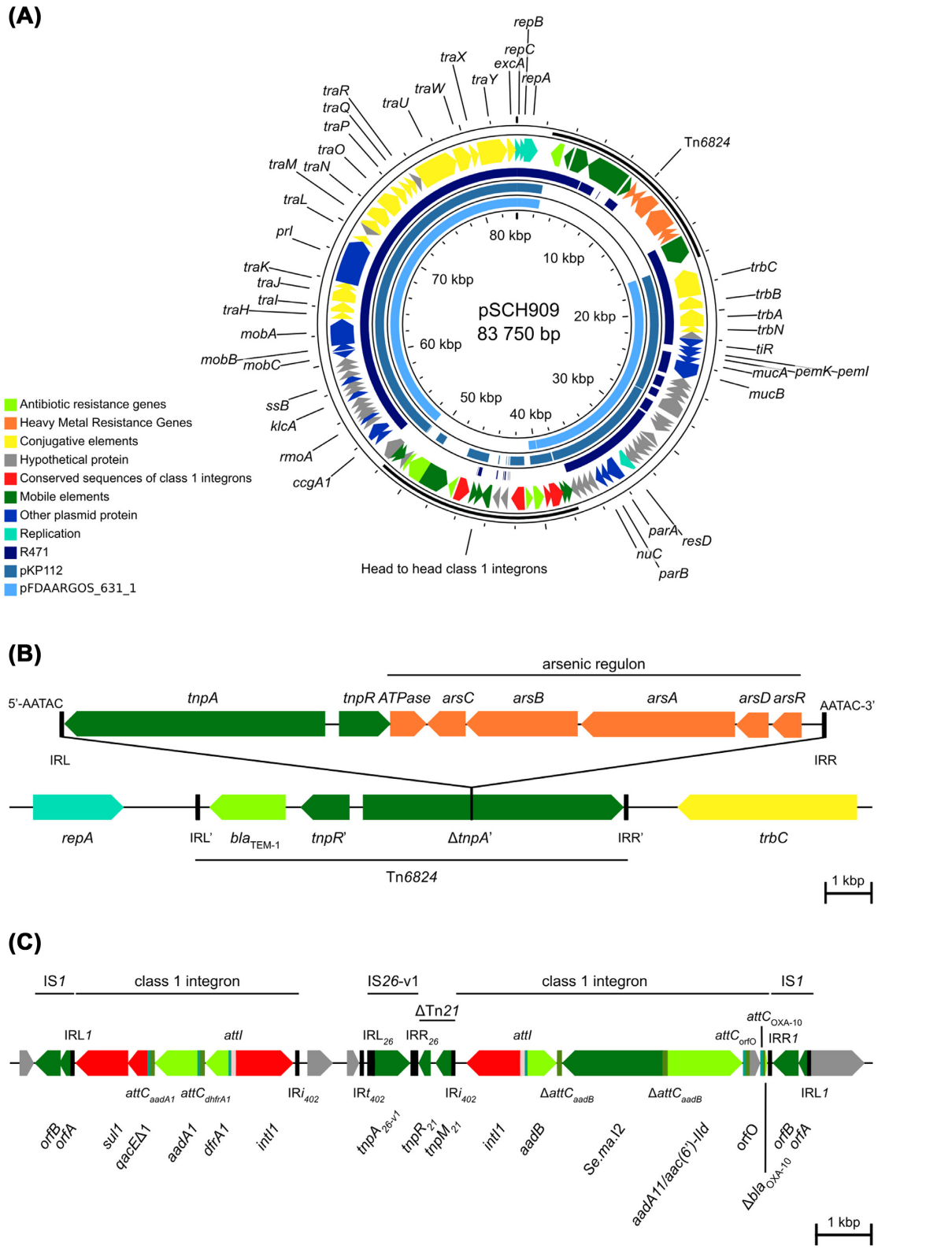

Figure 2. Plasmid pSCH909 (CP063239). **(A)** The most important genes and sites are shown on the map. The external circle indicates the positions of CDSs colored by functional category. The next three circles map Blastn pairwise alignments (expected threshold: $1e^{-20}$) with plasmids R471 (KM406489) in dark blue, pKP112 (LN864819) in blue and pFDAARGOS_631_1 (CP044032) in light blue. The scale (in kbp) is indicated on the innermost circle. **(B)** Tn*6824* is shown with the *ars* regulon genes on a Tn*3*-family transposon inserted into Tn*3*. **(C)** The two head to head class 1 integrons and intervening genes are shown. One class 1 integron possessed *dfrA1-aadA1* and the other *aadB- Se.ma.*I2-*aadA11/aac(6')-IId-* orfO-Δbla_{OXA-10}. The *attI* sites are shown by light pink and the *attC* sites of the gene cassettes are shown by pine green and hunter green bars. CGview software (Petkau *et al.* 2010) was used to construct the plasmid map.

SmaR, a novel chromosomal Genomic Island related to M-type plasmids

A genomic island (GI) which we named SmaR, with 46 705 bp length and a G + C content of 57%, and similar to a region found in M1b subtype plasmids (see below; Blackwell, Doughty and Moran 2021) was identified in the chromosome of *S. marcescens* SCH909. Bioinformatic analysis revealed that SmaR is a novel pseudo-compound transposon flanked by two directly-oriented IS*26* (Fig. 3).

SmaR has 99.93% identity with 97% coverage with the multidrug resistance region found in the M-type plasmid R1215 from *S. marcescens* NCTC 50 331 that was isolated in or prior to 1980 (Fig. 3B). The main difference is IS*4321* between Tn*21* and Tn*2670*, IS*26* within Tn*1* and Δ*tnpR* from Tn*1* surrounded by two IS*26* in the skeleton of SmaR GI. The general structure of SmaR GI between both IS*26* resembles the features of the Multiple Antimicrobial Resistance Region, or MARR region, described in AbaR0-types and AbGRI2-types GI from *A. baumannii* also identified in R1215-related plasmids (Blackwell, Nigro and Hall 2016). Blastn revealed that related sequences and similar architecture of SmaR with more than 65% coverage are only found in multidrug resistant regions from the chromosome of *A. baumannii* and M-type plasmids from *Enterobacteriaceae* strains. Bioinformatic analysis performed with Blastn revealed that related sequences and architecture similar to SmaR with more than 65% coverage were only found in multidrug resistant regions from the chromosome of *A. baumannii* and M-type plasmids from *Enterobacteriaceae* strains as shown by the distance tree of the sequences closest to the SmaR GI in Fig. 4.

The class 1 integron In618 with *aacC1*-orfP-orfP-orfQ-*aadA1* within the variable region found in R1215 and also usually found in the AbaR0-type genomic islands (GI) of Global Clone 1 from *A. baumannii* (Hamidian and Hall 2018), was also found located in SmaR.

As part of SmaR, downstream of the first IS*26* there is a region of 8255 bp in length harboring 14 ORFs plus Δ*mucB* (Fig. 3B). That region is only observed associated with plasmids except for a segment (1542 bp) embedded in the chromosome of an *Enterobacter hormaechei* strain (strain UM_CRE-14, CP023430). The region under consideration (8255 bp) has 100% identity and 100% coverage with plasmids of the IncM1b incompatibility group circulating in several species of *Enterobacteriaceae*; pEB-1 (KX230795) from *Enterobacter cloacae*, pIGT15 (KP294351.1) from *Escherichia coli*, pACM1 (KJ541681.1) from *Klebsiella oxytoca* and one uncultured strain contained the plasmid pARM26 (KP294350.1). An interesting group of highly related IncM1b plasmids are p202c also named pSEM (KM406490.1), R1215 (KU315015.1) and RCS51TR717_p (LT985264.1) (that have 99.98–99.99% identity with 100% coverage in 8255 bp length) where the *mucB* gene was interrupted by a multidrug resistant region, as well as pEB-1, pIGT15, pACM1 and pARM26 (all of them M1b subtype plasmids, Blackwell, Doughty and Moran 2021). These are examples of a lineage of M-type plasmids that have an insertion within the *mucB* gene, a region of multidrug resistant genetic elements similar to those seen in the genome of *A. baumannii* epidemic Global Clone 1 (GC1) and 2 (GC2) (see below) (Preston *et al*. 2014; Blackwell, Nigro and Hall 2016; Preston and Tine 2017).

Moreover, SmaR has 99.93% identity with 97% coverage with the multidrug resistant and M-type region found in R1215 from *S. marcescens* NCTC 50331 that was isolated in or prior to 1980 (Fig. 3B). The main difference is IS*4321* between Tn*21* and Tn*2670*, IS*26* within Tn*1* and Δ*tnpR* from Tn*1* surrounded by two IS*26* in the skeleton of SmaR GI. The general structure of SmaR GI between both IS*26* resembles the features of the Multiple Antimicrobial Resistance Region, or MARR region, described in AbaR0-types and AbGRI2-types GI from *A. baumannii* also identified in R1215-related plasmids (Blackwell, Nigro and Hall 2016). Bioinformatic analysis performed with BLASTn revealed that related sequences and similar architecture of SmaR with more than 65% coverage are only found in multidrug resistant regions from the chromosome of *A. baumannii* and M-type plasmids from *Enterobacteriaceae* strains. The distance tree of the sequences closest to the SmaR GI is shown in Fig. 4.

Multidrug resistant class 1 integrons are maintained in *S. marcescens* SCH909 along time

The maintenance of the three class 1 integrons from *S. marcescens* SCH909 strain in the absence of antibiotic pressure was evaluated in three independent experiments after serial subcultures for 30 days by PCR using specific primers (Centro´n and Roy 2002) for detecting i) *intI1* flanked by the *dfrA1* gene cassette, ii) *intI1* flanked by the *aadB* gene cassette for and iii) *intI1* flanked by the *aacC1* gene cassette for In618. No loss of *intI1-dfrA1*, *intI1-aadB* nor *intI1-aacC1* from *S. marcescens* SCH909 was observed. This experiment showed that the three class 1 integrons were fixed over time of at least 1 month without antimicrobial pressure.

DISCUSSION

Several novel genetic elements related to antimicrobial resistance were identified in *S. marcescens* SCH909. On one hand, the biggest multidrug resistant genetic element was a novel genomic island, that we named SmaR, with chromosomal location in *S. marcescens* SCH909 harboring seven antimicrobial resistance determinants that shares a common source with MARR region from plasmid R1215, as well as with well-documented GI in two major pandemic clones, as AbGRI2-0 in GC2 and AbaR0-type in GC1 *A. baumannii* strains (Fig. 4; Blackwell, Nigro and Hall 2016). Also, presence of several IS including IS*26*, IS*1* and Tn*3*-like transposons were involved in the genesis of novel putative mobile elements in the pSCH909 plasmid such as Tn*6824* carrying an arsenic regulon flanked by bla_{TEM-1} and two head to head class 1 integrons surrounded by two complete IS*1* conforming a novel composite transposon. The *Se.ma*.I2 Group II Class C-*attC* intron was found in pSCH909 (Centro´n and Roy 2002; Quiroga and Centrón 2009). It was proposed that group IIC-*attC* introns could be involved in gene cassette formation (Centro´n and Roy 2002; Le´on and Roy 2009). It was shown that while *Se.ma*.I2 preferentially inserts into *attC* sites (Quiroga, Roy and Centrón 2008), it also inserts, less efficiently, into transcriptional terminators (Le´on and Roy 2009), the usual targets of other group IIC introns (Robart, Seo and Zimmerly 2007). Recently, *Se.ma*.I2 has been found in multidrug resistant ST147 strains of *K. pneumoniae* (Nahid, Zahra and Sandegren 2017). Two strains, DA48896 and MS6771, are nearly isogenic, differing by the presence of two extra copies of *Se.ma*.I2 in DA48896, inserted not into *attC* sites but rather into transcriptional terminators. This presumptive *in vivo* transposition reflects the previously observed *in vitro* transposition into transcriptional terminators (Le´on and Roy 2009) and supports the hypothesis of a role of group IIC-*attC* introns in gene cassette formation.

The mosaic structure of SmaR is a novel pseudo-compound transposon flanked by two IS*26* with a similar architecture and

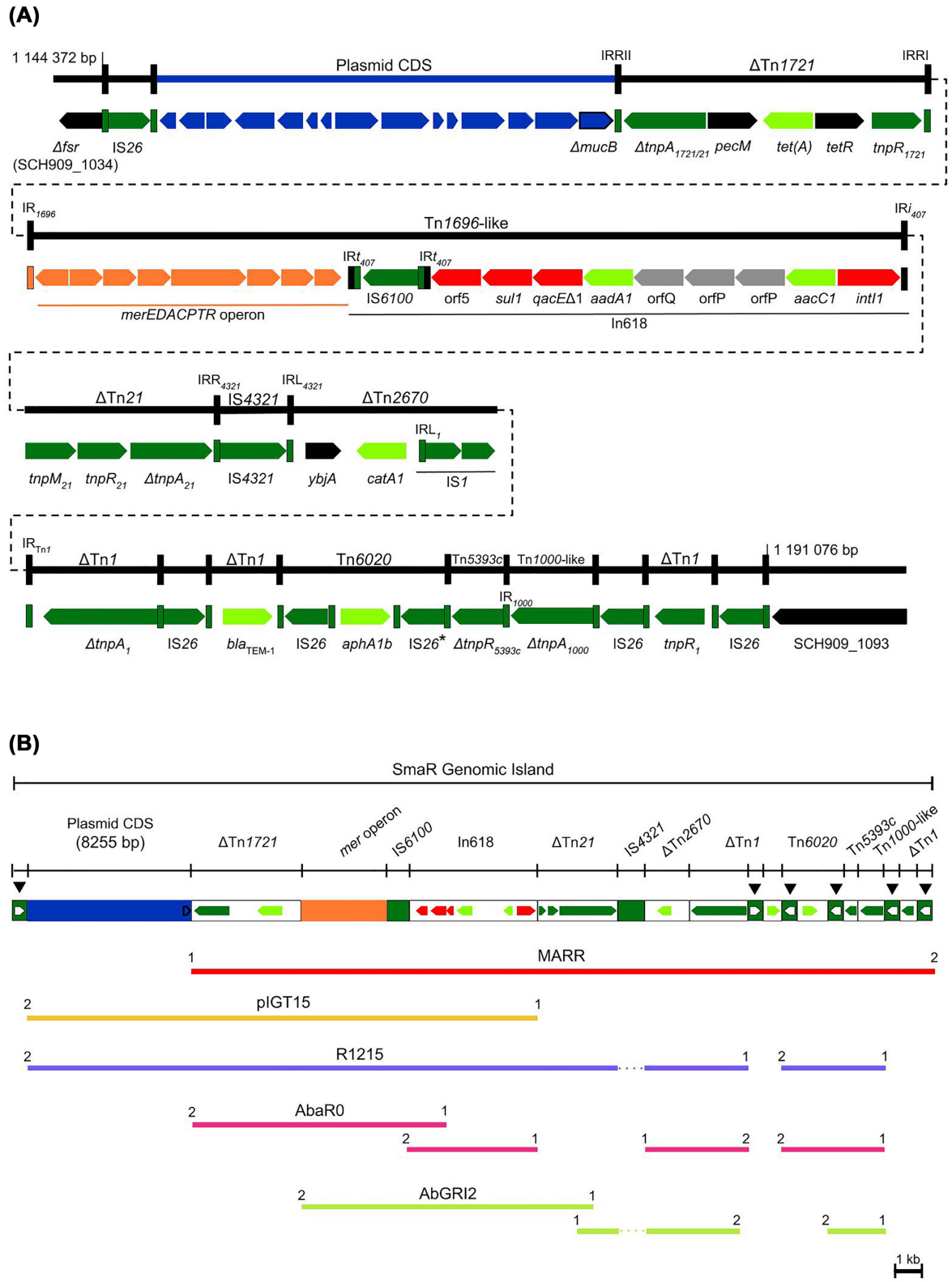

Figure 3. Map of SmaR Genomic Island. The SmaR genomic island is a pseudo-composite transposon with two terminal IS*26* insertion sequences. **(A)** SmaR is made up mostly of transposon (Tn) fragments; among them are Tn*1721*, Tn*1696*-like, Tn*21*, Tn*2670*, Tn*1*, Tn*6020*, Tn*5393c* and Tn*1000*-like; and insertion sequences (IS) IS*6100*, IS*4321*, IS*1* and six copies of IS*26*, one having three point mutations (*). The *mer* operon, encoding *merE, D, A, C, P, T* and *R* genes, is represented by orange arrows and the antibiotic resistance genes are shown with light green arrows. The blue arrows show R1215-associated plasmid genes. **(B)** Comparison of SmaR´s MARR region (red) with the pIGT15 (yellow), R1215 (lilac), AbaR0 (magenta) and AbGRI2 (green). The black triangles (▼) represent the IS*26* insertion sequences embedded along SmaR. Numbers 1 and 2 denote the directions of the DNA fragments.

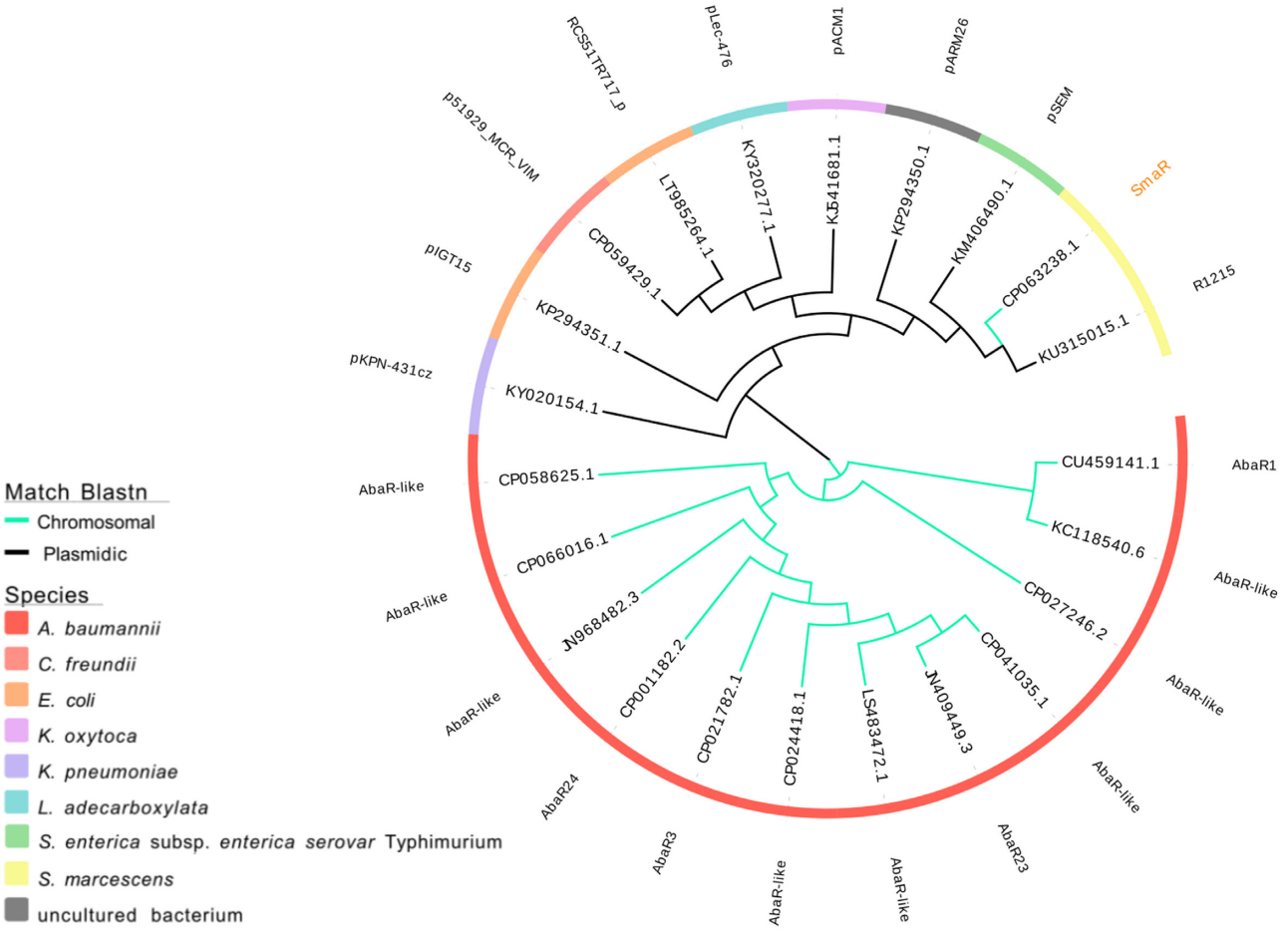

Figure 4. Distance Tree of SmaR. The outermost circle indicates the names of the sequences used for the tree (plasmid or genomic island). The SmaR Genomic Island is indicated in orange lettering. The next circle in the map showed the species from which the element was found. The innermost circle has the accession numbers of the BLASTn best results; *A. baumannii* AYE (CU459141.1), *A. baumannii* 11W359501 (CP041035.1), *A. baumannii* A1 (JN968482.3), *A. baumannii* WCHAB005078 (CP027246.2), *A. baumannii* AB0057 (CP001182.2), *A. baumannii* ATCC BAA1605 (CP058625.1), *A. baumannii* A85 (CP021782.1), *A. baumannii* NCTC13421 (LS483472.1), *A. baumannii* FDAARGOS_1036 (CP066016.1), *A. baumannii* A388 (CP024418.1), *A. baumannii* D81 (N409449.3), *A. baumannii* A85 (KC118540.6), *C. freundii* CIFR51929 (CP059429.1), *E. coli* CZD1527 (KP294351.1), *E. coli* 717 (LT985264.1), *K. oxytoca* ATCC 51983 (KJ541681.1), *K. pneumoniae* Kpn-431 (KY020154.1), *L. adecarboxylata* strain Lec-476 (KY320277.1), *S. enterica* subsp. *enterica* serovar *Typhimurium* (KM406490.1), *S. marcescens* NCTC 50331 (KU315015.1), *S. marcescens* SCH909 (CP063238.1) and uncultured bacterium (KP294350.1). The black lines indicated plasmid localization and light blue lines indicate chromosomal localization.

composition to the MARR. However, the closest genetic element to that of SmaR was found in the M-type plasmid R1215, which was previously identified as related to AbGRI2 and AbaR0-type GI (Blackwell, Nigro and Hall 2016). MARR is a mosaic region shaped by several complete and deleted transposons and insertion sequences including Tn*6020* (with *aphA1b*), usually several copies of IS*26*, and several characteristic antimicrobial resistance determinants such as the class 1 integron In*618* with *aacC1*-orfP-orfP-orfQ-*aadA1* within the variable region inserted in a Tn*21* backbone, *tet*(A) in ΔTn*1721*, a *mer* operon in Tn*1696-like*, *cat1* in Tn*2670* and bla_{TEM-1} in a fragmented Tn*1* (Nigro *et al.* 2013), suggesting that independent *de novo* formation is highly unlikely, and therefore they should share a common ancestor. This finding supports the previous discovery of Blackwell, Nigro and Hall (2016), showing that the mosaic structure resembling MARR has been evolving in M-type plasmids. SmaR is most closely related to R1215, followed by AbGRI2 and MARR from AbaR0 GI (Fig. 3B). The greater similarity to AbGRI2 is not only for the arrangement of the IS*26* sequences that have inserted into the Tn*1* transposon in a similar way but also for In*618* bordering an incomplete Tn*21* transposon. Interestingly, in the case of AbaR0-type GI, the MARR was captured by Tn*6019*, while in SmaR and AbGRI2 the capture was due to IS*26*. Then, several genetic elements of the mobilome can be involved in the process of mobilization of this mosaic region of multiple antimicrobial resistance determinants, from M-type plasmids to chromosomes of different genera of bacteria. It is not clear if the arrangements found in *S. marcescens* SCH909 or in *A. baumannii* are subjected to LGT or mostly to vertical inheritance.

Since the isolation of SCH909 was in Greece in 1988 (Centro´n and Roy 2002), several related multidrug resistant genetic elements were identified before or later in other genetic locations and other species (i.e. MARR-related sequences in R1215 from the 1980s, or MARR-related sequences in pS1929_MCR_VIM from 2001; Fig. 4). Although it is not possible to determine the steps involved in the evolution and transfer of these rearrangements found in *S. marcescens* SCH909, it is likely that this strain may be a source for potential LGT to other species. Knowing the remarkable number of niches that *S. marcescens* can occupy (Iguchi *et al.* 2014; Abreo and Altier 2019), it is feasible to speculate on its essential role as a species able to capture new antimicrobial resistance genes and/or plasmids from the environment

that can potentially be selected in the clinic (Park *et al.* 2009; Zou *et al.* 2011; Vilacoba *et al.* 2014; Wendel *et al.* 2017; Iasakov *et al.* 2019). Taking into account that R1215 was also isolated in *S. marcescens*, these examples support the idea that this species has a high genomic plasticity adapted to acquisition of novel genetic elements from different niches, including antimicrobial resistance determinants relevant for successful maintenance in nosocomial strains.

Interestingly, although M-type plasmids are circulating in several species of *Enterobacteriaceae* such as *E. coli*, *S. enterica*, *K. pneumoniae*, *S. marcescens* and others (Carattoli 2009; Ho *et al.* 2011; Partridge *et al.* 2012; Seiffert *et al.* 2014; Blackwell, Nigro and Hall 2016; Nigro and Hall 2016; Kubota *et al.* 2019), *in silico* analysis from this study revealed that the whole mosaic structure resembling MARR from M-type plasmids is found in a chromosomal location only in *A. baumannii* and *S. marcescens* SCH909. In agreement with these findings, we showed in the present work maintenance of In*618* which is located within SmaR, carried out for 1 month in studies without antimicrobial pressure for *S. marcescens* SCH909 and previously for *A. baumannii* A144 strain belonging to GC1 (Álvarez *et al.* 2020). It is plausible to speculate that the MARR region is not easily maintained in other species, denoting a great plasticity in *A. baumannii* and *S. marcescens* for acquisition of antimicrobial resistance determinants.

ACKNOWLEDGMENTS

ASG and VEA were supported by fellowships from CONICET. DC and MPQ are members of the Carrera del Investigador Científico, CONICET, Argentina. The authors' work was supported by the grant PUE 2522 from CONICET given to IMPaM (UBA/CONICET), and PICT 2430–2017 from the Agencia Nacional de Promoción de Ciencia y Técnica (ANPCYT) given to DC.

ASG was supported by a doctoral scholarship from the Government of Canada under The Emerging Leaders in the Americas Program 2019–2020 (ELAP).

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSLE* online.

Conflicts of Interest. None declared.

REFERENCES

Abreo E, Altier N. Pangenome of *Serratia marcescens* strains from nosocomial and environmental origins reveals different populations and the links between them. *Sci Rep* 2019;**9**:46.

Almuneef MA, Baltimore RS, Farrel PA *et al.* Molecular typing demonstrating transmission of Gram-negative rods in a neonatal intensive care unit in the absence of a recognized epidemic. *Clin Infect Dis* 2001;**32**:220–7.

Altschul S. Basic local alignment search tool. *J Mol Biol* 1990;**215**:403–10.

Álvarez VE, Quiroga MP, Galán AV *et al.* Crucial Role of the accessory genome in the evolutionary trajectory of *Acinetobacter baumannii* global clone 1. *Front Microbiol* 2020;**11**:342.

Arndt D, Grant JR, Marcu A *et al.* PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 2016;**44**:W16–21.

Bankevich A, Nurk S, Antipov D *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;**19**:455–77.

Bielli A, Piazza A, Cento V *et al.* In vivo acquisition and risk of inter-species spread of *bla*KPC-3-plasmid from *Klebsiella pneumoniae* to *Serratia marcescens* in the lower respiratory tract. *J Med Microbiol* 2020;**69**:82–6.

Blackwell GA, Doughty EL, Moran RA. Evolution and dissemination of L and M plasmid lineages carrying antibiotic resistance genes in diverse Gram-negative bacteria. *Plasmid* 2021;**113**:102528.

Blackwell GA, Nigro SJ, Hall RM. Evolution of AbGRI2-0, the progenitor of the AbGRI2 resistance island in global clone 2 of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2016;**60**:1421–9.

Camacho C, Coulouris G, Avagyan V *et al.* BLAST+: architecture and applications. *BMC Bioinformatics* 2009;**10**:421.

Carattoli A, Hasman H. PlasmidFinder and In Silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS). *Methods Mol Biol*. Humana Press Inc., 2020;**2075**:285–94.

Carattoli A, Seiffert SN, Schwendener S *et al.* Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. Janssen PJ (ed.). *PLoS ONE* 2015;**10**:e0123063.

Carattoli A, Zankari E, García-Fernández A *et al.* In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 2014;**58**:3895–903.

Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2009;**53**:2227–38.

Carver T, Berriman M, Tivey A *et al.* Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. *Bioinformatics* 2008;**24**:2672–6.

Centro´n D, Roy PH. Presence of a group II intron in a multiresistant *Serratia marcescens* strain that harbors three integrons and a novel gene fusion. *Antimicrob Agents Chemother* 2002;**46**:1402–9.

Davies OL. WHO publishes list of bacteria for which new antibiotics are urgently needed. *WHO Newsletters* 2017.

Flyg C, Kenne K, Boman HG. Insect pathogenic properties of *Serratia marcescens*: phage-resistant mutants with a decreased resistance to cecropia immunity and a decreased virulence to *Drosophila*. *Microbiology* 1980;**120**:173–81.

Galata V, Fehlmann T, Backes C *et al.* PLSDB: a resource of complete bacterial plasmids. *Nucleic Acids Res* 2019;**47**:D195–202.

Hamidian M, Hall RM. The AbaR antibiotic resistance islands found in *Acinetobacter baumannii* global clone 1 – structure, origin and evolution. *Drug Resist Updat* 2018;**41**:26–39.

Hedges RW, Jacob AE. Transposition of ampicillin resistance from RP4 to other replicons. *MGG Mol Gen Genet* 1974;**132**:31–40.

Hejazi A, Falkiner FR. *Serratia marcescens*. *J Med Microbiol* 1997;**46**:903–12.

Ho PL, Lo WU, Yeung MK *et al.* Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. *PLoS ONE* 2011;**6**:e17989

Hyatt D, Chen G-L, LoCascio PF *et al.* Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010;**11**:119.

Iasakov TR, Anisimova LG, Zharikova N V. *et al.* Evolution and comparative genomics of the pSM22 plasmid of the IncF/MOBF12 group. *Mol Biol (Mosk)* 2019;**53**:600–12.

Iguchi A, Nagaya Y, Pradel E *et al.* Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen. *Genome Biol Evolut* 2014;**6**:2096–110.

Kubota H, Suzuki Y, Okuno R *et al.* IMP-68, a novel IMP-type metallo-β-lactamase in imipenem-susceptible *Klebsiella pneumoniae*. Castanheira M (ed.). *mSphere* 2019;**4**:e00736–19.

Le´on G, Roy PH. Potential role of group IIC-*attC* Introns in integron cassette formation. *J Bacteriol* 2009;**191**:6040–51.

Levesque C, Piche L, Larose C *et al.* PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 1995;**39**:185–91.

McArthur AG, Waglechner N, Nizam F *et al.* The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother* 2013;**57**:3348–57.

Merkier AK, Rodriguez MC, Togneri A *et al.* Outbreak of a cluster with epidemic behavior due to *Serratia marcescens* after colistin administration in a hospital setting. *J Clin Microbiol* 2013;**51**:2295–302.

Moffatt JH, Harper M, Harrison P *et al.* Colistin resistance in *Acinetobacter baumannii* is mediated by complete loss of lipopolysaccharide production. *Antimicrob Agents Chemother* 2010;**54**:4971–7.

Nahid F, Zahra R, Sandegren L. A bla OXA-181-harbouring multiresistant ST147 *Klebsiella pneumoniae* isolate from Pakistan that represent an intermediate stage towards pan-drug resistance. Chang Y-F (ed.). *PLoS ONE* 2017;**12**:e0189438.

Nakamura T, Shibata N, Doi Y *et al.* IMP-1 type metalo-beta-lactamase producing *Serratia marcescens* strains isolated from blood culture between 1991 to 2000. *J Jpn Assoc Infect Dis* 2002;**76**:246–53.

Nigro SJ, Farrugia DN, Paulsen IT *et al.* A novel family of genomic resistance islands, AbGRI2 clone 2 *Acinetobacter baumannii* in Australia via modification of genomic resistance islands and acquisition of plasmids. *J Antimicrob Chemother* 2013;**71**:2432–40.

Nigro SJ, Hall RM. Loss and gain of aminoglycoside resistance in global clone 2 *Acinetobacter baumannii* in Australia via modification of genomic resistance islands and acquisition of plasmids. *J Antimicrob Chemother* 2016;**71**:2432–40.

Park YJ, Kim SY, Yu JK *et al.* Spread of *Serratia marcescens* coharboring *aac(6 ') -Ib-cr*, *bla* CTX-M, *armA*, and *bla* OXA-1 carried by conjugative IncL/M type plasmid in Korean hospitals. *Microb Drug Resist* 2009;**15**:97–102.

Partridge SR, Ginn AN, Paulsen IT *et al.* pEl1573 Carrying *bla* IMP-4, from Sydney, Australia, is closely related to other IncL/M plasmids. *Antimicrob Agents Chemother* 2012;**56**:6029–32.

Petkau A, Stuart-Edwards M, Stothard P *et al.* Interactive microbial genome visualization with GView. *Bioinformatics* 2010;**26**:3125–6.

Pong CH, Harmer CJ, Ataide SF *et al.* An *IS26* variant with enhanced activity. *FEMS Microbiol Lett* 2019;**366**:31.

Preston KE, Hitchcock SA, Aziz AY *et al.* The complete nucleotide sequence of the multi-drug resistance-encoding IncL/M plasmid pACM1. *Plasmid* 2014;**76**:54–65.

Preston KE, Tine JA. Acquisition of a second multidrug resistance-encoding element by IncM1 plasmid pACM130 abolished conjugative transfer. *Plasmid* 2017;**92**:26–9.

Quiroga C, Centrón D. Using genomic data to determine the diversity and distribution of target site motifs recognized by class C-*attC* group II introns. *J Mol Evol* 2009;**68**:539–49.

Quiroga C, Roy PH, Centrón D. The *S.m a.*I2 class C group II intron inserts at integron attC sites. *Microbiology* 2008;**154**:1341–53.

Rami´rez MS, Pineiro S, Centro´n D. Novel insights about class 2 integrons from experimental and genomic epidemiology. *Antimicrob Agents Chemother* 2010;**54**:699–706.

Richards H, Datta N. Reclassification of incompatibility group L (IncL) plasmids. *Plasmid* 1979;**2**:293–5.

Robart AR, Seo W, Zimmerly S. Insertion of group II intron retroelements after intrinsic transcriptional terminators. *Proc Natl Acad Sci* 2007;**104**:6620–5.

Roberts AP, Chandler M, Courvalin P *et al.* Revised nomenclature for transposable genetic elements. *Plasmid* 2008;**60**:167–73.

Rodriguez-R L, Konstantinidis K. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ* 2016;**4**:e1900v1.

Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;**4**:406–25.

Seiffert SN, Perreten V, Johannes S *et al.* OXA-48 carbapenemase-producing *Salmonella enterica* serovar Kentucky isolate of sequence type 198 in a patient transferred from Libya to Switzerland. *Antimicrob Agents Chemother* 2014;**58**:2446–9.

Siguier P. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006;**34**:D32–6.

Subramanian B, Gao S, Lercher MJ *et al.* Evolview v3: a webserver for visualization, annotation, and management of phylogenetic trees. *Nucleic Acids Res* 2019;**47**:W270–5.

Tansirichaiya S, Rahman MA, Roberts AP. The transposon registry. *Mobile DNA* 2019;**10**, DOI: 10.1186/s13100-019-0182-3.

Vilacoba E, Quiroga C, Pistorio M *et al.* A *bla* VIM-2 plasmid disseminating in extensively drug-resistant clinical *Pseudomonas aeruginosa* and *Serratia marcescens* isolates. *Antimicrob Agents Chemother* 2014;**58**:7017–8.

Wendel AF, Kaase M, Autenrieth IB *et al.* Protracted regional dissemination of GIM-1-producing *Serratia marcescens* in Western Germany. *Antimicrob Agents Chemother* 2017;**61**:e01880–16.

Zankari E, Hasman H, Cosentino S *et al.* Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 2012;**67**:2640–4.

Zou L, Pan X, Wu Q *et al.* First detection of OKP-A β-lactamase in two *Serratia marcescens* isolates in China. *New Microbiol* 2011;**34**:371–8.