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Analysis of the Mobilization Region of the Broad-Host-Range IncQ-Like Plasmid pTC-F14 and Its Ability To Interact with a Related Plasmid, pTF-FC2

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Plasmid pTC-F14 is a 14.2-kb plasmid isolated from *Acidithiobacillus caldus* that has a replicon that is closely related to the promiscuous, broad-host-range IncQ family of plasmids. The region containing the mobilization genes was sequenced and encoded five Mob proteins that were related to those of the DNA processing (Dtr or Tra1) region of IncP plasmids rather than to the three-Mob-protein system of the IncQ group 1 plasmids (e.g., plasmid RSF1010 or R1162). Plasmid pTC-F14 is the second example of an IncQ family plasmid that has five *mob* genes, the other being pTF-FC2. The minimal region that was essential for mobilization included the *mobA*, *mobB*, and *mobC* genes, as well as the *oriT* gene. The *mobD* and *mobE* genes were nonessential, but together, they enhanced the mobilization frequency by approximately 300-fold. Mobilization of pTC-F14 between *Escherichia coli* strains by a chromosomally integrated RP4 plasmid was more than 3,500-fold less efficient than the mobilization of pTF-FC2. When both plasmids were coresident in the same *E. coli* host, pTC-F14 was mobilized at almost the same frequency as pTF-FC2. This enhanced pTC-F14 mobilization frequency was due to the presence of a combination of the pTF-FC2 *mobD* and *mobE* gene products, the functions of which are still unknown. Mob protein interaction at the *oriT* regions was unidirectionally plasmid specific in that a plasmid with the *oriT* region of pTC-F14 could be mobilized by pTF-FC2 but not vice versa. No evidence for any negative effect on the transfer of one plasmid by the related, potentially competitive plasmid was obtained.

Plasmid pTC-F14 was recently isolated from the moderately thermophilic (50°C), acidophilic, sulfur-oxidizing bacterium *Acidithiobacillus caldus* (11). The strain of *A. caldus* in which the plasmid was found was one of two dominant organisms in a bacterial consortium undergoing pilot-scale testing for the commercial extraction of nickel from ores (17). The 14.2-kb plasmid pTC-F14 was shown to have an IncQ-like replicon that was closely related to, but compatible with, the broad-host-range 12.2-kb plasmid pTF-FC2 (9, 10). Plasmid pTF-FC2 had been previously isolated from a different, but related, iron- and sulfur-oxidizing bacterium, *Acidithiobacillus ferrooxidans* (6, 16, 19).

Although IncQ and IncQ-like plasmids are not self-transmissible, they are efficiently mobilized by conjugative plasmids of the *Escherichia coli* IncPα and IncPβ groups. By using IncP plasmids or IncP-based helper plasmids, the IncQ plasmids have been successfully mobilized to a large number of hosts, including a wide range of gram-negative bacteria; several gram-positive bacteria, including *Arthrobacter* spp., *Streptomyces lividans*, and *Mycobacterium smegmatis*; and cyanobacteria, such as *Synechococcus*, as well as being mobilized into plant and animal cells (reviewed in reference 18). Likewise, pTF-FC2 has been mobilized from *E. coli* to several gram-negative bacteria and from *Agrobacterium tumefaciens* to plant cells (D. E. Rawlings, unpublished observations). This, together with the broad-host-range properties of their replicons, makes these plasmids highly promiscuous and interesting to study from the fundamental biology, ecology, and applied biology points of view.

There are two major groups of IncQ and IncQ-like plasmids, the most distinguishing characteristic between the groups being whether they possess a three-gene, IncQ-like mobilization system or a five-gene, IncP-like mobilization system (18). Examples of the three-*mob*-gene plasmid family are the IncQ plasmids RSF1010, R1162, and R300B and the IncQ-like plasmids pIE1107, pIE1115, pIE1130, and pDN1 (25, 27). Plasmid pTF-FC2 was the only example of an IncQ-like plasmid with a five-*mob*-gene system (19) until the discovery of plasmid pTC-F14. Recently, the sequence of another IncQ-like plasmid, pRAS3, with a five-*mob*-gene system has been observed (14), although no biology of this system has been reported. The amino acid sequences of the Mob proteins from the two groups of plasmids belonging to the IncQ family are not related to each other.

As part of this study, we report that like pTF-FC2, the mobilization genes of pTC-F14 are of the IncP type. Because pTC-F14 and pTF-FC2 are promiscuous plasmids that were isolated from acidiphilic, iron- and/or sulfur-oxidizing, chemolithotrophic bacteria that share a similar habitat, it is not unlikely that the plasmids may come into contact with each other. Plasmids pTC-F14 and pTF-FC2 have diverged sufficiently for their replicons to be compatible, which should allow them to coexist in the same host cell (9, 10). This raised questions such as have the *mob* genes diverged sufficiently to be plasmid specific, or will they complement the mobilization activity of each other? Was there competition between plasmids at the level of mobilization? That is, had one of the plasmids evolved a mobilization system that would allow it to dominate the

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TABLE 1. Strains, plasmids, and primers used in this study

Strain, plasmid, or primer	Genotype or description	Source or reference
Strains		
DH5α	F′/*endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1 reacA1 gyrA*(Nal^r) *relA1* Δ(*lacZYA-argF*)*U169* (ϕ80*dlac*Δ(*lacZ*)*M15*)	Promega Corp., Madison, Wis.
S17.1	*recA pro hsdR* (RP4-2 Tc::Mu Km::Tn*7*)	23
CSH56	F$^-$ *ara* Δ(*lac pro*) *supD nalA thi*	Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
HB101	F$^-$ Δ(*mcrC-mrr*) *hsdS20 recA13 ara-14 proA2 lacY1* λ$^-$ *galK2 rpsL20*(Sm^r) *xyl-5 mtl-1 supE44*	3
Plasmids		
pUC19	Amp^r *lacZ′*; ColE1 replicon, cloning vector	28
pACYC184	Tc^r Cm^r; p15A replicon, cloning vector	5
pBR322	Amp^r Tc^r; ColE1 replicon, cloning vector	2
pKK223-3	Amp^r; ColE1 replicon, expression vector	4
pTC-F14Cm	Cm^r; natural pTC-F14 plasmid with a chloramphenicol resistance gene inserted at the single *Bam*HI site	9
pTC-F14Km	Km^r; pTC-F14Cm with the chloramphenicol resistance gene replaced by the kanamycin resistance gene from Tn*5*	This study
pDER412	Cm^r; natural pTF-FC2 plasmid with chloramphenicol resistance gene cloned into the Tn*5467* transposon	16
pMmob	Amp^r, 5,554-bp *Bam*HI-*Xba*I fragment of pTC-F14 containing all mobilization genes and the *repB* primase, cloned into pUC19	This study
pMmob1-pMmob9	Amp^r; PCR-based deletions of the mobilization region of pTC-F14 cloned into pUC19; refer to Fig. 1	This study
pMmob1184	Cm^r; minimum mobilization region one of pTC-F14 cloned into the tetracycline resistance marker of pACYC184	This study
pMmob1322	Amp^r; minimum mobilization region one of pTC-F14 cloned into pBR322	This study
pmobE	Amp^r; PCR product of *mobE* gene of pTF-FC2 cloned into pKK223-3	This study
pmobDE	Amp^r; PCR product of *mobDE* genes of pTF-FC2 cloned into pKK223-3	This study
pmobCDE	Amp^r; PCR product of *mobCDE* genes of pTF-FC2 cloned into pKK223-3	This study
pAC105	Cm^r; exonuclease III shortening of the pTF-FC2 mobilization region containing *mobC*, *-D*, and *-E* cloned into pACYC184	20
pAC209	Cm^r; exonuclease III shortening of the pTF-FC2 mobilization region containing *mobA*, *-B*, *-C*, and *-D* and a truncated *mobE* cloned into pACYC184	20
pAC218	Cm^r; exonuclease III shortening of the pTF-FC2 mobilization region containing *mobA*, *-B*, and *-C* with *mobD* and *-E* removed also in pACYC184	20
pAC221	Cm^r; exonuclease III shortening of the pDER412 mobilization region containing *mobA* and *-B* and the *oriT* cloned into pACYC184	20
pOriTF14	Amp^r; a 203-bp *Hin*dIII-*Nco*I fragment of pTC-F14 containing the *oriT* cloned into pUC19	This study
pOriTFC2	Amp^r; the *oriT* of pTF-FC2 cloned into pUC19	This study
Primers		
mobEF2	(*Eco*RI) 5′-TACAGAATTCAGCAAGCGCATGAGC-3′	This study
mobDEF2	(*Eco*RI) 5′-TACAGAATTCCCAAAACCCGACAGC-3′	This study
mobCDEF2	(*Eco*RI) 5′-TATAGAATTCCCACGTGGCGAAGCC-3′	This study
mobER2	(*Xba*I) 5′-TACATCTAGAATGTTGAGCGCGTCG-3′	This study
mobAR14	(*Eco*RI) 5′-TACAGAATTCCGGTCCATGTCGTCG-3′	This study
repBR14	(*Eco*RI) 5′-TACAGAATTCGGGTAATCGGATGGC-3′	This study
mobC′R14	(*Pst*I) 5′-TATACTGCAGCTTTCCCGCCTTTGC-3′	This study
mobCR14	(*Pst*I) 5′-TATACTGCAGTTGCCACCACCGACG-3′	This study
mobDR14	(*Pst*I) 5′-TATACTGCAGTCGGGTGTCGGTTCC-3′	This study
mobER14	(*Pst*I) 5′-TACACTGCAGCTGTCCGAAAGTAGG-3′	This study
mobAR14#2	5′-TGGCGTCGCTTGTTTGGTTC-3′	10

horizontal transfer process, thereby giving it a selective advantage in preference to the other? Here we characterize the mobilization genes of pTC-F14 and report on the ability of the mobilization systems of pTC-F14 and pTF-FC2 to interact with each other.

MATERIALS AND METHODS

Bacterial strains, plasmids, and primers. The strains, plasmids, and primers used in this study are listed in Table 1.

Media and growth conditions. *E. coli* strains were grown in either Luria-Bertani broth or on LA plates (21) at 37°C, supplemented as required with antibiotics at the following concentrations: ampicillin, 100 μg/ml; chloramphenicol, 34 μg/ml; streptomycin, 50 μg/ml; kanamycin, 50 μg/ml; and nalidixic acid, 50 μg/ml.

Mating assays. Donor and recipient (CSH56) cells were cultured separately overnight with appropriate antibiotic selection. Cells were washed three times in 0.85% (wt/vol) NaCl solution and mixed in a donor/recipient ratio of 1:10. An LA plate was spotted with 100 μl of this mixture and incubated at 37°C for 1 h. The agar plug was excised, suspended in 5 ml of 0.85% NaCl solution, and vigorously shaken to dislodge mating cells. Cells were pelleted by a 2-min spin in a micro-

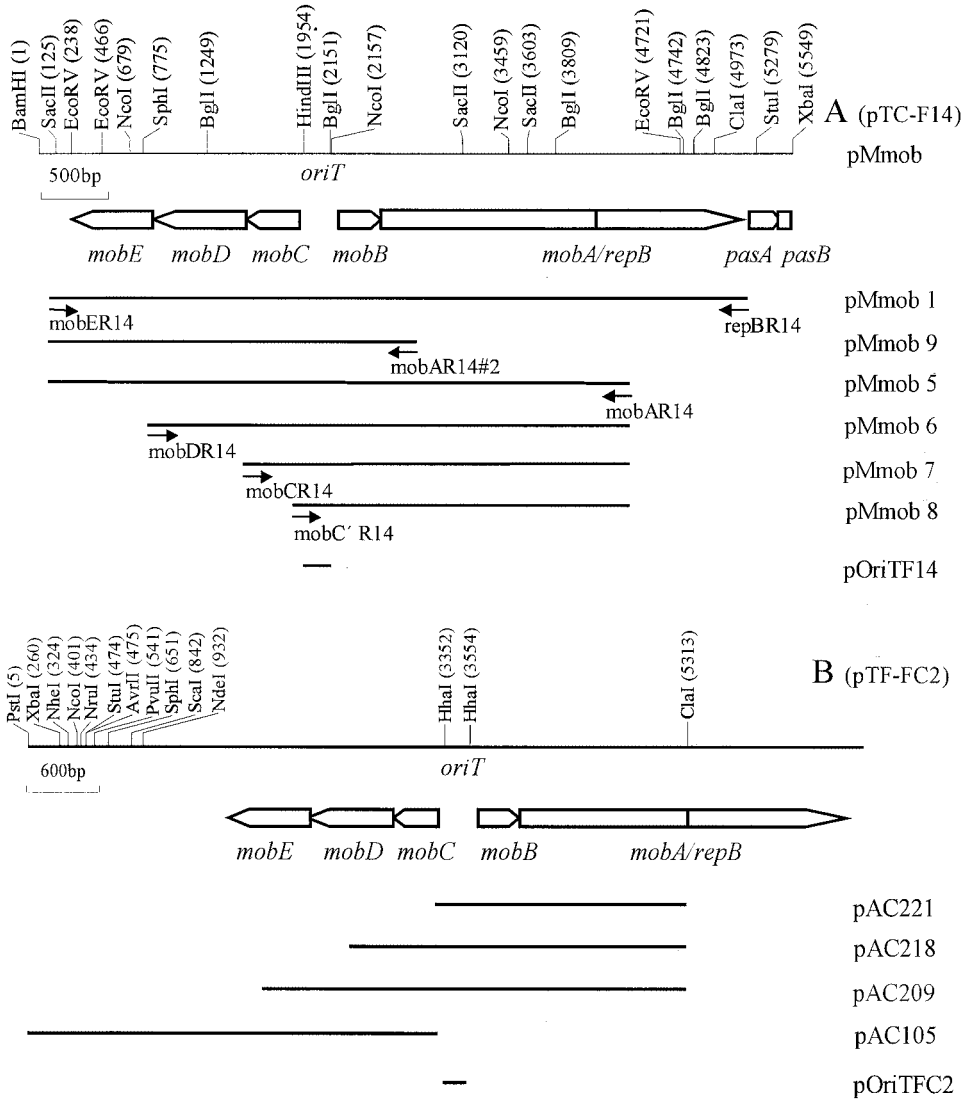

FIG. 1. Genetic and restriction endonuclease cleavage maps of the mobilization regions of plasmids pTC-F14 and pTF-FC2. (A) The 5.55-kb *Bam*HI-*Xba*I region of pTC-F14 showing the locations of the *mob*, *repB*, and *pas* genes as well as the *oriT* gene. The positions of the primers used to amplify and construct certain subclones are shown by short horizontal arrows. (B) The previously reported *mob* region of pTF-FC2 and subclones (20) used in this study.

centrifuge and resuspended in 1 ml of 0.85% NaCl solution. Serial dilutions were then plated onto media that selected for donor and transconjugant cells. The transfer frequency was calculated as the number of transconjugants per donor during the 1-h mating period.

DNA manipulations, sequencing, and bioinformatics. General techniques were performed according to standard procedures (21) or the manufacturers' recommendations. DNA was digested to give fragments with sizes of 700 bp to 1.2 kbp and cloned into the pUC19 vector. The DNA sequence was determined by a combination of sequencing from the ends of a number of subclones and synthesis of specific primers to obtain overlapping sequence from both strands. Sequencing was performed by the dideoxy chain termination method with an ABI PRISM 377 automated DNA sequencer, and the sequence was analyzed with a variety of software programs (mainly the PC-based DNAMAN [version 4.1] package from Lynnon Biosoft). Searches for sequences related to Mob proteins were performed by using the gapped-BLAST program of the National Center for Biotechnology Information at www.ncbi.nih.nlm.gov (1). Sequence alignments (based on CLUSTAL W) were carried out with the multiple alignment program, and amino acid sequence homology trees were constructed with the tree output program within the DNAMAN package.

PCRs. PCRs were performed with the Expand high-fidelity *Taq* DNA polymerase from Roche with a Hybaid PCR Sprint cycler. Plasmid pDER412 was used as template with primers mobEF2, mobDEF2, mobCDEF2, and mobER2 (listed in Table 1) to produce pmobCDE, pmobDE, and pmobE, respectively. Primers mobAR14, mobAR14#2, repBR14, mobC′R14, mobCR14, mobDR14, and mobER14 (Table 1 and Fig. 1) with pMmob as a template were used to give the mobilization region fragments (pMmob1 to pMmob9). After an initial denaturation of 60 s at 94°C, 25 cycles of 30 s at 55 to 60°C (depending on primer set) and an elongation step of up to 4 min (approximately 1 min per 1,000 bp) at 72°C were performed. A final extension step of 120 min at 72°C before cooling to 4°C completed the reaction.

Nucleotide sequence accession number. The nucleotide sequence of the 5.5-kbp region sequenced has been submitted to the EMBL-GenBank database under accession no. NC_004734/AF325537.

RESULTS

Mobilization of pTC-F14. Selectable chloramphenicol and kanamycin resistance genes were cloned into plasmid pTC-F14

TABLE 2. Mobilization frequency of plasmids and constructs

Test plasmid[a]	Plasmid present in *trans*[a]	Mobilization frequency of test plasmid[b]
pTC-F14Km		$(2.7 \pm 1.5) \times 10^{-3}$
pTC-F14Cm		$(2.8 \pm 1.8) \times 10^{-3}$
pTC-F14Cm[c]	R751	1.3×10^{-5}
pTC-F14Cm[c]	R388	$<10^{-6}$
pTF-FC2 (pDER412)		≥ 10
pTF-FC2 (pDER412)	pTC-F14Km	≥ 10
pTC-F14Km	pTF-FC2 (pDER412)	8.4 ± 0.52
pMmob (F14, *mobEDCBA repB pasA*)		≥ 10
pMmob1 (F14, *mobEDCBA repB*)		≥ 10
pMmob5 (F14, *mobEDCBA*)		$(3.3 \pm 3.1) \times 10^{-1}$
pMmob6 (F14, *mobDCBA*)		$(1.2 \pm 1.0) \times 10^{-1}$
pMmob7 (F14, *mobCBA*)		$(5.4 \pm 3.3) \times 10^{-3}$
pMmob8 (F14, *mobBA*)		$<10^{-6}$
pMmob9 (F14, *mobEDCB*)		$<10^{-6}$
pMmob8 (F14, *mobBA*)	pTC-F14Cm	2.4 ± 1.4
pMmob1184 (as for pMmob1)		$(2.1 \pm 0.8) \times 10^{-1}$
pMmob1322 (as for pMmob1)		$(2.3 \pm 1.6) \times 10^{-1}$
pTC-F14Cm	pAC105 (FC2, *mobEDC*)	$(7.9 \pm 2.1) \times 10^{-1}$
pTC-F14Cm	pAC209 (FC2, *mobDCBA*)	$(2.3 \pm 3.5) \times 10^{-3}$
pTC-F14Cm	pAC221 (FC2, *mobBA*)	$(8.9 \pm 2.4) \times 10^{-4}$
pTC-F14Cm	pmobE (FC2)	$(4.4 \pm 2.1) \times 10^{-3}$
pTC-F14Cm	pmobDE (FC2)	≥ 10
pTC-F14Cm	pmobCDE (FC2)	$(4.7 \pm 2.9) \times 10^{-1}$
pTC-F14Cm	pAC218 (FC2 *mobC*) + pmobE (FC2)	$(1.1 \pm 2.6) \times 10^{-3}$
pOriTF14		$<10^{-6}$
pOriTF14	pTC-F14Cm	1.5 ± 1.0
pOriTF14	pTF-FC2 (pDER412)	$(3.5 \pm 0.1) \times 10^{-2}$
pOriTF14	pTC-F14Km + pTF-FC2 (pDER412)	≥ 10
pOriTFC2		$<10^{-6}$
pOriTFC2	pTC-F14Cm	$<10^{-6}$
pOriTFC2	pTF-FC2 (pDER412)	≥ 10
pOriTFC2	pTC-F14Km + pAC105 (FC2, *mobEDC*)	$(3.5 \pm 4.7) \times 10^{-1}$
pOriTFC2	pTC-F14Km + pAC221 (FC2, *mobBA*)	$(1.7 \pm 0.8) \times 10^{-2}$

[a] Where relevant, genes or plasmids are indicated in parentheses.
[b] Mobilization frequency is the number of transconjugants per donor during a 60-min mating with a donor/recipient ratio of 1:10 using *E. coli* S17-1 as the donor and *E. coli* CSH56 as the recipient. A mating frequency of ≥10 is indicated when the number of transconjugants equaled the number of recipients. Mating frequencies were the average of at least three independent experiments, and standard deviations are indicated.
[c] *E. coli* HB101 was used as the donor strain.

to produce plasmids pTC-F14Cm and pTC-F14Km, respectively (Table 1). These plasmids were transformed into an *E. coli* S17-1 donor strain that has an RP4 plasmid derivative integrated into the chromosome to provide the conjugative functions required for plasmid mobilization. Both pTC-F14Cm and pTC-F14Km were mobilized to an *E. coli* CSH56 recipient strain at similar frequencies of approximately 2.8×10^{-3} transconjugants per donor. To determine whether the type of conjugative plasmid affected the mobilization frequency, we compared mobilization frequencies by using two self-transmissible plasmids different from the RP4 (IncPα) that was integrated into the chromosome of *E. coli* S17-1. Plasmid pTC-F14Cm was mobilized by R751 (IncPβ) from *E. coli* HB101 at a frequency about 100-fold lower than that by the RP4 derivative in *E. coli* S17-1, while mobilization by R388 (IncW) was not detectable (Table 2). A 5.55-kb *Bam*HI-*Xba*I fragment from pTC-F14 was subcloned into the nonmobilizable vector pUC19 and was found to be mobilized by *E. coli* S17-1 at frequencies that approached saturation. Saturation indicates that after 1 h of mating at a donor/recipient ratio of 1:10, the number of transconjugants was approximately equal to the number of recipients. This 5.55-kb fragment therefore contained all of the components needed for mobilization and was sequenced.

Analysis of the mobilization region of pTC-F14 and comparison with related plasmids. Five open reading frames were identified, arranged in a manner similar to those of pTF-FC2 (Fig. 1). However, some of the predicted amino acid sequences and characteristics of the mobilization proteins of pTC-F14 differed substantially from their counterparts in pTF-FC2. The MobA-RepB fusion and MobB proteins were the best conserved at 75.4 and 77.8% amino acid sequence identity, while the MobC, MobD, and MobE proteins were poorly conserved at 26.5, 39.8, and 21.2% amino acid sequence identity, respectively. Although all pairs of Mob proteins were of comparable sizes, the predicted pI values of the MobE proteins differed by almost 3 pH units. Surprisingly, plasmid pRAS3.1, isolated from *Aeromonas salmonicida* in Norway, has Mob proteins (GenBank accession no. AY043299.1/NC_003124.1) that are considerably more closely related to pTF-FC2 than pTC-F14 is to pTF-FC2. The sequences of the MobA, MobB, MobC, MobD, and MobE proteins of pRAS3.1 are 93.8, 88.8, 94.1, 97.4, and 88.8% identical to that of pTF-FC2, respectively, whereas they are only 75.0, 74.5, 25.8, 40.7, and 20.8% identical

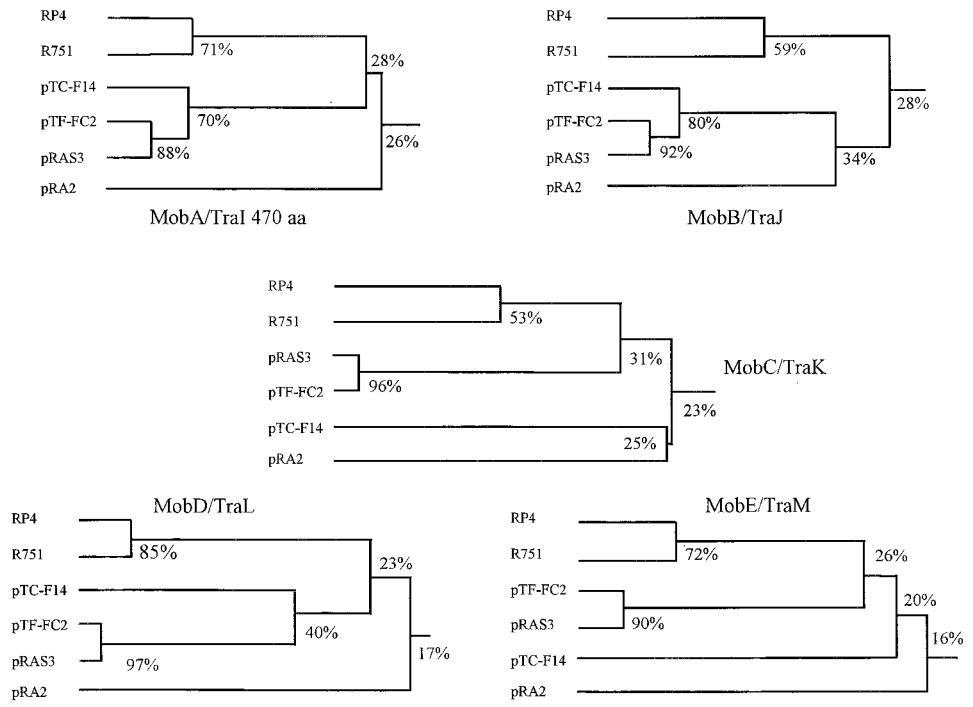

FIG. 2. Phylogenetic relationship between the MobA/TraI, MobB/TraJ, MobC/TraK, MobD/TraL, and MobE/TraM proteins of the IncP and IncQ group 2 plasmids as well as pRA2. Since in the IncQ-related plasmids the MobA plasmids exist as a MobA-RepB fusion, only the N-terminal 470 amino acids were considered for comparison. The percentages shown represent amino acid sequence identities. Accession numbers are as follows: RP4, L27758; R751, U67194; pTC-F14, AF325537; pTF-FC2, M57717; pRAS3, AY043298; and pRA2, U88088.

to that of pTC-F14. Three of the Mob proteins (MobA, MobB, and MobC) of pTC-F14, pTF-FC2, and pRAS3 had a greater than 20% amino acid sequence identity to the N-terminal 400-amino-acid portion of TraI and the complete TraJ and TraK proteins of the IncPα plasmid RP4 and the IncPβ plasmid R751, respectively. MobD and MobE had weaker but detectable sequence identity (17 to 18%) to TraL and TraM of RP4 and R751. These Mob proteins clearly belong to the IncP-like family of conjugation-associated, DNA processing proteins (Dtr), and a dendrogram showing the relationship between proteins of this family is presented in Fig. 2 (15, 24).

The *oriT* region of pTC-F14 was identified by sequence analysis and was found to be located on a 203-bp *Nco*I-*Hin*dIII fragment. This fragment was cloned into the nonmobilizable pUC19 vector (pOriT-F14) and transformed into *E. coli* S17-1, which contained a resistant pTC-F14Cm. pOriT-F14 was mobilized at a frequency that was about 500-fold greater than that of pTC-F14Cm. The *oriT* regions of IncPα and IncPβ plasmids as well as the four plasmids that have mobilization regions related to the IncP plasmids are compared in Fig. 3. The four mobilizable plasmids each contained an inverted repeat sequence that has been shown to be the site at which the relaxosome of plasmid RP4/RK2 binds prior to nicking at the *oriT* gene (26, 29). The highly conserved nucleotide hexamer that immediately precedes the *nic* site is also shown. In contrast to the mobilization proteins, for which plasmids pTF-FC2 and pRAS3 were the most closely related, the *oriT* regions of pTC-F14 and pRAS3 were considerably more closely related (matches at 42 of 50 bp) than those of pRAS3 and pTF-FC2 (32 of 50 bp) or pTC-F14 and pTF-FC2 (30 of 50 bp) (Fig. 3).

Determination of which *mob* genes are essential or nonessential for mobilization. A series of PCR-based deletions of the pTC-F14 mobilization region were made (Fig. 1). These were designed to test which genes were required for mobilization, as well as to determine the smallest region that is mobilized at the frequency of the intact *mobE-repB* region. When the entire *mobE-repB* region of pTC-F14 was cloned into the pUC19 vector (pMmob1), the mobilization frequency was at the level of saturation. This was an increase of more than 3,000-fold relative to the frequency obtained with the mobilization genes linked to its natural replicon (pTC-F14Km or pTC-F14Cm). To test whether this increase in mobilization

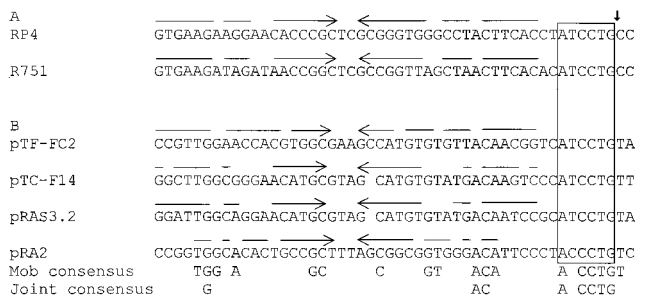

FIG. 3. Comparison of the *oriT* regions of the IncP conjugative plasmids (A) and the IncQ group 2 and pRA2 mobilizable plasmids (B). Imperfect inverted repeat sequences are shown by horizontal arrows, while the highly conserved hexamer preceding the nick site is boxed. The small vertical arrow shows the nick site as determined for RP4/RK2.

frequency was due to placement of the *mobE-repB* region in the pUC19 vector, the *mobE-repB* region of pMmob1 was cloned into vectors pACYC184 and pBR322. Both of these constructs (pMmob1184 and pMmob1322) had mobilization frequencies ~50-fold less than that of the pUC19 construct (pMmob1) but still ~100-fold higher than that of the parent plasmid (Table 2). This suggested that the increase in mobilization frequency was associated with the placement of the mobilization region within the high-copy-number vector pUC19. Deletion of most of *repB* (pMmob5) reduced the mobilization frequency by about 30-fold. The mobilization frequency was restored to saturation levels by placing a *repB*-expressing construct in *trans* with pMmob5 (data not shown). This indicated that the *repB* gene assisted but was not essential for mobilization. Using the *mobE-mobA* (pMmob5) construct as a starting point, sequential deletion of the *mobE* (pMmob6), *mobED* (pMmob7), and *mobEDC* (pMmob8) genes was carried out. Deletion of *mobE* had no discernible effect on the mobilization frequency, while deletion of both *mobE* and *mobD* (pMmob7) reduced the mobilization frequency by approximately 600-fold, whereas there was no detectable mobilization of the *mobE-mobC* deletion (pMmob8). Deletion of most of *mobA* from pMmob5 (pMmob9) resulted in a construct with a mobilization frequency below the detection limit.

Comparison of the mobilization efficiencies and interaction between the mobilization systems of pTC-F14 and pTF-FC2. The mobilization frequencies of plasmids containing the *mob* genes of pTC-F14 and the pTF-FC2 when associated with their natural replicons were compared. Plasmid pTC-F14Km was mobilized from *E. coli* S17-1 donor cells to CSH56 recipient cells at a frequency of 2.83×10^{-3} transconjugants per donor, which was more than 3,000-fold less than that of plasmid pDER412, which contained the pTF-FC2 mobilization genes (Table 2). To test whether mobilization of one plasmid was affected by coresidence of the other, both pTC-F14Km and pTF-FC2 (pDER412) were placed into *E. coli* S17-1 cells, and the frequency of transfer was measured. The frequency of mobilization of pTC-F14Km was enhanced almost to saturation in the presence of pTF-FC2, while the presence of pTC-F14 had no discernible effect on the mobilization of pTC-FC2. To determine what property of pTF-FC2 was required for this enhancement of mobilization frequency, plasmid constructs containing combinations of pTF-FC2 *mob* genes subcloned into the vector pACYC184 were introduced into *E. coli* S17-1(pTC-F14Cm) cells. Coresident plasmids pAC221 (containing pTF-FC2 *mobA* and *mobB*) and pAC209 (containing *mobA*, *mobB*, *mobC*, *mobD*, and a truncated *mobE* gene) did not increase the frequency of mobilization. In contrast, pAC105, which contained *mobC*, *mobD*, and a complete *mobE* gene, enhanced the mobilization frequency of pTC-F14 by about 100-fold, although this was about 10-fold less than when the whole of pDER412 was present. To determine whether this result was due to the *mobE* of pTF-FC2, the gene was amplified by PCR and cloned behind the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *tac* promoter in plasmid pKK223-3 (construct pmobE). This construct did not improve the mobilization frequency of pTC-F14Cm. When a combination of pTF-FC2 *mobD* and *mobE* genes (pmobDE) were placed in *trans* and induced with IPTG, mobilization of pTC-F14Cm reached saturation. IPTG induction of a combination of the *mobCDE* genes (pmobCDE) enhanced pTC-F14Cm mobilization by approximately 150-fold to about the same level as with pAC105. This indicated that it was the combination of *mobD* and *mobE* from pTF-FC2 that enhanced pTC-F14 mobilization.

Interaction at the *oriT* regions. To test whether the mobilization proteins of the two plasmids could act specifically on the *oriT* regions of each other, plasmids containing one of the cloned *oriT* fragments (pOriT-F14 and pOriT-FC2) were transformed into *E. coli* S17-1 containing either pTC-F14Km or pDER412. Both cloned *oriT* regions were functional, because they were mobilized by their respective parent plasmids at a frequency comparable to or greater than that of the parent plasmid (Table 2). The construct containing the *oriT* region of pTC-F14 (pOriT-F14) was mobilized at a frequency of 1.48 transconjugant per donor when pTC-F14 was placed in *trans*, but only at 3.48×10^{-2} with pDER412 in *trans*. With both pTC-F14 and pDER412 in *trans*, the mobilization frequency of pOriT-F14 reached saturation.

In the pTF-FC2 *oriT* complementation experiments, pDER412 was able to mobilize a construct containing its own *oriT* gene (pOriT-FC2) at a saturation frequency, while mobilization by pTC-F14 was below the limit of detection. Complementation of the *oriT* regions was therefore unidirectional, with pTF-FC2 able to mobilize the *oriT* of pTC-F14, but not vice versa. We tested whether there was any detectable interaction between the *mob* genes of pTC-F14 and the *oriT* of pTF-FC2 by providing two subsets of the *mob* genes of pTF-FC2 in *trans*. Plasmid pTC-F14Km was able to mobilize pOriT-FC2 at a frequency of 3.54×10^{-1} when in the presence of the pTF-FC2 *mobCDE* genes (pAC105) and at a frequency of 1.67×10^{-2} when the *mobAB* genes (pAC221) were present. This result was surprising and suggested that at least one of the pTF-FC2 *mobCDE* gene products, as well as one of the *mobAB* gene products, is able to independently assist in the recognition of the heterologous pTF-FC2 *oriT* by pTC-F14.

DISCUSSION

Plasmid pTC-F14 is the second member of the five-*mob*-gene, IncQ-like plasmid family to have its mobilization system investigated. Two other members of this IncQ-like plasmid family, now designated IncQ group 2, are pTF-FC2 and pRAS3, although no report on the biology of pRAS3 mobilization has been published. The observation that the amino acid sequence relationship between the mobilization proteins of plasmids pTF-FC2 and pRAS3 is much closer than that between the proteins of pTF-FC2 and pTC-F14 is remarkable (Fig. 2). The implication is that all three *mob* regions shared the same common ancestor, but pTF-FC2 and pRAS3 diverged more recently than pTF-FC2 and pTC-F14. Since divergence, pTF-FC2 and pRAS3 are now found in bacteria as different as the obligately acidophilic chemolithotrophic *A. ferrooxidans* strain FC isolated in South Africa and the neutrophilic *A. salmonocida* strain isolated in Norway. This serves to illustrate the highly promiscuous nature of the IncQ plasmid family. The 32.7-kb plasmid pRA2 is a fourth example of a plasmid containing a set of five *mob* genes that are related to the Tra1 system of the IncP plasmids (13). Plasmid pRA2 has a unique replicon with no similarity to those of the IncQ-like

plasmids, and this suggests that the five-*mob*-gene system is a mobilization module that may also be acquired by different, otherwise unrelated plasmids.

To facilitate an investigation into the minimum region required for mobilization, the *mobE-repB* region of pTC-F14 was cloned into the nonmobilizable vector pUC19. The mobilization frequency of this construct (pMmob1) was at saturation level, which was about 10^4-fold higher than when the mobilization system was linked to its natural IncQ replicon (pTC-F14Km). An increase in mobilization frequency was not likely to be due to derepression of the IncP helper plasmid, because the mobilization frequency of the pTC-F14Km plasmid was not increased (data not shown). We considered whether this increase in mobilization frequency was associated with the high copy number of the pUC19 vector (up to 500 copies) and therefore transferred the *mobE-repB* region into the lower-copy-number vectors pACYC184 (20 to 30 copies) and pBR322 (25 to 50 copies). The mobilization frequency of these constructs (pMmob1184 and pMmob1322) was reduced 20- to 30-fold but was still 100-fold higher than that of pTC-F14Km (copy number 12 to 16). The increase in mobilization frequency was consistent with an increase in vector copy number, although this observation cannot be taken as proof that the mobilization frequency was affected by copy number.

We wished to detect whether all five of the *mob* genes were required for mobilization and whether the presence of the *repB* gene affected the mobilization frequency of pTC-F14. A characteristic of all IncQ and IncQ-like plasmids is that the *mobA* and *repB* genes are fused in such a way that the MobA (nickase) and the RepB (primase) may be synthesized as separate proteins or as MobA-RepB fusion protein. All three polypeptides have been detected for plasmids RSF1010 (22) and pTF-FC2 (7; Rawlings, unpublished). A fortuitous *Cla*I site was present in plasmid pTF-FC2 that allowed deletion of the *repB* primase gene (Fig. 1). This deletion did not affect the frequency of mobilization between *E. coli* strains. In the case of pTC-F14, a PCR-generated fragment was used to delete *repB* (pMmob5), and this reduced the mobilization frequency about 30-fold compared with that of a plasmid containing an intact *repB* (pMmob1). Placement of a *repB*-expressing construct in *trans* with the RepB-truncated MobA restored mobilization frequencies to levels similar to those when MobA-RepB was present, indicating that the decrease in mobilization was not due to an increase in structural instability of the truncated MobA. The *repB* gene was therefore not essential for mobilization of pTC-F14, although unlike pTF-FC2, the presence of *repB* did enhance mobilization. This is in sharp contrast with the three-*mob*-gene IncQ plasmid R1162, in which the MobA-linked RepB primase was essential for the recovery of plasmids in recipient cells (12). These authors argue that the *mobA-repB* gene fusion of R1162 most likely occurred after the IncQ replicon acquired mobilization genes and may be unique among IncQ-like plasmids. Deletion of the pTC-F14 *mobE* gene had no noticeable effect on plasmid mobilization, while the additional deletion of *mobD* reduced mobilization (600-fold) and the further deletion of *mobC* abolished mobilization. This is in contrast to pTF-FC2, in which deletion of *mobE* reduced mobilization 150-fold, with no mobilization detected on deletion of both *mobE* and *mobD*.

The discovery that when pTF-FC2 was coresident with pTC-F14, the mobilization of the latter plasmid was increased by about 3,000-fold was unexpected. We further discovered that the presence of the combination of the pTF-FC2 *mobD* and *mobE* genes, but not the individual *mobD* and *mobE* genes, was responsible for this increase. This suggests that the apparently dispensable pTC-F14 *mobE* gene does play a role in mobilization, but the pTC-F14 *mobE* gene is not optimally functional in the mating system used (described below). The functions of MobD and MobE proteins are unknown, and the same applies to the related TraL and TraM proteins of the IncPα and IncPβ plasmids. TraL has been found to have an ATP- or GTP-binding Walker A box (24), and this box is present and highly conserved in the MobD proteins of the IncQ-like plasmids (data not shown). The role of MobD and MobE in facilitating the mobilization of one plasmid by another found in this study emphasizes the need to discover the function of the proteins.

In the present study, plasmid pTF-FC2 was clearly much more readily mobilized between *E. coli* strains than plasmid pTC-F14. Furthermore, a coresident pTF-FC2 could mobilize a plasmid containing the *oriT* of pTC-F14 (although not as efficiently as pTC-F14), while a coresident pTC-F14 could not mobilize a plasmid containing the *oriT* gene of pTF-FC2. Based on these results, plasmid pTF-FC2 might be expected to be a more promiscuous plasmid than pTC-F14. However, the fact that mobilization studies were carried out between *E. coli* strains by using the chromosomally located IncP plasmid RP4 as a conjugative helper plasmid must be taken into account. It is possible that pTF-FC2 is more suited to mobilization by this plasmid than pTC-F14, while there may be an as yet unknown helper plasmid that mobilizes pTC-F14 better than pTF-FC2. The reason for the unexpected observation that the *mobD* and *mobE* genes of pTF-FC2 were better able to assist pTC-F14 mobilization than its own genes could be because the MobD and MobE proteins are better suited to work with RP4, while the equivalent proteins of pTC-F14 may be better suited to function with a different conjugative plasmid.

The interpretation of experiments on the ability of plasmids containing the cloned *oriT* regions to be mobilized by the *mob* genes of the other plasmid is not fully clear. The *oriT* of pTC-F14 could be mobilized by its own *mob* proteins, and this mobilization frequency was enhanced in the presence of pTF-FC2. This result was consistent with the ability of pTF-FC2 to enhance the mobilization frequency of pTC-F14. Plasmid pTC-F14 was not able to mobilize a plasmid containing the *oriT* of pTF-FC2, unless some of the pTF-FC2 genes were present. What was surprising is that when we attempted to determine which of the pTF-FC2 *mobAB* or *mobCDE* genes were required, we found that either set of genes partly enhanced mobilization. A possible explanation is that more than one of the products of the pTF-FC2 *mob* genes are likely to enhance binding of the mobilization complex to the *oriT* of pTF-FC2. The nicking and processing of DNA prior to plasmid transfer by conjugation are frequently plasmid specific. For example, despite the high degree of similarity between the DNA-processing transfer proteins and the *oriT* regions of the IncP plasmids RP4/RK2 and R751, the *oriT* of RP4/RK2 cannot be transferred by R751 (8). Transfer of the RP4/RK2 *oriT* took place only when the specific *traJ* and *traK* genes of RK2/RP4 were present, with *traI* also being required, although this was

not plasmid specific. Plasmid RP4 TraJ (29) and TraK (30) proteins bind specifically to different features of the *oriT* region. In the case of pTF-FC2, it is likely that MobB (related to TraJ) and MobC (related to TraK) of pTF-FC2 could bind to its own *oriT* and thereby assist the otherwise *oriT*-specific proteins of pTC-F14 to recognize the *oriT* of pTF-FC2.

Part of the motivation for this study was to gain an understanding of the evolution of mobilization systems. The sequence similarity between the proteins associated with plasmid replication and mobilization suggests that plasmids pTC-F14 and pTF-FC2 share a common ancestor. It has been reported that plasmids pTC-F14 and pTF-FC2 are compatible in *E. coli*, and this indicates that replicons of the plasmids have diverged sufficiently for them to function as independent units. Pressure for the replicons to diverge may have arisen because the two broad-host-range, promiscuous plasmids occur in bacteria that share a similar ecological niche. This means they may have frequently encountered each other, and divergence to the point of compatiblity would mean that the plasmids will not exclude each other from the same host cell and thereby would each have an increased "replication space." It was of interest to discover whether the mobilization systems of these related plasmids would compete with each other. If one plasmid has a more dominant mobilization system, it would presumably be transferred horizontally to more host cells than the competing plasmid and thereby dominate an ecosystem. No reduction in mobilization frequency of one plasmid when coresident with the other plasmid was detected. In contrast, a coresident pTF-FC2 appeared to assist the mobilization of pTC-F14. Similarly, the cloned *oriT* region of pTF-FC2 could be mobilized by a coresident pTC-F14 when some but not all of the pTF-FC2 *mob* genes were present.

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