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Complete Nucleotide Sequence of the pCTX-M3 Plasmid and Its Involvement in Spread of the Extended-Spectrum β-Lactamase Gene $bla_{CTX-M-3}$ ▽

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Here we report the nucleotide sequence of pCTX-M3, a highly conjugative plasmid that is responsible for the extensive spread of the gene coding for the CTX-M-3 extended-spectrum β-lactamase in clinical populations of the family *Enterobacteriaceae* in Poland. The plasmid belongs to the IncL/M incompatibility group, is 89,468 bp in size, and carries 103 putative genes. Besides $bla_{CTX-M-3}$, it also bears the bla_{TEM-1}, *aacC2*, and *armA* genes, as well as integronic *aadA2*, *dfrA12*, and *sul1*, which altogether confer resistance to the majority of β-lactams and aminoglycosides and to trimethoprim-sulfamethoxazole. The conjugal transfer genes are organized in two blocks, *tra* and *trb*, separated by a spacer sequence where almost all antibiotic resistance genes and multiple mobile genetic elements are located. Only $bla_{CTX-M-3}$, accompanied by an IS*Ecp1* element, is placed separately, in a DNA fragment previously identified as a fragment of the *Kluyvera ascorbata* chromosome. On the basis of sequence analysis, we speculate that pCTX-M3 might have arisen from plasmid pEL60 from plant pathogen *Erwinia amylovora* by acquiring mobile elements with resistance genes. This suggests that plasmids of environmental bacterial strains could be the source of those plasmids now observed in bacteria pathogenic for humans.

Bacterial plasmids constitute an important part of the so-called horizontal gene pool, the pool of genes that can be acquired by various bacterial strains by means of horizontal gene transfer (56). The ability of plasmids to spread by conjugation greatly enhances their impact on the genetic plasticity, metabolic potential, and environmental adaptability of bacteria, which in the case of plasmids carrying virulence or antimicrobial resistance genes is a source of serious clinical and epidemiological problems (see references 35, 56, and 61 and references therein).

Among the genes that are often located in conjugative plasmids are those coding for extended-spectrum β-lactamases (ESBLs), which in large part are responsible for the resistance of the members of the family *Enterobacteriaceae* to newer β-lactams (11, 24, 35). The epidemiology of ESBLs has recently been dominated by the extremely rapid and worldwide spread of organisms producing enzymes of the CTX-M family, both in nosocomial environments and in the community (10, 36, 45). To date, over 60 CTX-M-type β-lactamases have been identified, showing the intensive evolution of this group (http://www.lahey.org/studies/webt.stm). bla_{CTX-M} genes are derivatives of the chromosomal β-lactamase genes of the genus *Kluyvera* (17, 29, 43, 46, 49). In transmissible plasmids found in clinical isolates, they usually reside in fragments of *Kluyvera* sp. chromosomes in association with either IS*Ecp1*-like insertion sequences (30, 34, 37, 47) or CR1 elements inserted in *sul1*-type class 1 integrons (3, 19, 41, 50, 58). The role of IS*Ecp1*-like sequences in the mobilization of *Kluyvera* genes and details of this transposition process have been demonstrated recently (35, 47, 48); moreover, these elements are also frequently responsible for the high-level expression of bla_{CTX-M} genes (10, 13, 47).

In Poland, the first gene of the bla_{CTX-M} type, $bla_{CTX-M-3}$, was identified in 1996 in clinical isolates of *Citrobacter freundii* and *Escherichia coli* from one of the hospitals in Warsaw. It was associated with a large plasmid that could be easily transferred to *E. coli* via conjugation and that conferred resistance to penicillins, cephalosporins, and aztreonam, as well as aminoglycosides, gentamicin, and tobramycin (25). Later, the rapid spread of CTX-M-3-producing organisms was observed in the same center (44) and all over the country (7, 8), and it has mainly been attributed to the horizontal transmission of plasmids. Of the several types of plasmids with $bla_{CTX-M-3}$ genes identified by restriction fingerprinting, one family of similar molecules in particular has been widely disseminated into multiple locales and multiple species, including *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *C. freundii*, *Morganella morganii*, *Serratia marcescens*, and *Salmonella enterica* (7, 8). The most prevalent variant of that family was the plasmid first observed in 1996 in *C. freundii* isolates, in which CTX-M-3 had been originally identified (25). In Taiwan, where CTX-M-3 is the most common ESBL, it has been encoded by large, transmissible IncI1-like plasmids (37),

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while in Spain the dissemination of CTX-M-3 and other CTX-M-1-like β-lactamases is ascribed to the broad-host-range plasmids of the IncA/C2, IncL/M, and IncN groups (42).

In this paper we report the sequence of the pCTX-M3 plasmid originating from *C. freundii* isolate 2526 from the Praski Hospital in Warsaw (25). The results of our study demonstrate the physical link between the $bla_{CTX\text{-}M\text{-}3}$ gene, the IncL/M replicon, and an efficient conjugal transfer system. We also discuss the possible evolution of pCTX-M3 from a putative ancestor pEL60 from a plant pathogen, *Erwinia amylovora* (22).

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* A15(pCTX-M3), a transconjugant of *C. freundii* 2526 (25), was the initial source of pCTX-M3 in this study. Clinical isolates *K. pneumoniae* 179, *S. marcescens* 12, and *E. coli* 279 were described previously (44); and the other clinical strains or transconjugants used in the study included *E. coli* 2112, *E. coli* 3624, *E. coli* 1145, *E. coli* 8350, *E. coli* 1775, *K. pneumoniae* 9172, and *K. pneumoniae* 2113, which were also described previously (7). *E. coli* DH5α (27) was used as a host strain in all cloning experiments. In the matings, *E. coli* DH5α(pCTX-M3) and *Agrobacterium tumefaciens* UBAPF(pCTX-M3) (39) were used as the donors and *E. coli* JE2571 Rif^r (*leu thr thi lacY thy pil fla* Rif^r [12]) and DH5α(pACYC184) were used as the recipients, respectively. The shotgun library was prepared in vector pCR4BluntTOPO (Invitrogen, Carlsbad, CA). Plasmid pMI3, the pUC19 derivative with the *bla* gene replaced by *cat* of pACYC184 (the AvaII-NarI fragment of pUC19 blunt ended by use of the Klenow fragment and ligated with FnuDII-digested pACYC184), was used as the vector for cloning of the IncL/M and IncI1 *oriT* sequences. Plasmid pLG221, a ColIb-P9 derivative (14), was used as an IncI1 helper plasmid in *oriT* mobilization experiments.

Plasmid conjugal transfer. One-milliliter volumes of cultures of the donor and recipient strains (10^9 CFU of each strain per ml) grown in Luria-Bertani (LB) broth (Biocorp, Warsaw, Poland) were mixed and incubated for 30 min at 37°C. Conjugation was stopped by vigorous vortexing for 30 s and placing the mating mixture on ice. Transconjugants were selected on LB agar (Biocorp) supplemented with cefotaxime (5 μg/ml; Polfa Tarchomin, Warsaw, Poland) and rifampin (50 μg/ml; Polfa Tarchomin). Solid mating was performed likewise, with an additional step of filtering of the mating mixture through a sterile HA 45-μm-pore-size filter (Millipore, Billerica, MA), which was then incubated on an LB agar plate. In the control experiments, the frequencies of spontaneous mutation of both the donor and the recipient to the phenotype of the transconjugants were measured.

DNA cloning and sequencing. Plasmid DNA was isolated by the alkaline lysis method, and cloning procedures were performed by standard protocols (53). The pCTX-M3 DNA for construction of a shotgun library was purified by CsCl-ethidium bromide gradient ultracentrifugation (53). The shotgun library was prepared in the pCR4BluntTOPO plasmid with the use of a TOPO shotgun subcloning kit (Invitrogen). The plasmid was sequenced by using a combination of shotgun sequencing and primer walking methods. BigDye (version 2.0; Applied Biosystems, Foster City, CA) chemistry on an ABI377 automated sequencer (Applied Biosystems) was used. All enzymes used for cloning were from MBI Fermentas, Vilnius, Lithuania.

Plasmid sequence assembly and analysis. The assembly of the sequence was performed with the use of the phred/phrap/consed software suite (20, 21), and the consed software autofinish function was used for gap closing and finishing of the sequence. The following programs were used for bioinformatic analysis: for gene identification, the Glimmer program (version 2.0) trained on *E. coli* long open reading frames (ORFs) was used (18, 52); for homology searches, the BLAST (version 2.0) program was used (2); and for alignment construction, the ClustalW (version 1.8) program was used (57).

PCR detection of *repA* and *traU* genes and mapping of $bla_{CTX\text{-}M\text{-}3}$-containing regions. Primers repAu (5′-CACTTTGGCCGGTCAGAGAT-3′) and repAd (5′-CGATCATCTTCCAGAGGCG-3′) were used for the amplification of *repA*, and primers traUC (5′-ATCTGACGCATCATTGCGCC-3′) and traUN (5′-TGTAGGGCTTACGTCGCAGG-3′) were used for the amplification of *traU*. The presence of IS*Ecp1* and its distance to $bla_{CTX\text{-}M\text{-}3}$ were analyzed by PCR with primers ALA-3 and ALA-4, as described previously (6). The 3′ flank of $bla_{CTX\text{-}M\text{-}3}$ was mapped with primer P1A (7), which anneals to the more distal part of $bla_{CTX\text{-}M\text{-}3}$, and primer IRR (5′-GCGCACGTAGGTCCCAGG-3′), which anneals to the very 3′ end of the *Kluyvera ascorbata* DNA present in pCTX-M3 (49).

Nucleotide sequence accession number. The complete nucleotide sequence of the pCTX-M3 plasmid has been submitted to the GenBank database under accession no. AF550415.

RESULTS AND DISCUSSION

Overall sequence analysis. The complete nucleotide sequence of plasmid pCTX-M3 was verified by comparison of in silico-generated restriction maps with the experimental ones. The plasmid is a circular molecule of 89,468 bp and contains 103 putative genes (Fig. 1), of which 22 (21.4%) have no homologs in public databases, 44 (42.7%) have homologs of unknown functions, and the remaining 37 (35.9%) have homologs with known functions. In sixty-seven percent, the plasmid consists of a known backbone, described before in plasmid pEL60 from the plant pathogen *E. amylovora* (21), enriched with two additional regions that contain all pCTX-M3 antimicrobial resistance genes and mobile genetic elements (see below). The mean G+C content of pCTX-M3 is 51%, but it varies from 32% to 65%, depending on the sequence block (Fig. 1). The high G+C content (ca. 60%) is characteristic of a region located at position 3.2 to 14.0 kb, with the sequence of pCTX-M3-specific *orf8* (positions 9639 to 9911) displaying the highest G+C content (65%). Two fragments of exceptionally low G+C content, 34% and 35%, are present at positions 1.3 to 3.2 kb and 72.0 to 80.0 kb, respectively, and are located inside the two regions with resistance genes and mobile elements. Thus, pCTX-M3 probably evolved from an environmental plasmid similar to pEL60 through the stepwise integration of mobile genetic elements associated with antimicrobial resistance genes (see below).

Plasmid backbone. Only one replication region was identified in the sequence of pCTX-M3. This replication cassette (positions 54341 to 55979) was confirmed to be identical to that of the prototypical IncL/M replicon of pMU407.1 (4, 5). The segregational stability of the plasmid is ensured by an active partition system encoded by *parAB* genes (position 17203 to 18633) related to those of IncI1 plasmid ColIb-P9 (*parA*, 64% similarity; *parB*, 34% similarity). The presence of a single replicon in pCTX-M3, as well as the functionality of the *parAB* system, were confirmed in a separate analysis (39). Two other regions possibly involved in stable plasmid maintenance were identified; and these included (i) region *pemIK* (positions 757 to 1348), which is identical to that of IncFII plasmid R100 and which encodes a postsegregational killing system (59), and (ii) the *resD* gene, which codes for a putative resolvase (positions 16229 to 16972) that is most similar to the resolvase of pYVe8081 (GenBank accession no. AF074611; 76% similarity). pCTX-M3 also bears *mucAB* genes (positions 4698 to 6435), which are members of the *umuDC*-like family of UV resistance genes (28).

Conjugal transfer system. Genes coding for the pCTX-M3 conjugal transfer system are organized in two blocks, *tra* (positions 31620 to 54603) and *trb* (positions 89418 to 83109). The system is related to that of IncI1 plasmid ColIb-P9 in terms of the amino acid sequences of specific proteins (30% to 60%) and of a conserved gene order (Fig. 2). Nevertheless, significant differences are observed between the two systems. First, the *oriT* region (*oriT*, *nikAB*) in ColIb-P9 is located downstream of the *trb* cluster, and they are both convergently tran-

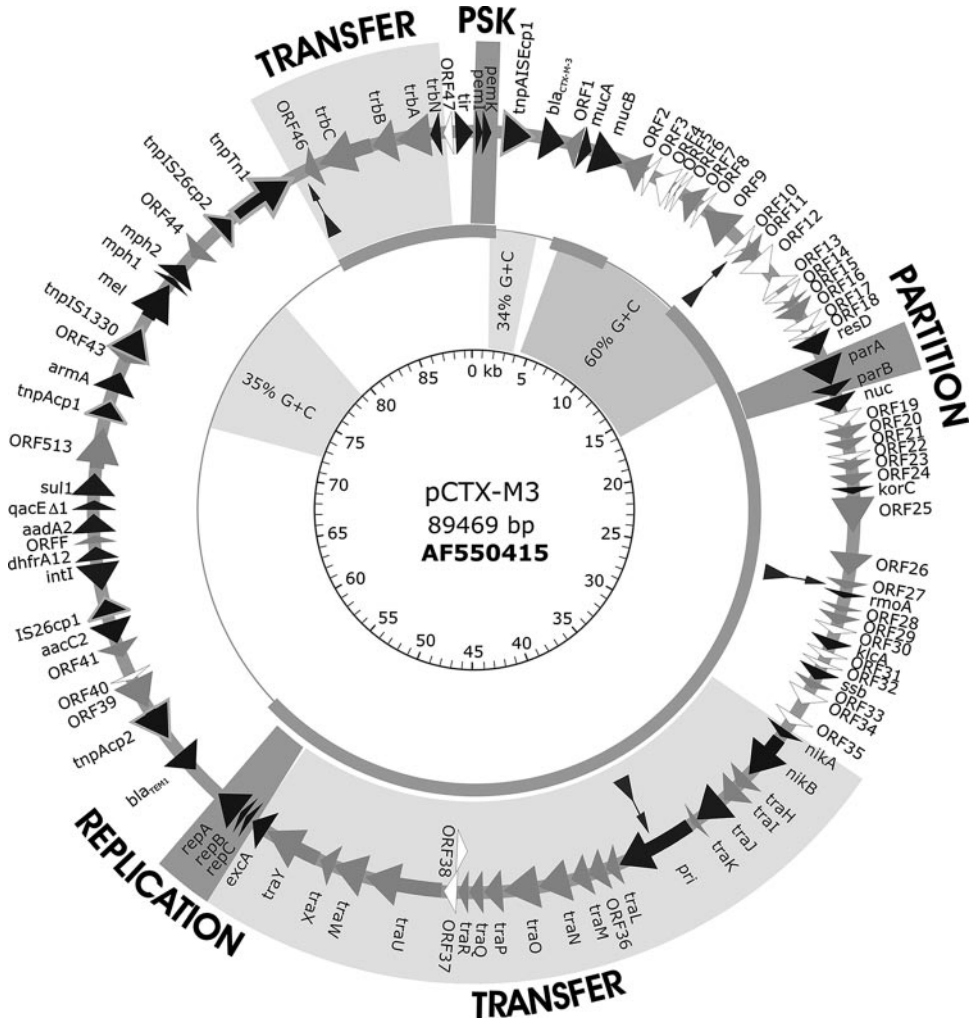

FIG. 1. Overview of the pCTX-M3 sequence. ORFs are shown by arrows: pCTX-M3-specific ORFs are in white, ORFs homologous to genes of known function are in black, and ORFs homologous to genes of unknown function are in gray. Functional sequence blocks are highlighted with shades of gray bands and are indicated on the outside: replicon, stabilizing cassettes (PSK and partition), and conjugal transfer system. The middle circle with a thick line indicates the pCTX-M3 sequence identical to that of plasmid pEL60. The inner circle is a schematic representation of blocks of low and high G+C contents. Arrows inside the outer circle indicate the positions in which pEL60 has additional sequences that are absent from pCTX-M3.

scribed, while in pCTX-M3 this region is a part of the *tra* operon. Second, the *trb* operon in the IncI1 system is placed next to *tra* and is transcribed in the same direction, whereas in pCTX-M3, *trb* lies 28.5 kb apart from the *tra* genes and is transcribed in the opposite direction. Third, some genes present in one of the plasmids are missing from the other; e.g., pCTX-M3 lacks *traEFG*, the three initial genes of the *tra* operon, and *traV*, which was found to be essential for the conjugal transfer of ColIb-P9 (33). In contrast, ColIb-P9 lacks a counterpart of *orf36*, which in pCTX-M3 separates *traL* and

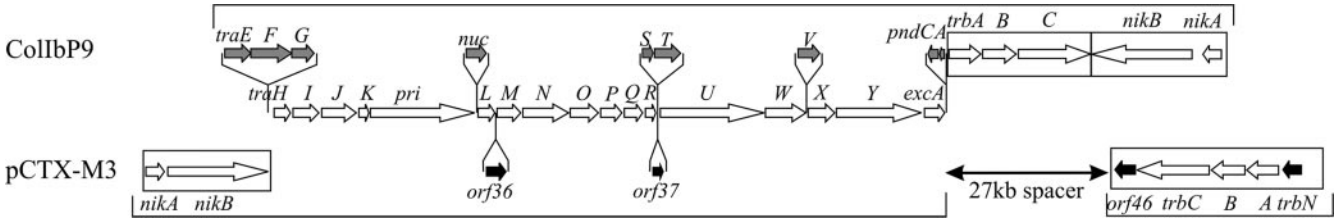

FIG. 2. Comparison of regions coding for the conjugal transfer systems of pCTX-M3 and IncI1 plasmid ColIb-P9. ORFs are presented as arrows: those present in both plasmids are not filled, and ORFs present only in pCTX-M3 or ColIb-P9 are black and gray, respectively, and are shown under or above the scheme of the common structure region.

traM, and the *traST* genes of ColIb-P9 are replaced in pCTX-M3 by a single gene, *orf37*. Interestingly, no homology between *orf37* and either *traS* or *traT* was detected. It is noteworthy that *pil* genes have not been found in pCTX-M3; thus, its host should not produce thin pili, which in IncI1 carriers enable liquid mating (31, 32). Therefore, it should be underlined that pCTX-M3 is able to transfer in liquid medium (7, 25, 44) at the same frequency as it does on solid support, reaching a value of 0.1 transconjugants/donor (the frequency of spontaneous mutations of donor and recipient strains to the transconjugant phenotype was estimated to be lower than 10^{-8}).

As shown recently by Mierzejewska et al. (39), the broad host range of pCTX-M3 is not limited to the family *Enterobacteriaceae* but also includes members of the classes *Alpha-*, *Beta-*, and *Gammaproteobacteria*. We managed to transfer pCTX-M3 back from a soil bacterium, *A. tumefaciens* (a member of the class *Alphaproteobacteria*), to *E. coli* at a frequency of 10^{-5} per donor cell. This demonstrated the functionality of the transfer system in an *Agrobacterium* sp. and confirmed the possibility that environmental bacteria are a reservoir of pCTX-M3 ancestors.

***oriT* identification.** We have identified a region of pCTX-M3 (positions 31616 to 31721) that resembles *oriT* of IncI1 plasmid ColIb-P9 (23). The 105-bp fragment, which contains the putative $oriT_{\text{pCTX-M3}}$, was cloned into the pMI3 vector to yield the construct pOriT, which was tested for mobilization by pCTX-M3 in both liquid and solid matings. The plasmid was mobilized at a frequency of 0.1 transconjugant/donor cell in both cases, demonstrating the full functionality of the cloned *oriT*. In parallel, $oriT_{\text{IncI1}}$ of IncI1 plasmid pLG221 (GenBank accession no. AP005147; positions 67061 to 66944) cloned in pMI3 (pOriTIncI1) was mobilized by pLG221 at a frequency of 2.9×10^{-4}. Moreover, pOriT ($oriT_{\text{pCTX-M3}}$) was mobilized by pLG221 and pOriTIncI1 was mobilized by pCTX-M3 at frequencies of 5.9×10^{-5} and 8.7×10^{-6}, respectively. The results indicate that the mobilization of $oriT_{\text{pCTX-M3}}$ by a heterologous transfer system (IncI1) is possible, albeit at low frequency, thus suggesting an even increased possibility of plasmid dissemination in microbial populations.

The 27-kb replicon-*trb* region. A 27-kb region separating the replicon and the *trb* operon (positions 55980 to 83109; Fig. 1) is one of the two segments that differentiate plasmid pCTX-M3 from plasmid pEL60 from *E. amylovora* (22). It contains almost all of the pCTX-M3 mobile elements and antimicrobial resistance genes, including $bla_{\text{TEM-1}}$, which codes for the broad-spectrum β-lactamase TEM-1; *aadA2*, *aacC2*, and *armA*, which confer resistance to aminoglycosides; and *dfrA12* and *sul1*, which are responsible for resistance to trimethoprim-sulfamethoxazole.

A fragment of transposon Tn*1* containing the $bla_{\text{TEM-1}}$ gene is located (positions 56790 to 57990) next to the replicon. The direct repeat (DR) created by the Tn*1* insertion and the Tn*1* left inverted repeat (IRL) are present directly at the boundary with the replicon. The second part of Tn*1*, which includes the truncated *tnp* gene, the right inverted repeat, and the second DR (positions 80415 to 83069), is found at the other end of the 27-kb insert, close to the *trb* genes. Tn*1* was disrupted by other mobile elements (see below), which removed a part of its *tnp* and the whole *res* gene. A putative insertion sequence (IS; positions 58454 to 60070) is located behind $bla_{\text{TEM-1}}$, with complete inverted repeats (IRs) and DRs. Its *tnp* reveals 97% similarity to that in plasmid pADP-1 (38). This IS is followed by *orf39* to *orf41* and the *aacC2* gene, and the entire segment from Tn*1* to *aacC2* is almost identical to a fragment of recently described plasmid pU302L of *S. enterica* serovar Typhimurium (15).

The first of two IS*26* copies present in pCTX-M3 (positions 63800 to 64622) and a class 1 integron (positions 64640 to 69064) are located downstream of the *aacC2* gene. This integron bears three gene cassettes in its variable region, namely, *dfrA12*, *orfF*, and *aadA2*. An identical array of gene cassettes was found in an integron of plasmid pLEW517 from *E. coli* (60) and in several others (16) (GenBank accession no. AF188331). The pCTX-M3 integron contains a complete 5′ conserved sequence (5′-CS) with the terminal IR (IRi), whereas its 3′-CS is truncated 24 bp after the *sul1* stop codon due to the insertion of a CR1 element with *orf513* (positions 71184 to 71218) (54). Downstream of CR1 there is a putative IS homologous to IS*R391B* (9) with truncated IRL (positions 71219 to 72092), the aminoglycoside resistance gene *armA*, an IS*1330*-like element (positions 73766 to 75094), and genes reported before to be associated with resistance to macrolides: *mel* (1) and *mph1* and *mph2* (40, 55). This region ends with the second copy of IS*26* (positions 79595 to 80417). The whole segment flanked by two IS*26* copies might be considered a putative large composite transposon, and a similar structure was found before in pMUR050, an IncN group plasmid (26). It differs from that in pCTX-M3 only by the lack of two integronic gene cassettes (*dfrA12* and *orfF*) and by a peculiar structure of the integron's 5′-CS, where the integrase gene *intI1* is truncated, duplicated, and surrounded by two IS*26* copies. pMUR050 is of "animal" origin (it was isolated from an *E. coli* strain from a diarrheic pig), and it has been implicated in the conjugative dissemination of the *armA* gene (26).

The mosaic structure of the 27-kb replicon-*trb* region clearly suggests that it arose from multiple insertions (Fig. 3). As both DRs created by the Tn*1* insertion are present at boundaries of the region, the first event had to be the Tn*1* transposition. The subsequent integration events disrupted Tn*1* by removing 156 initial codons of *tnp* and the whole *res* gene but leaving $bla_{\text{TEM-1}}$ intact. These events caused the acquisition of several mobile elements, some of which were acquired together with other resistance genes, including those present in the class 1 integron. It is impossible to determine if all these elements were acquired by pCTX-M3 in a series of separate events or as bigger modules that were preformed earlier. Since no DRs were found on the flanks of the IS*26* elements, the composite transposon-like structure emerged in pCTX-M3 rather not by transposition but by another recombination mechanism.

The IS*Ecp1*- and $bla_{\text{CTX-M-3}}$-containing region. IS*Ecp1* (positions 1457 to 3112) and $bla_{\text{CTX-M-3}}$ (positions 3161 to 4151) are the only mobile element and resistance gene, respectively, that are located outside the 27-kb replicon-*trb* region of pCTX-M3. The presence of IS*Ecp1* at a distance 128 bp upstream from the $bla_{\text{CTX-M-3}}$ and the $bla_{\text{CTX-M-15}}$ genes in pCTX-M3 and a similar plasmid, respectively, has been reported earlier (6). The pCTX-M3 fragment placed directly downstream from IS*Ecp1* was identified by Rodríguez et al. (49) to be a chromosomal fragment from a *K. ascorbata* strain. Apart from the

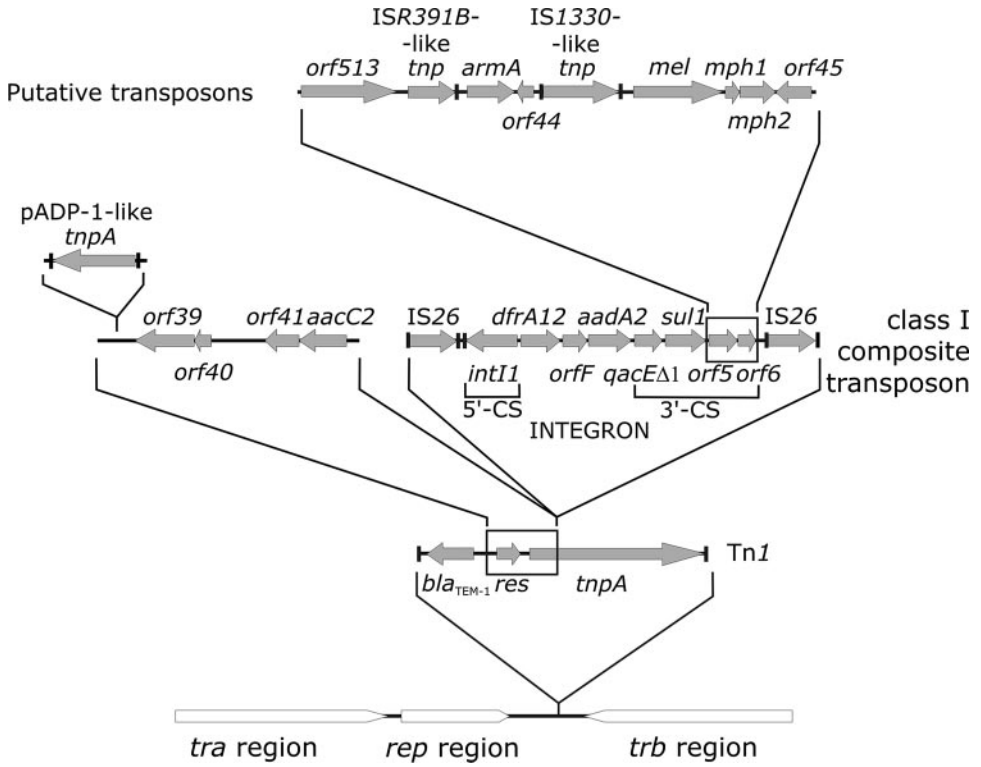

FIG. 3. Organization of the 27-kb region of the replicon and the *trb* genes. The partial pCTX-M3 sequence is drawn schematically out of scale. Arrows, ORFs; black lines, the sites of integration of particular sequence segments; black frames, hypothetical fragments of Tn*1* and the integron that are not observed in pCTX-M3, probably due to integration events; vertical bars, IRs of the respective mobile elements and IRi of the integron; gray blocks, the regions of the plasmid backbone.

128-bp spacer and the entire coding region of $bla_{CTX\text{-}M\text{-}3}$, it contains 343 bp of a *Kluyvera orf477* that, together with 204 bp of the pCTX-M3 sequence, constitutes an *orf1* of unknown function. The 3′ end of the insert is terminated by an 18-bp sequence which is a part of *orf477* that most probably mimicked the right IR of IS*Ecp1*. The duplication of a pentanucleotide (TGCAG), the IS*Ecp1* target sequence (positions 1452 to 1456), was found next to this boundary (positions 4508 to 4512) and most probably was generated in the one-ended transposition event that mobilized $bla_{CTX\text{-}M\text{-}3}$ from the *K. ascorbata* chromosome (48).

Cooccurrence of the $bla_{CTX\text{-}M\text{-}3}$ gene with the IncL/M replicon and the conjugal transfer system. Multiple plasmids purified from Polish CTX-M-3 producers were compared before by PstI restriction fingerprinting and were split into several types, with the highly predominant type A (pCTX-M3 family). Several pCTX-M3-like plasmid variants and the plasmid of type B were nonconjugative (7, 8, 44). We decided to check whether these differences could be due to differences in the conjugal transfer genes and whether the $bla_{CTX\text{-}M\text{-}3}$ gene has always been linked to the IncL/M replicon and located in the same context as it is in pCTX-M3. The *repA* gene was chosen as an IncL/M marker, and the *traU* gene was chosen as a marker of the transfer system. *repA* is essential for IncL/M plasmid replication (4, 5), whereas *traU* codes for one of the most conserved and essential proteins of the transfer system (25a, 51), The results of PCR tests carried out with several pCTX-M3-like variants, as well as plasmids of types B, C, and D, are shown in Table 1. All plasmids of types pCTX-M3, C, and D contained the *repA* and *traU* genes, whereas the plasmid of type B had neither of these. PCR mapping of the $bla_{CTX\text{-}M\text{-}3}$ locus revealed that all of the types of plasmids carried the same fragment of the *K. ascorbata* chromosome described above. The results demonstrated clearly the major role of conjugative

TABLE 1. Cooccurrence of $bla_{CTX\text{-}M\text{-}3}$, IncL/M *traU*, and *repA*

Isolate[a]	Plasmid type/ variant by PstI fingerprinting[a]	Occurrence			
		Mating[b]	*traU*[c]	*repA*[c]	$bla_{CTX\text{-}M\text{-}3}$ locus[d]
C. freundii 2526*	A1–pCTX-M3	+	++	++	+
K. pneumoniae 179	A4	−	++	++	+
S. marcescens 12	A5	−	++	++	+
E. coli 2112*	A11	+	++	++	+
K. pneumoniae 2113*	A12	+	++	++	+
E. coli 3624*	A18	+	++	++	+
E. coli 1145*	A33	+	++	++	+
K. pneumoniae 9172	A34	−	++	++	+
E. coli 279	B	−	−	−	+
E. coli 8350*	C	+	+	++	+
E. coli 1775*	D	+	++	++	+

[a] Clinical isolates and their plasmid fingerprints were described previously (6, 24, 45); in cases marked with an asterisk, plasmids were purified from transconjugants for this analysis.

[b] + and −, positive and negative mating results, respectively.

[c] ++, intense band of a PCR product; +, weak band, −, no band present.

[d] Defined as IS*Ecp1*, $bla_{CTX\text{-}M\text{-}3}$, (128 bp from IS*Ecp1*), and *orf477* (373 bp) (50).

IncL/M plasmids in the spread of the $bla_{CTX-M-3}$ gene in populations of the family *Enterobacteriaceae* in Poland and suggested the possible transfer of the $bla_{CTX-M-3}$-containing element to other plasmids.

Conclusions. We have sequenced plasmid pCTX-M3, which seems to be in large part responsible for the rapid dissemination of CTX-M-3-producing microorganisms in Poland (7, 8, 25, 44). The $bla_{CTX-M-3}$ gene observed in the country most probably emerged by a single IS*Ecp1*-mediated mobilization from the *K. ascorbata* genome (49), and our results suggest that it could have been transmitted to other plasmids of the same or different replicon types. The pCTX-M3 plasmid bears the IncL/M type of replicon, which enables replication in a broad range of hosts (39) and which codes for the conjugal transfer system that is most similar to that of IncI1 plasmids. Despite the lack of the *pil* genes, pCTX-M3 is able to transfer with a high efficiency both in liquid and on solid media, and the transfer system is also functional in *A. tumefaciens*. Moreover, the *oriT* sequence of pCTX-M3 may serve as a transfer origin both for its cognate IncL/M system and for the heterologous IncI1 system. The backbones of pCTX-M3 and pEL60, a plasmid from bacterial species pathogenic for plants, reveal extended identity, with differences clustered in two regions packed with mobile genetic elements and antibiotic resistance genes. Our results allow us to speculate that an ancestral plasmid similar to pEL60 might have originated from an environmental bacterium and that the plant bacterial community may be the source of plasmids utilized by species pathogenic for human in their rapid adaptation to quickly changing clinical environments.

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