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Small Plasmids Harboring *qnrB19*: a Model for Plasmid Evolution Mediated by Site-Specific Recombination at *oriT* and Xer Sites

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Plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4, isolated from *Salmonella* and *Escherichia coli* clinical strains from hospitals in Argentina, were completely sequenced. These plasmids include the *qnrB19* gene and are 2,699, 3,082, 2,989, and 2,702 nucleotides long, respectively, and they share extensive homology among themselves and with other previously described small *qnrB19*-harboring plasmids. The genetic environment of *qnrB19* in all four plasmids is identical to that in these other plasmids and in transposons such as Tn*2012*, Tn*5387*, and Tn*5387*-like. Nucleotide sequence comparisons among these and previously described plasmids showed a variable region characterized by being flanked by an *oriT* locus and a Xer recombination site. We propose that this arrangement could play a role in the evolution of plasmids and present a model for DNA swapping between plasmid molecules mediated by site-specific recombination events at *oriT* and a Xer target site.

Qnrs are pentapeptide repeat proteins that mediate resistance to quinolones by protecting type II DNA topoisomerases (14, 28). They are known since 1998 when the first *qnr* gene was found in the multiresistance plasmid pMG252 harbored by a *Klebsiella pneumoniae* strain isolated from the urine of a patient at the University of Alabama (20). Since then five *qnr* families (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) have been found, usually hosted in large plasmids (31). The first *qnrB* gene (*qnrB1*) was identified in a plasmid from a *K. pneumoniae* strain isolated in South India (16), and 38 members of the family quickly followed (http://www.lahey.org/qnrStudies/) (13). The *qnrB19* gene has been found in several genera of *Enterobacteriaceae* isolated from humans (healthy people and clinical isolates), animals, and food of animal origin in numerous geographical regions (6, 9, 12, 17, 21, 22, 27). An interesting characteristic of the *qnrB19* allele is that it has been found within large plasmids, associated to IS*Ecp1C*-based transposons (6, 9, 27), and in small plasmids (~3 kbp) lacking IS*Ecp1C* or any other insertion sequence (12, 17, 22) (Table 1). However, in spite of being located in such dissimilar elements, the *qnrB19* genes share a conserved genetic environment (22).

We have recently analyzed a collection of clinical enterobacterial isolates with decreased quinolone susceptibility, and we found four small plasmids harboring *qnrB19* (2). We describe here their molecular features and characterize their relationships with other *qnrB19*-harboring genetic platforms. Furthermore, we propose possible pathways of evolution of the *qnrB19* environment as well as a site-specific recombination-based model for DNA modifications at a variable region found in these plasmids.

MATERIALS AND METHODS

Bacterial strains and plasmids. The plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 analyzed in the present study were isolated from *Salmonella enterica* serovar Infantis M7849, *Escherichia coli* M9996, *E. coli* M9888, and *Salmonella* sp. strain M9397, respectively (Table 1). *Salmonella* Infantis M7849 was isolated at the Hospital Castro Rendón, Province of Neuquén (March 2006). *E. coli* strains M9888 and M9996 were isolated at the Policlínico Central de San Luis, Province of San Luis (May 2007 and August 2008, respectively), and *Salmonella* sp. strain M9397 was isolated at the Hospital de Niños Alassia, Province of Santa Fe (July 2007). These isolates are included in a group of 20 *Salmonella* and 25 *E. coli* strains that is part of a collection of 105 strains with decreased susceptibility to quinolones obtained from 31 hospitals in the city of Buenos Aires and 11 provinces of Argentina (2). A thorough characterization of these strains will be published elsewhere. Recombinant plasmids pPBR1 and pPBR2 were generated by ligating HindIII-digested pPAB19-1 or pPAB19-2 to HindIII-digested pUC19 (38). Dimers of plasmids pES and pKS492 were used as controls in dimer resolution experiments (4). *E. coli* DS941 (AB1157 *recF143 lacI*^{q} *lacZ*; possesses wild-type *xerC*, *xerD*, *argR*, and *pepA*) (33), *E. coli* DS9028 (DS941 *xerD3*::*fol*) (30), and the hyper-recombinogenic *E. coli* JC8679 (DS945 *recBC sbcA*) (32) were used to carry out the Xer recombination experiments.

DNA sequencing and analysis. Plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 were screened by PCR using the divergent primers *qnrB*-Fout (5′-GACGTTCAGTGGTTCAGATCTCTC) and *qnrB*-Rout (5′-GACTAAAATTGCACCCTTTCTGACT) that bind to the *qnrB19* gene, leading to amplifications of its surrounding plasmid sequences. The amplicons were sequenced using BigDye terminator methodology and sequence-based primers (DNA walking), with an ABI 3130*xl* genetic analyzer (Applied Biosystems/Perkin-Elmer, Foster City, CA). Nucleotide sequence editing and analyses were performed using ClustalX2 or ClustalW2 (v2.0.9; ftp://ftp.ebi.ac.uk/pub/software/clustalw2) (18), BioEdit (v7.0.9; http://www.mbio.ncsu.edu/bioedit/bioedit.html)

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TABLE 1 *qnrB19*-harboring genetic elements

Genetic element	Size (bp)	Country	Host[c]	Origin	Source or reference (accession no.)
IS*Ecp1C*-based transposons[a]					
Tn*2012* (pR4525, 40 kb)	2,738	Colombia	*E. coli*	Clinical isolate	6
Tn*5387* (pLRM24, 80 kb)	2,966	USA	*K. pneumoniae*	Clinical isolate	27
Tn*5387*-like (p61/9, >21 kb)	2,828	Italy	*S. enterica*	Clinical isolate	9
Small plasmids[b]					
pPAB19-1	2,699	Argentina	*Salmonella* Infantis M7849*	Clinical isolate	This study (GQ412195)
pPAB19-2	3,082	Argentina	*E. coli* M9996*	Clinical isolate	This study (JN979787)
pPAB19-3	2,989	Argentina	*E. coli* M9888*	Clinical isolate	This study (JN985534)
pPAB19-4	2,702	Argentina	*Salmonella* sp. strain M9397*	Clinical isolate	This study (JN995611)
pSGI15	2,699	Netherlands	*S. enterica*	Clinical isolate	12
pECY6-7	2,699	Bolivia, Peru	*E. coli*, *E. fergusonii*, *E. hermanii*, *E. aerogenes*, *K. ascorbata*, *K. pneumoniae*	Healthy people	21, 22
pECC14-9	3,071	Bolivia, Peru	*E. coli*	Healthy people	21, 22
pMK100	2,699	Colombia	*S. enterica*	Retail poultry	17
pMK102	2,750	Colombia	*S. enterica*	Ground beef	17

[a] The plasmids hosting the transposons and their sizes are indicated in parentheses.
[b] pPAB19-1 was found in 16 of the 45 *E. coli* and *Salmonella* isolates (8 *E. coli*, 1 *Salmonella* Infantis, and 7 *Salmonella* spp.); pPAB19-2 was found in 3 of the 45 *E. coli* and *Salmonella* isolates (2 *E. coli* and 1 *Salmonella* sp.); pPAB19-3 was found in 1 of the 45 *E. coli* and *Salmonella* isolates (*E. coli*); pPAB19-4 was found in 4 of the 45 *E. coli* and *Salmonella* isolates (*Salmonella* spp.).
[c] *, Strains from which the individual sequenced plasmid was isolated.

(10), and the basic local alignment search tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST/) (1).

Xer recombination assays. Xer recombination assays were carried out as described previously (36). Briefly, dimers of recombinant plasmids to be tested were prepared by transforming *E. coli* JC8679, culturing the transformed strains and extracting plasmid DNA, which was electrophoresed in 0.7% agarose gels. The DNA corresponding to the dimer was purified from the agarose gels using a QIAquick gel extraction kit (Qiagen). Since dimers run very close to the position of open circular monomer DNA, the isolated samples were used to transform the XerD-deficient *E. coli* DS9028. Since this strain cannot resolve the dimers, some colonies carry the monomer, and some others carry the dimers, allowing the isolation of transformants that have a plasmid dimer. Purified plasmid dimers were introduced by chemical transformation into *E. coli* DS941, the transformants were cultured overnight at 37°C in Lennox L broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) or medium containing the same concentrations of tryptone and yeast extract with no added NaCl, and the plasmid content was analyzed by agarose gel electrophoresis.

Nucleotide sequence accession numbers. The nucleotide sequences of plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 have been deposited in GenBank under accession numbers GQ412195, JN979787, JN985534, and JN995611, respectively.

RESULTS AND DISCUSSION

***qnrB19* genetic environment.** Four *qnrB19*-containing plasmids, pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4, isolated from clinical *Salmonella* spp. or *E. coli* isolates were sequenced and comparison analyses showed that they are identical or highly related to other known small *qnrB19*-carrying plasmids isolated from enterobacteria (Fig. 1A and B, also see Table 1 for information about these other plasmids). Plasmid pPAB19-1 is identical to a plasmid that has been isolated from *E. coli*, *E. fergusonii*, *E. hermanii*, *Enterobacter aerogenes*, *K. pneumoniae*, *S. enterica*, and *Kluyvera ascorbata* strains from the Netherlands, Bolivia, Peru, and Colombia by different research groups and has received several names (pSGI15, pECY6-7, and pMK100) (12, 17, 21, 22). Plasmids pPAB19-2, pPAB19-3, and pPAB19-4 are highly related to pPAB19-1 and other plasmids such as pECC14-9 (Bolivia and Peru) and pMK101 (Colombia) isolated from *E. coli* and *S. enterica*, respectively (17, 21, 22). A ColE1-type replication region locus is within the fragment that is common to all plasmids and share 93% identity with other non-*qnrB*-carrying plasmids from enterobacteria such as pJHCMW1 (29).

The genetic variations observed in this group of plasmids can be fitted into two pathways of plasmid evolution starting from the most common plasmid pPAB19-1/pSGI15/pECY6-7/pMK100. In pathway A, the plasmids pPAB19-2 and pECC14-9 could have been generated by a first event consisting of a replacement of a DNA region that we named variable region 1 (VR1), flanked by the *oriT* locus and a Xer site-specific recombination site, followed by a second event consisting of a point mutation and a deletion of one 11-bp copy from a region that includes 12 11-bp imperfect repeats (Fig. 1A). We cannot discard the possibility that pECC14-9 is the direct result of a DNA replacement in pPAB19-1 and in that case pPAB19-2 was generated by a point mutation and the addition of a copy of the 11-bp imperfect repeat (Fig. 1A). Plasmids pMK101 and pPAB19-4 could have resulted from a replacement and a deletion in a separate region that we called variable region 2 (VR2) (Fig. 1B). Plasmid pPAB19-3 has undergone modifications in both VR1 and VR2 (Fig. 1A).

The genetic environment of *qnrB19* in these plasmids, as well as in the IS*Ecp1C*-based transposons Tn*2012*, Tn*5387*, and Tn*5387*-like, which have been proposed as important players in *qnrB19* mobilization (6, 9, 27), are very well conserved (shaded area in Fig. 1). IS*Ecp1* has 14-bp inverted repeats (IRs) and preferentially uses 5-bp AT-rich sites as the insertion targets. IS*Ecp1* is characterized by its ability to mobilize DNA fragments located adjacent to the IR right (IRR) of the element most probably by using alternative IRR-like sequences (named as IRR1, IRR2, IRR3, and so on) that have partial identity with IRR and might be recognized as such by the IS*Ecp1* transposase (24). Our analyses also showed that the

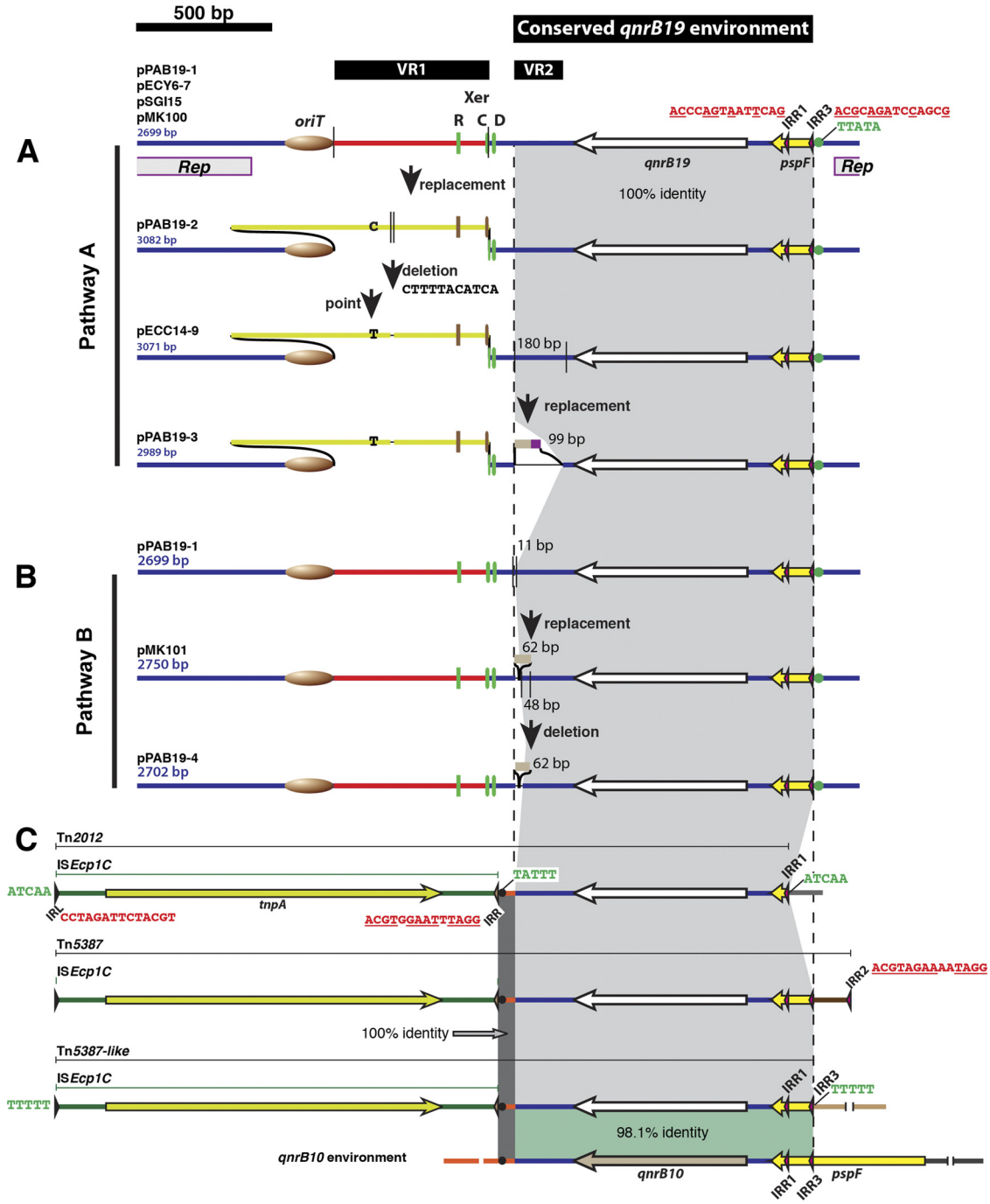

FIG 1 Comparative diagram of *qnrB19*-harboring elements. Colors indicate identical nucleotide sequences. For the sake of clarity, in two regions across the different elements a gray shading was added to indicate the portions with identical sequences. In the case of the *qnrB10* environment, a green shading was added to show the 98.1 identity region. Variable regions VR1 and VR2, as well as the conserved *qnrB19* environment, are indicated by black bars at the top of the genetic maps. The thin vertical lines in the plasmid maps indicate the edges of the DNA fragments replaced or deleted in each rearrangement. The Xer recombination sites components are indicated as follows: R, ARG box (Arg-binding region); C, XerC-binding site; D, XerD-binding site. The different colors of XerC, XerD, and ArgR binding sites indicate that they have different sequences. The *oriT* is indicated as a brown oval. IRL, IRR, IRR1, IRR2, and IRR3 are indicated by slender arrowheads, and their sequences are shown in red (underlined nucleotides correspond to a perfect reverse complement of the IRL sequence). The target site duplications of IS*Ecp1C* or IS*Ecp1C*-based transposons are shown in green (the TTATA sequence after IRR3 in the different plasmids and the TATTT after IRR in the transposons are emphasized by green and black dots, respectively). The location of the replication region (Rep) of all plasmids is shown below the pPAB19-1 genetic map.

common *qnrB19* genetic environment is highly homologous (98.1% identity) to that of the *qnrB10* allele (Fig. 1C), which was recently described in a study on clinical enterobacteria from Argentina (25), where it was found to be associated to IS*CR1*. In that study it was also proposed that several IS*CR1*-associated *qnrB* alleles could have been originally located in similar chromosomal contexts, i.e., downstream of *pspF*, the transcriptional activator of the stress-inducible *psp* operon (25). This hypothesis is supported

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pPAB19      TGAGCGAGGAAGCGGAATATATCC-TGTACTACATATCCTGCTG-ACGCGCTGGTGCCGCCT
pJHCMW1     TGAGCGAAGAAGCGTCATATCACCGTAACCTCTATTTTCTCCTGTACGCTCTAGCGCTGCAT
            ******* ******  ****  ** *   **  ** * ** *** **** ** * ** ** *

pPAB19      TTTTTCTCCTGCTACATGAAGCACTT---CACTGATTTCCACATCCGTGCCAACATAGTCAG
pJHCMW1     TTTCCCT-CTGCCATATGAAGCACTTTCGCGAAGTGTTACTCCTCCGT---AACATATTTAG
            ***  ** **** * ***********   *   *  ** * * *****   ****** * **
                                                          nick site
pPAB19      CCAGTATACACTCCGCTAGCGCTACGTGACTGGTTCAGGGCTGCGCCCCGAAACC
pJHCMW1     CCCGTGTACACTCTGCTAGTGTTACATGACTGATTCAGGGCTGCGCCCCGAAACC
            ** ** ******* ***** * *** ****** **********************
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FIG 2 Comparison of *oriT* regions. Comparison was carried out using CLUSTAL W2. The *oriT* sites from pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 are identical.

by recent results indicating that the chromosome of *Citrobacter* spp. is the likely source of plasmid-mediated *qnrB* (15).

On the basis of the findings described above, we hypothesize that the original environment of *qnrB19* could have been similar to that of *qnrB10*, i.e., it was located downstream of *pspF*. Then, IS*Ecp1C* could have transposed to the TATTT site downstream of *qnrB19* (see Fig. 1C) and captured this gene through subsequent transposition events by using the alternative IRR-like sequences IRR1 or IRR3, giving rise to Tn*2012* or Tn*5387*/Tn*5387*-like, respectively. In turn, Tn*5387*-like could have transposed to an ancestor of the small ColE1-type plasmids analyzed here. As recently suggested by Palecchi et al. (22), the presence of a TTATA site at the edge of the conserved *qnrB19* environment in all plasmids (Fig. 1A and B) supports this assumption. However, these plasmids lack IS*Ecp1C*. It has been proposed that this insertion sequence could have been lost by excision using another IRR-like site located a few base pairs beyond IRR (22). However, we think that this is just one of many possibilities. One point of excision is at the edge of a region with high variability (VR2, Fig. 1), which could indicate other mechanisms of excision at this end of the insertion sequence. Furthermore, in Tn*5387* and Tn*5387*-like we did not find any putative 14-bp sequence at the left edge of the conserved *qnrB19* environment that matched the signatures of an IRR-like site (≥3 nucleotide identities with IRR, including a GG or CG at its 3′ end) (24). Therefore, other rearrangements, including the possibility of another sequence playing a role similar to the IRRs but that has the potential to induce further modifications, may have occurred during excision of IS*Ecp1C*.

The VR1 region: an *oriT*/Xer recombination-based mechanism of plasmid evolution? VR1 regions are flanked by an *oriT* locus and a Xer site-specific recombination-like site (Fig. 1). The *oriT* locus of the plasmids described here shares extensive homology with others that can utilize the ColE1 *mob* functions such as that in the plasmid pJHCMW1 (Fig. 2). The pJHCMW1 *oriT* was shown to be functional in mating experiments using as donor strain *E. coli* DH5α harboring a recombinant plasmid that includes *oriT* and a helper plasmid carrying the ColE1 *mob* genes and the RK2 *tra* genes (8).

A diagram of the general structure of plasmids' Xer recombination sites is shown in Fig. 3A. They usually consist of a core recombination site were the strand exchanges occur and a stretch of ~180 bp known as accessory sequences that possess binding sites for the architectural proteins PepA and ArgR (Fig. 3B). The core recombination site includes two 11-nucleotide binding sites for the tyrosine recombinases XerC and XerD, separated by a 6- to 8-nucleotide central region. Binding of the architectural proteins to the accessory sequences facilitates formation of a synaptic com-

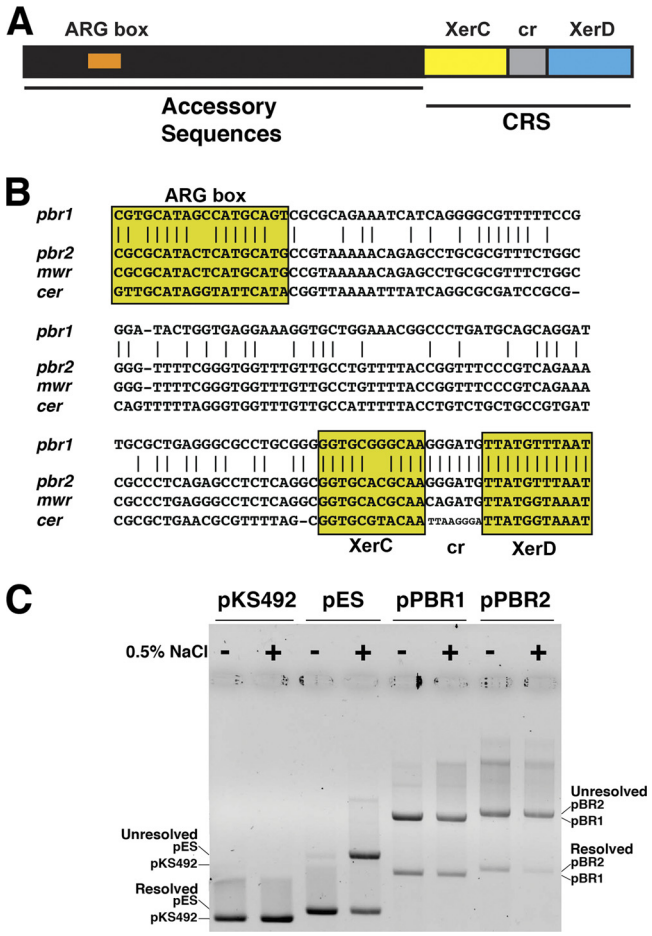

FIG 3 (A) Schematic diagram of plasmid's Xer recombination sites. The sites contain a core recombination region that includes the 11-bp XerC and XerD binding sites and a central region (6 to 8 bp),and accessory sequences (180 bp) with which the architectural proteins ArgR and PepA interact. The diagram is not drawn to scale. (B) Comparison of the nucleotide sequences of *pbr1* (present in pPAB19-1 and pPAB19-4), *pbr2* (present in pPAB19-2 and pPAB19-3), *mwr*, and *cer*. The ArgR-binding site (ARG box) and different regions of the core recombination site (CRS) are shown. XerC, XerC-binding site; XerD, XerD-binding site; cr, central region. Identical nucleotides between *pbr1* and *pbr2* are indicated by vertical lines. (C) Dimer resolution assay. Dimers of plasmids pKS492 (*cer*), pES (*mwr*), pPBR1 (*pbr1*), and pPBR2 (*pbr2*) were introduced by transformation into *E. coli* DS941. The cells were cultured in medium containing 0 or 0.5% added NaCl in the presence of 100 μg of ampicillin per ml for 20 generations. Plasmid DNA was isolated and subjected to agarose gel electrophoresis. The positions of dimers and monomers are indicated at the sides.

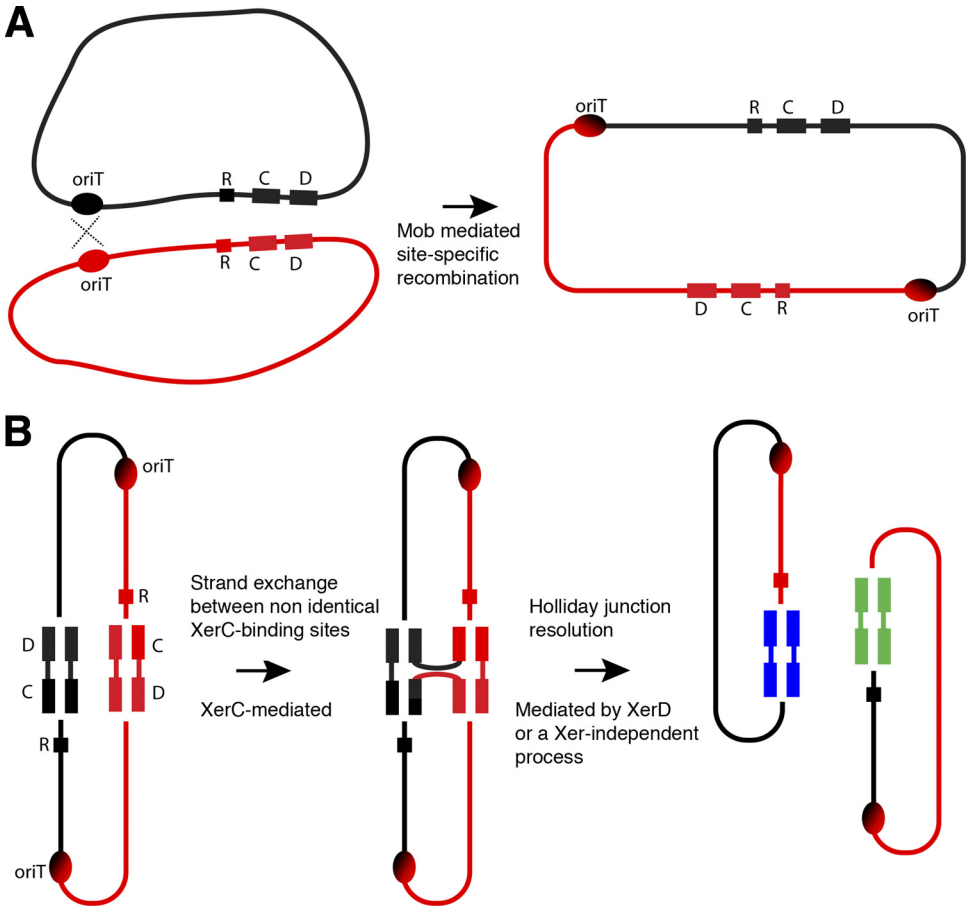

FIG 4 Model of exchange of the DNA region flanked by *oriT* and Xer recombination sites. (A) Two plasmids (black and red) form a cointegrate through *oriT* site-specific recombination mediated by the nickase supplied by a resident plasmid that includes a *mob* gene. R, ARG box; C, XerC-binding site; D, XerD-binding site. (B) The cointegrate is resolved through Xer site-specific mediated recombination. Whether the accessory sequences are needed to facilitate formation of a synaptic complex is still unknown. Double-stranded DNA is shown only at the core recombination site to show the exchange of strands. XerC mediates the exchange of the first pair of strands at an undetermined nucleotide inside the XerC-binding site and forms a Holliday junction. Resolution of the Holliday junction may occur through XerD-mediated exchange of the second pair of strands or through a Xer-independent process such as replication. Since we do not know the mechanism of resolution of the Holliday junction we cannot predict the final nucleotide sequence of the newly formed Xer recombination sites. For this reason we indicate them in blue and green. Resolution of the Holliday junction results in two plasmids that have swapped the region limited by *oriT* and the Xer recombination site.

plex (26), where XerC is activated through interaction with XerD and catalyzes the exchange of the first pair of strands, which results in the formation of a Holliday junction (11) that, in the case of *cer* (ColE1) or *mwr* (pJHCMW1), is resolved by Xer-independent processes (3, 34). Two different versions of Xer recombination sites were found at one end of the VR1 in plasmids pPAB19-1, pPAB19-2, pPAB19-3, and pPAB19-4 (schematically shown in Fig. 1). The nucleotide sequence of these sites, from here on called *pbr1* (pPAB19-1 Xer recombination site present in pPAB19-1 and pPAB19-4) and *pbr2* (pPAB19–2 Xer recombination site present in pPAB19-2 and pPAB19-3), as well as a comparison among themselves and to the nucleotide sequences of other well-known Xer recombination sites are shown in Fig. 3B. The *pbr1* and *pbr2* sites include identical central regions and XerD-binding sites but seem to derive all or part of the XerC-binding site and the accessory sequences from a different source (Fig. 3B). To test whether they are functional, we generated dimers of recombinant clones pPBR1 and pPBR2, which include *pbr1* or *pbr2*, respectively, and carried out resolution assays. Dimers of recombinant clones including *mwr* or *cer*, the Xer recombination sites from pJHCMW1 and ColE1, were used as controls. We have shown before that the concentration of NaCl in the medium where *E. coli* harboring the dimers is cultured affects the efficiency of resolution for some Xer recombination sites. Therefore, we carried out these assays using culture medium containing 0 or 0.5% added NaCl. It is known that dimers containing *cer* are efficiently resolved regardless of the NaCl concentration in the culture medium and dimers containing *mwr* increase the resolution efficiency as the NaCl in the medium decreases (23, 36). Figure 3C shows that the level of resolution of dimers containing *pbr1* or *pbr2* is considerably lower than those of the controls containing *cer* or *mwr*. We have shown before that levels of resolution as low as that one exhibited by *mwr*-harboring dimers in cells grown in L broth containing 0.5% NaCl are not enough to confer stability by multimer resolution (34, 35). As shown in Fig. 3C, the levels of resolution of dimers containing *pbr1* or *pbr2* are not significantly modified when the cells are cultured in the absence of added NaCl, and in both cases the efficiency is significantly lower than those of *mwr*, suggesting that

they are unable to stabilize the host plasmid by multimer resolution under the conditions commonly tested in the laboratory. It is possible that *pbr1* and *pbr2*, as well as other Xer recombination sites that present low efficiency, may not have as a main role plasmid stabilization, but they contribute to plasmid evolution by working in concert with the nearby *oriT* locus. Based on their comparison of the pECY6-7 and pECC14-9 nucleotide sequences, Pallechi et al. recently suggested that the Xer recombination sites found in these two plasmids could play a role in plasmid evolution (22). We postulate here a mechanism for swapping of DNA fragments flanked by an *oriT* locus and a Xer recombination site (Fig. 4). In this model the first event involves site-specific recombination at *oriT*, a process that has been described before for ColE1 and other plasmids' *oriT* loci and depends exclusively on the presence of the *oriT* site and the nickase activity (19, 39). Site-specific recombination at the *oriT* sites of two plasmids, represented as red and black in Fig. 4, leads to integration (Fig. 4A). The cointegrate includes two directly positioned nonidentical Xer recombination sites that may serve as substrate for a second site-specific recombination event mediated by Xer (Fig. 4B). Although Xer site-specific recombination usually occurs between directly repeated identical Xer sites, recombination events between nonidentical Xer sites has been described before (7, 37). It is not possible for us at this time to determine whether the accessory sequences play a role in positioning the XerC and XerD binding sites to form a synaptic complex. In the second recombination event the recombinase XerC mediates the strand exchange of one pair of strands to form a Holliday junction. In Fig. 4B the point of action of XerC has been drawn at the position it usually happens, but we do not know whether the nick and religation in this particular reaction can occur at another position. The Holliday junction is then resolved by XerD-catalyzed strand exchange of the second pair of strands or by a Xer-independent process such as replication (Fig. 4B). Since we do not know the site of action of XerC or the mechanism of resolution of the Holliday junction, we cannot predict the final nucleotide sequence of the newly formed Xer recombination sites. However, if the XerC binding sites and/or the central regions are not identical, we can predict that the newly formed Xer recombination sites will have a modified structure with respect to the original ones (represented by the different colors of the newly formed sites in Fig. 4B).

As a consequence of the two successive site-specific recombination reactions, the plasmids have exchanged the fragment that includes the whole region between *oriT* and the Xer recombination site, and the Xer recombination site in each plasmid has also been modified (see Fig. 4B). The extension of the modification of the Xer recombination site is dependent on the similarities of the recombination sites, the point of action of XerC, and the mechanism of resolution of the Holliday junction. Future studies will permit us to test the model and define it with more exactitude. The combination *oriT*-Xer recombination site could be considered one more element used by plasmids to evolve by swapping DNA regions. Unlike integrons that mediate acquisition or shedding of genes flanked by specific target sites (*attI* and *attC*) by site-specific recombination mediated by an integrase (5), this *oriT*-Xer element swaps DNA regions that do not necessarily include genes and do not present any requirements other than being located between an *oriT* and a Xer recombination site.

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