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Complete Sequencing of IncI1 Sequence Type 2 Plasmid pJIE512b Indicates Mobilization of bla_{CMY-2} from an IncA/C Plasmid

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Sequencing of pJIE512b, a 92.3-kb IncI1 sequence type 2 (ST2) plasmid carrying bla_{CMY-2}, revealed a bla_{CMY-2} context that appeared to have been mobilized from an IncA/C plasmid by the insertion sequence IS*1294*. A comparison with published plasmids suggests that bla_{CMY-2} has been mobilized from IncA/C to IncI1 plasmids more than once by IS*1294*-like elements. Alignment of pJIE512b with the only other available IncI1 ST2 plasmid revealed differences across the backbones, indicating variability within this sequence type.

Plasmid-mediated AmpC β-lactamases, particularly in *Escherichia coli* and in *Salmonella* species, represent a significant public health concern, as they confer resistance to the globally important cephalosporin antibiotics and β-lactamase inhibitors (1). The most prevalent AmpC β-lactamase, CMY-2, encoded by bla_{CMY-2}, was first reported in 1990 (1, 2). bla_{CMY-2} and its minor variants are now globally disseminated among a number of species, most likely through a combination of clonal expansion of strains bearing bla_{CMY-2}-carrying plasmids and horizontal transfer of the plasmids themselves (3–6).

Sequence comparisons indicate that a region including bla_{CMY-2} was mobilized from the *Citrobacter freundii* chromosome onto a plasmid (7) by the insertion sequence IS*Ecp1* (Fig. 1a and b) (1, 8). Variations in the context of bla_{CMY-2}-like genes are due to differences in the size of the *C. freundii* region mobilized and/or the result of recombination (3, 8, 9).

Various plasmid types, including IncA/C, IncI1, IncI2, and IncFII, are associated with the carriage of bla_{CMY-2} (10–15). However, IncA/C and IncI1 plasmids are often reported as the most common carriers of bla_{CMY-2} (10, 13–15), particularly IncI1 sequence type 2 (ST2), ST12, and ST23 (10, 13, 16), as defined by plasmid multilocus sequence typing (pMLST) (17). Despite their importance, no ST2 or ST23 plasmid sequences have been published. Here, we completely sequenced pJIE512b, an IncI1 ST2 plasmid carrying bla_{CMY-2}.

Clinical *E. coli* isolate JIE512b was identified as carrying bla_{CMY-2} during routine PCR screening for plasmid-borne *ampC* genes (see Table S1 in the supplemental material) at the Centre for Infectious Diseases and Microbiology at Westmead Hospital, New South Wales, Australia. Conjugation of JIE512b with DH5αRf, with selection on 16 μg/ml cefoxitin plus 80 μg/ml rifampin, performed as previously described (18), gave transconjugants carrying bla_{CMY-2} and a single plasmid that was named pJIE512b. pMLST (17) indicated that pJIE512b is ST2. Plasmid DNA was purified using the HiSpeed plasmid midikit (Qiagen, Germany), and 1 ng was used for library preparation with the Nextera XT DNA sample preparation kit (Illumina, Inc., USA). Sequencing by Illumina MiSeq technology was performed at the Australian Genome Research Facility (Melbourne, Australia), and sequence reads were processed using FLASh (http://ccb.jhu.edu/software/FLASH/) and were assembled into contigs with Velvet (http://www.ebi.ac.uk/~zerbino/velvet/), SPAdes (http://bioinf.spbau.ru/spades), and CLC Genomics Workbench (http://www.clcbio.com/products/clc-genomics-workbench/). Seven contigs (~900-fold average coverage) were assembled using SeqMan (DNASTAR, Madison, WI, USA) with PCR amplification (see Table S1 in the supplemental material) and Sanger sequencing to confirm contig boundaries. Analysis and annotation of the resulting sequence were performed using the RAST server (19), BLASTn searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi), ISfinder (https://www-is.biotoul.fr//), and the Gene Construction Kit program (Textco BioSoftware, Inc., USA).

Four contigs corresponded to components of the IncI1 shufflon. This multiple inversion system is responsible for generating variation in the C terminus of the PilV tip adhesin of the thin pilus, leading to differences in recipient cell specificity in liquid mating (20). The shufflon contains up to four segments separated by seven inverted repeats, and a shufflon-specific recombinase (encoded by *rci*) catalyzes recombination between these repeats (20). Sequencing of the amplicons obtained with primers in the *rci* and *pilV* genes flanking the shufflon (see Table S1 in the supplemental material) revealed mixed bases in this region, suggesting a population of plasmids harboring different arrangements. We assembled the four shufflon contigs according to the arrangement that appeared to be dominant.

The resulting 92,339-bp plasmid was similar in organization to other IncI1 plasmids, with a complete conjugal transfer region, including the *traABCD* gene cluster, thin pilus formation region *pilI* through *pilV*, *traL* through *traY*, and *trb* transfer regions, and DNA-processing *nikAB* genes (21). Genes involved in the inhibition of the bacterial SOS response (*psiAB*) and in plasmid addiction (*pndCA*) were also identified (16, 21). pJIE512b harbored a 4,831-bp insert containing 161 bp of the right end of IS*Ecp1* and a 2,823-bp region of the *C. freundii* chromosome, including bla_{CMY-2} (Fig. 1c). IS*Ecp1* was truncated by IS*1294*, and the *C. freundii* region was followed by a 159-bp fragment of IncA/C backbone. IS*1294* transposes by a

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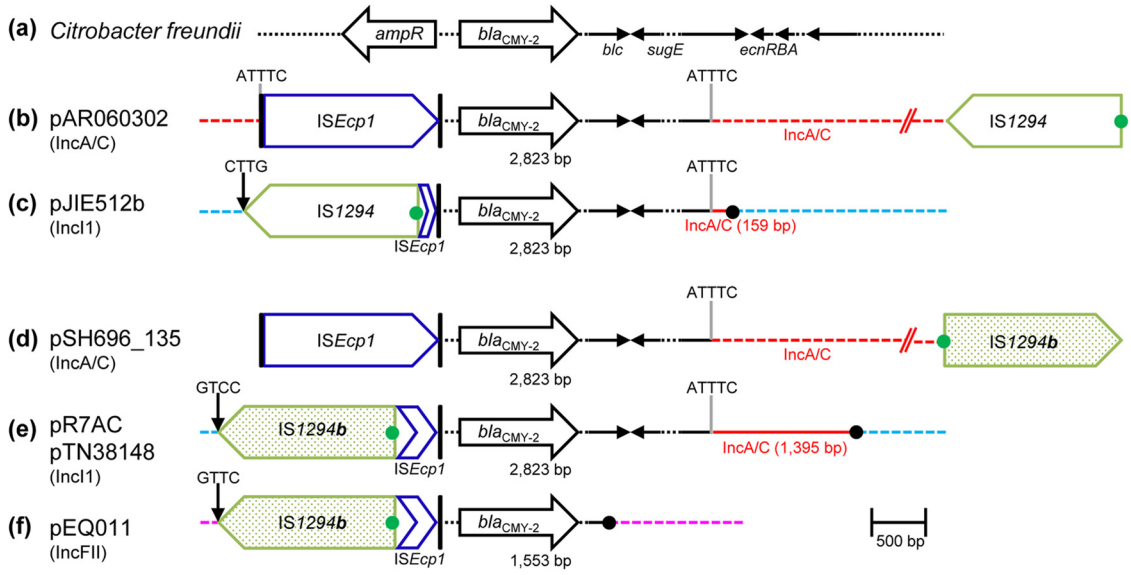

FIG 1 Different bla_{CMY-2} contexts. IS*Ecp1* is indicated by dark blue, inverted repeats are indicated by vertical black bars, and direct repeats are labeled and indicated by vertical gray bars. IS*1294* and IS*1294*-like elements are depicted with green and stippled green, respectively, target sites are labeled with black arrows, *ter*IS sequences are depicted with green dots, and *ter*IS look-alike sequences are depicted with black dots. Plasmid backbones are indicated by dashed red (IncA/C), cyan (IncI1), and pink (IncFII) lines. IncA/C backbone regions that have been mobilized are indicated by solid red lines. The lengths of the *Citrobacter freundii* part of the insertions are shown. (a) *C. freundii* chromosomal region, including bla_{CMY-2} (GenBank accession no. U21727 and AY125469). (b) The 2,823-bp bla_{CMY-2} region in IncA/C plasmid pAR060302 (GenBank accession no. FJ621588) is associated with a complete IS*Ecp1*, and the whole insert is flanked by direct repeats. IS*1294* is present elsewhere in the backbone. (c) bla_{CMY-2} context in pJIE512b. The insert is flanked by the IS*1294* target site CTTG at the 5′ end and bounded by the IS*1294* *ter*IS look-alike sequence GTTC at the 3′ end. (d) IncA/C plasmid pSH696_135 (GenBank accession no. JN983048) contains a complex IS*Ecp1*-bla_{CMY-2} arrangement, part of which is shown. An IS*1294b* element is present elsewhere in the plasmid. (e) The IS*1294b*-ΔIS*Ecp1*-bla_{CMY-2} segment in pR7AC (GenBank accession no. KF434766) and pTN38148 (FM246883) is flanked at the 5′ end by the imperfect target site GTCC and bounded at the 3′ end by the *ter*IS look-alike sequence GTTC. (f) The shorter bla_{CMY-2} insert in the IncFII plasmid pEQ011 (GenBank accession no. KF582523) is flanked by the IS*1294* target site GTTC and the imperfect *ter*IS look-alike sequence CTTG.

rolling-circle mechanism and can capture adjacent regions by inefficient replication through its termination site (*ter*IS) to an alternative *ter*IS look-alike sequence (22). The IncA/C fragment in pJIE512b ends in GTTC, which matches the last 4 bp of *ter*IS. This implies that IS*1294* inserted into IS*Ecp1* in an IncA/C plasmid and subsequently mobilized the adjacent region, including bla_{CMY-2} plus 159 bp of the IncA/C backbone. An IncA/C plasmid such as pAR060302 (GenBank accession no. FJ621588) (23), which carries an intact IS*Ecp1*, the 2,823-bp bla_{CMY-2} region, and IS*1294*, might be the source (Fig. 1b).

A similar bla_{CMY-2} context was identified in five partially sequenced IncI1 plasmids isolated from *E. coli* in France, including pTN38148 (GenBank accession no. FM246883) (9), and in a recently sequenced IncI1 ST2 plasmid from Denmark, pR7AC (KF434766). However, in these plasmids, an IS*1294*-like element, designated IS*1294b* here, truncates IS*Ecp1* at a different position, leaving a longer 372-bp fragment. The IncA/C fragment at the 3′ end of the insert (1,395 bp) is also substantially longer than that in pJIE512b and again ends with the IS*1294* *ter*IS look-alike sequence GTTC (Fig. 1e). IS*1294b* is ~95% identical to much of IS*1294*, but a 332-bp region that overlaps the start of the transposase gene is unrelated. IS*1294b* appears to be mobile, as it is found in at least eight different contexts in plasmid sequences in GenBank, including in IncA/C plasmids that also carry bla_{CMY-2}. One of these, e.g., pSH696_135 (GenBank accession no. JN983048; Fig. 1d) (24), may be the source of the insert transferred to pR7AC. An IS*1294b*-ΔIS*Ecp1*-bla_{CMY-2} insert with the same 372 bp of IS*Ecp1* but a shorter (1,553-bp) *C. freundii* region and with no IncA/C fragment is present in the recently published IncFII plasmid pEQ011 (GenBank accession no. KF582523) (25) (Fig. 1f). This insert ends in an imperfect *ter*IS look-alike sequence (CTTG) and thus appears to have been mobilized by IS*1294b* from a pR7AC-like IncI1 plasmid or directly from the IncA/C progenitor, both of which have longer *C. freundii* regions.

In pJIE512b, the bla_{CMY-2} insertion is upstream from *yafB* (unknown function), and an adjacent deletion removed part of the *cia* (colicin) gene (Fig. 2). In pR7AC, the insertion is downstream of *yafB*, a larger adjacent region is deleted, and *cia* is intact. Despite high homology across other regions of the backbones and identity at the pMLST target sites, a number of regions display <90% identity, including a short region encoding a single-stranded-DNA binding protein and several hypothetical proteins. An ~1.2-kb region of pJIE512b (at ~kb 10.5 in Fig. 2) (unknown function) shows no similarity to pR7AC but displays 100% identity to two IncI1 ST19 plasmids (16). These differences between the backbones and the different bla_{CMY-2} inserts reflect variability within pMLST ST2 and indicate, for at least this sequence type, that a more discriminatory plasmid typing method may be necessary for epidemiological purposes.

The novel bla_{CMY-2} context identified in this study, in addition to the contexts reported in the literature, suggests at least two separate mobilization events of bla_{CMY-2} from an IncA/C plasmid to an IncI1 plasmid mediated by an IS*1294*-like element. A recent paper also reported the IS*Ecp1*-mediated transfer of a bla_{CMY-2} region that includes 4,276 bp of backbone from an IncA/C plasmid to an IncX1 plasmid during conjugation experiments (26).

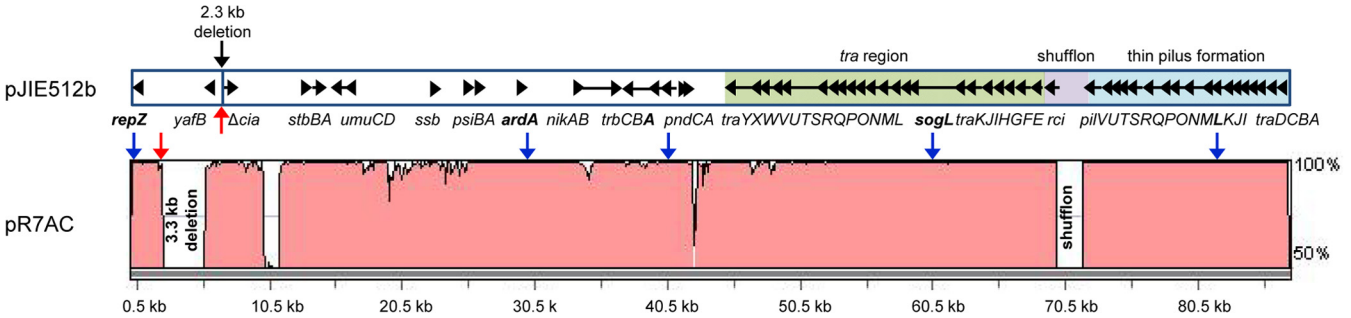

FIG 2 Comparison of the pJIE512b and pR7AC backbones, with bla_{CMY-2} inserts removed. Percent identity is displayed on the right of the diagram. The lengths and directions of selected genes are indicated by horizontal black arrows labeled with the gene name. pMLST target genes are shown in bold type, and pMLST amplicon positions are indicated by vertical blue arrows. The positions of the bla_{CMY-2} inserts in pJIE512b and pR7AC are indicated by red arrows. Important regions are shaded in green (*tra* region), purple (shufflon), or blue (thin pilus formation region). The location of the 2.3-kb deletion in pJIE512b is indicated by a vertical black arrow, and the position of the 3.3-kb deletion in pR7AC is labeled. The shufflon region is not included in the pR7AC GenBank entry. This diagram was drawn using mVISTA (http://genome.lbl.gov/vista/index.shtml) (28) using a calculation window of 100 bp.

These examples demonstrate movement of bla_{CMY-2} from a broad-host-range (IncA/C) plasmid to narrow-host-range (IncI1, IncF, IncX) plasmids that are more likely to be well adapted to their ecological niches (27), shedding light on the possible pathways by which bla_{CMY-2} (and potentially other resistance genes) have been mobilized into *E. coli*.

Nucleotide sequence accession number. The complete nucleotide sequence of pJIE512b has been submitted to the European Nucleotide Archive (ENA) under the accession number HG970648.

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