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Mercury Resistance Determinants Related to Tn*21*, Tn*1696*, and Tn*5053* in Enterobacteria from the Preantibiotic Era

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Three *mer* transposons from the Murray collection of preantibiotic enterobacteria show >99% sequence identity to current isolates. Tn*5073* is most closely related to Tn*5036* and Tn*1696*, and Tn*5074* is most closely related to Tn*5053*. Tn*5075* is most closely related to Tn*21* but lacks integron In2 and is flanked by insertion elements.

Tn*21* encodes mercuric ion resistance (Hg^r) and contains the class I integron In2, encoding resistance to sulfonamides (*sul*) and streptomycin-spectinomycin (*aadA*) (9, 18). It is carried by the conjugative plasmid NR1 (R100), which was isolated in Japan in the 1950s (21). More recently, Tn*21* (18) and other Tn*21*-like transposons carrying integron-associated antibiotic resistance (7) have been detected in *Escherichia coli* from agricultural (1, 28) and nonclinical sources (19), as well as from mercury amalgam-exposed, gram-negative gut bacteria (16, 17, 33), clinical bacterial isolates (13, 31, 36), and intercontinental plasmids carried by clinical isolates (10, 22).

It is now thought that Tn*21* evolved by the insertion of an In2 ancestor (lacking IS*1353*) into the *urf2M* gene of a hypothetical mercury resistance transposon, Tn*21*Δ (18) (also called Tn*X* [25]), probably catalyzed by transposition proteins encoded in *trans* (2, 18). A similar event led to the formation of Tn*1696* from plasmid R1033, where In4 inserted at the *res* site of a Tn*5036*-like *mer* transposon (25).

Hughes and Datta identified three Hg^r bacterial strains, M426, M567, and M634, from a total of 433 strains from the Murray collection of preantibiotic era enterobacteria (11). In this study, we sequenced the *mer* operons from M426, M567, and M643 in order to investigate the relationships between Hg^r sequences from clinical bacteria that had been isolated before antibiotics came into widespread use and present-day Hg^r sequences.

The plasmids, bacterial strains, antimicrobial resistance of these strains, and the 16S ribosomal DNA sequence identifications of the three Hg^r strains from this study (determined as previously described [32]) are shown in Table 1. All bacteria were grown at 37°C in Luria broth (LB) or on LB agar (27). Hg^r plasmids from M426, M567, and M634 were mated with *E. coli* TG2 (20), and Hg^r transconjugants were grown overnight on LB agar plates containing tetracycline (15 μg/ml) and $HgCl_2$ (20 μg/ml).

The *E. coli* TG2 Hg^r transconjugants from each of the three Murray strains contained an ~60-kb plasmid that conferred Hg^r. Plasmid DNA was isolated by standard methods (27), and PCR was performed with part of the *mer* operon from each plasmid as described elsewhere (3). PCR products purified by using a QIAquick PCR purification kit (Qiagen, Ltd., Crawley, United Kingdom) were sequenced with the Big Dye terminator cycle sequencing kit (PE Applied Biosystems, Warrington, United Kingdom) and an Applied Biosystems 3700 sequencer, according to the manufacturer's protocols. Further sequence analysis was performed by using primers designed from the sequences so obtained and from *merA* gene primers (5) (the primers used are described at http://www.biosciences.bham.ac.uk/labs/brown/mer_primers.htm). The transposon terminal inverted repeat DNA and flanking sequences were amplified by inverse PCR (26). Genetic maps of the sequenced *mer* operons are shown in Fig. 1A. DNA alignments and analysis were performed with the University of Wisconsin Genetics Computer Group version 9.0 suite of programs at the University of Birmingham.

The three *mer* operons that we sequenced represent different lineages and are not closely related to each other, but they are closely related to *mer* transposons isolated since the 1950s. Table 2 shows the percent identities between the sequences of the genes from Tn*5073* (*Klebsiella pneumoniae* M426), Tn*5074* (*Morganella morganii* M567), Tn*5075* (*E. coli* M634) and published DNA sequences.

The sequenced *merRTPCAD* genes (3,788 bp) of the Tn*5073* *mer* operon (Fig. 1A) had the highest identity at the DNA level to those from Tn*5036* (35), a Tn*5036*-like *mer* transposon from *Salmonella enterica* serovar Typhi CT18 plasmid pHCM1 (24), and to the sequenced *merAD* genes of Tn*1696*, which carries In4 (25) (Table 2). In comparison to the sequence encoded by Tn*5036*, there were two amino acid differences in the sequence encoded by Tn*5073*: MerR A17→V and MerA V250→A. The Tn*5073* *merT* gene carries five GTCTGAACCACAAAA duplications at the 5′ end (Fig. 1A). Multiple repeats of this sequence have also been observed in enterobacterial mercury resistance determinants from primates (16) and in Tn*5036*, Tn*5059*, and pKLH272 (35).

The sequenced *merRTPFADE* genes (3,647 bp) from the Tn*5074* *mer* operon (Fig. 1A) had the highest identity at the DNA level to those from Tn*5053* (14) and pMER327/419 (8) (Table 2). In comparison to the sequence encoded by Tn*5053*, there were four amino acid differences in the sequence encoded by Tn*5074*: MerR A119→S and K121→Q and MerA

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TABLE 1. Bacterial strains and plasmids, antibiotic resistance phenotypes, 16S rRNA sequences, and sources of the strains used in this study

Strain or plasmid	Antibiotic resistance[a]	Original tube date (yr)	Genotype	16S rRNA identification	Source or reference[b]
Murray collection					
Klebsiella sp. strain M426	Hg^r	1940		*Klebsiella pneumoniae* with 99% identity to sequence of accession no. AF144323.1	Urine NCTC
Proteus morganii M567	Hg^r	1935		*Morganella morganii* with 98% identity to sequence of accession no. AJ301681	Stool sample, child with dysentery NCTC
Escherichia coli M634	Hg^r	1931		*Escherichia coli* K-12 with 100% identity to sequence of accession no. NC000913	Cerebrospinal fluid NCTC
Laboratory strains					
Escherichia coli TG2	Tc^r		K-12 *lac-pro*Δ*srl-recA 306*::Tn*10 supE thi hsdD5* [F′ *traD36 proA*$^+$ *B*$^+$ *lacI*^{q} *lacZ*ΔM15]		Laboratory stock (4)
Escherichia coli KH802	Rif^r		F$^-$ *gyrA rpoB metB hsdS*$_B$ (r_K^- m_K^+)		P. Strike (34)
Plasmid RK2	Ap^r Tc^r Km^r				C. Thomas

[a] Tc^r, tetracycline resistance; Rif^r, rifampicin resistance; Ap^r, ampicillin resistance; Km^r, kanamycin resistance.
[b] NCTC, National Collection of Type Cultures, Colindale, London, United Kingdom.

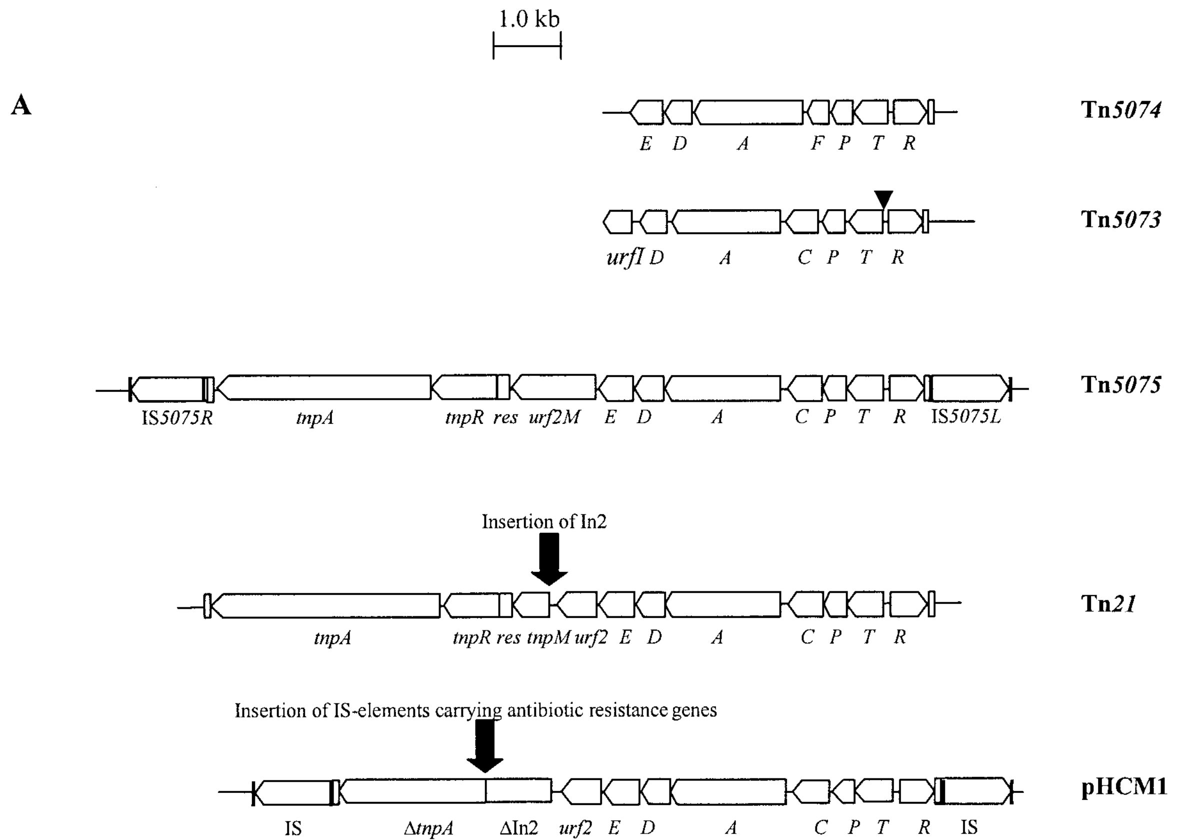
V232→A and S289→G. The Tn*5053* type *mer* operon was first isolated in environmental bacteria (8, 14) and has also been found in the fecal flora of primates and humans exposed to dental amalgam (16, 20).

In total, 11,298 bp of Tn*5075* were sequenced. The 3,962-bp *mer* operon from Tn*5075* carrying *merRTPCADE* has the highest identity at the DNA level to the equivalent regions of Tn*21* (18) and the Tn*21*-like *mer* transposon from *S. enterica* serovar Typhi CT18 plasmid pHCM1 (24) (Fig. 1A) (Table 2). The Tn*5075 res* site and the transposition genes (3,529 bp) *tnpR* and *tnpA* again had the highest identity to those of Tn*21*. In comparison to the sequence encoded by Tn*21*, there were three amino acid differences in the sequence encoded by Tn*5075*: MerA, Q558→H; TnpR, T165→A; and TnpA R455→A. Most importantly, Tn*5075* did not carry the integron In2, which in Tn*21* is located between *urf2* and the putative transposition gene *tnpM* (12) (Fig. 1A). Instead, in Tn*5075*, there is a single 945-bp open reading frame (*urf2M*) of unknown function rather than the predicted 987-bp hypothetical *urf2M* from Tn*21*Δ proposed by Liebert et al. (18). Tn*5075 urf2M* is 97.1% identical at the DNA level to the hypothetical *urf2M* that Liebert et al. (18) postulated to exist in the ancestor of Tn*21*. The reason for this lower percent identity is a 23-bp deletion in the Tn*5075 urf2M* sequence compared to the *tnpM* sequence in Tn*21*, resulting in a frameshift and a 314-amino-acid protein, rather than the 328-amino-acid protein predicted previously (18).

The insertion (IS) elements IS*5075*L and IS*5075*R (which are 1,351 bp and >99.5% identical to each other) flank Tn*5075*, forming a composite transposon (Fig. 1A). IS*5075*L and IS*5075*R belong to the IS*110* family and are >99.6% identical to the IS elements flanking the Tn*21*-like *mer* transposon from plasmid pHCM1 (Fig. 1A). IS*5075*L and IS*5075*R are between 91 and 93% identical to IS element sequences flanking other mercury resistance-encoding genes from gram-negative bacteria (Table 2).

Transposition of the Hg^r phenotype was determined by a mate-out assay (15) with *E. coli* TG2 carrying plasmid RK2, conjugated with Hg^r plasmids from M426, M567, or M634, into *E. coli* KH802. Transconjugants were selected on LB agar plates containing $HgCl_2$ (20 μg/ml) and carbenicillin (200 μg/ml), and the donor strain was counterselected with rifampin (50 μg/ml). Purified plasmid RK2 DNA from the transconjugants was analyzed for transposon insertion by *Pst*I digestion and gel electrophoresis (27). Tn*5073* transposed into plasmid RK2 at a frequency of 6.3×10^{-5} per donor cell. Tn*5074* transposed into RK2 at a frequency of 2.9×10^{-4} per donor

FIG. 1. (A) Genetic maps of the sequenced regions of Tn*5073*, Tn*5074*, Tn*5075*, Tn*21* (18), and the Tn*21*-like transposon from pHCM1 (24). Vertical black bars represent the 10- to 12-bp inverted repeat sequences flanking the IS elements. Vertical unfilled bars represent the 38bp inverted repeat sequences at the ends of the class II transposition module. The transposition (*tnp*) regions consist of the transposase gene (*tnpA*), the resolvase gene (*tnpR*), the putative transposon regulator (*tnpM*), and the resolution site, *res. urf2M* in Tn*5075* is of unknown function. The points at which In2 or antibiotic resistance-encoding IS elements have inserted into Tn*21* and pHCM1 are marked with black arrows. The mercury resistance-encoding operons consist of the regulatory genes *merR* and *merD*, the mercury transport genes *merT*, *merP*, and *merC* or *merF*, the putative transporter *merE*, and the gene encoding mercuric reductase, *merA*. *urfI* and *urf2* are of unknown function. All *mer* genes are marked in the figure with a single letter. The position of the multiple DNA sequence repeats found in *merT* from Tn*5073* are indicated (▼). (B) Postulated evolutionary pathway for Tn*5075*, Tn*21*, and the Tn*21*-like transposon in pHCM1. An ancestral *mer* transposon (a) could acquire either IS*5075*L and IS*5075*R to become Tn*5075* (b) or an integron related to In2, resulting in the formation of Tn*21* (c). Gene insertions and deletions in In2 would lead to Tn*21* variants (d), or Tn*21* could have acquired IS elements, leading to the formation of a precursor to the Tn*21*-like transposon in pHCM1 (e). Deletions of transposition genes and partial deletion of In2, followed by insertion of antibiotic resistance gene-carrying IS elements, would result in the formation of the Tn*21*-like transposon in pHCM1 (f).


1.0 kb
A
Tn5074
E D A F P T R
Tn5073
urfI D A C P T R
Tn5075
IS5075R tnpA tnpR res urf2M E D A C P T R IS5075L
Insertion of In2
Tn21
tnpA tnpR res tnpM urf2 E D A C P T R
Insertion of IS-elements carrying antibiotic resistance genes
pHCM1
IS ΔtnpA ΔIn2 urf2 E D A C P T R IS

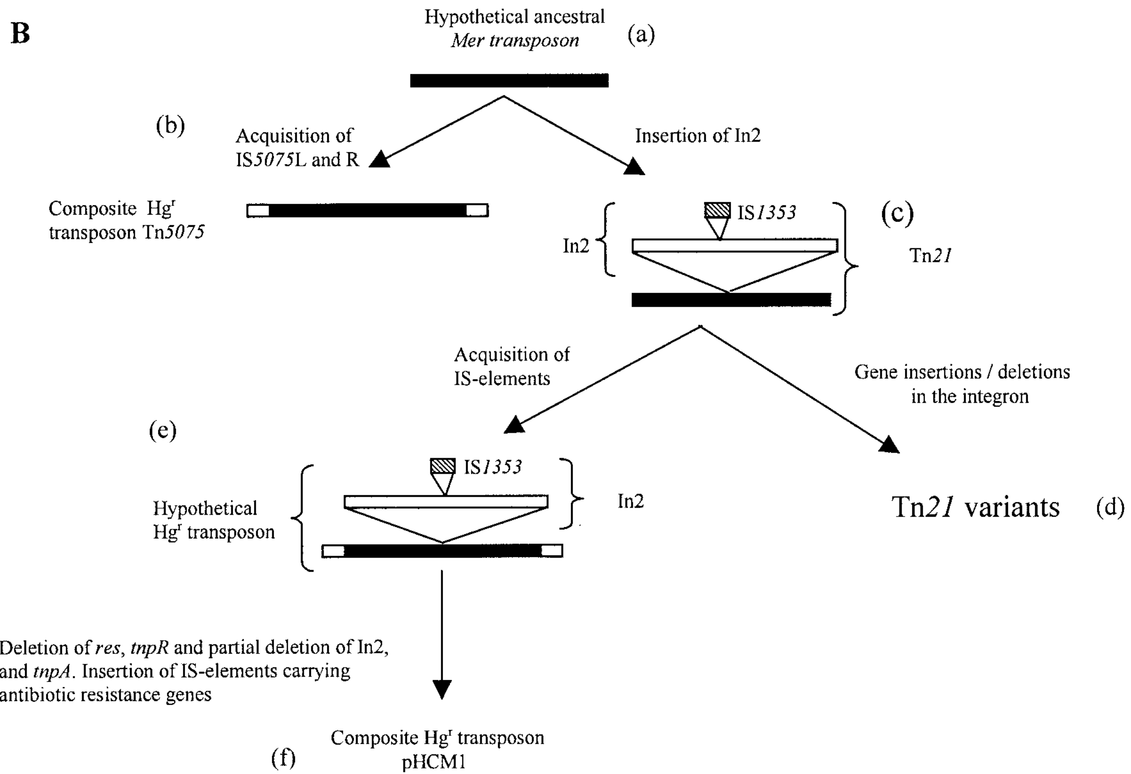

B
Hypothetical ancestral Mer transposon (a)
(b)
Acquisition of IS5075L and R
Insertion of In2
Composite Hg^r transposon Tn5075
IS1353
(c)
In2
Tn21
Acquisition of IS-elements
Gene insertions / deletions in the integron
(e)
IS1353
Hypothetical Hg^r transposon
In2
Tn21 variants (d)
Deletion of res, tnpR and partial deletion of In2, and tnpA. Insertion of IS-elements carrying antibiotic resistance genes
Composite Hg^r transposon pHCM1
(f)

TABLE 2. Comparison of DNA sequence identities between Tn*5073,* Tn*5074,* Tn*5075,* and published DNA sequences from other transposons

Transposon	Genes	% Identity to *mer* transposon (genes)[a]	Accession no.	Reference
Tn*5073*	*merRTPCAD*	>99.9 Tn*5036*-like transposon pHCM1	AL513383	24
		>99.9 Tn*1696* (*merAD*)	U12338	25
		>99.8 Tn*5036* (*merRTPCAD*)	Y09025	35
		>99.8 pKLH272 (*merRTPCAD*)	Y08992	35
Tn*5074*	*merRTPFADE*	>99.3 Tn*5053* (*merRTPFADE*)	L40585	14
		>99.2 pMER327/419 (*merRTPFADE*)	X73112	8
Tn*5075*	*merRTPCADE*	>99.9 Tn*21* (*merRTPCADE*)	AF071413	18
		>99.9 Tn*21*-like transposon from pHCM1 (*merRTPCADE*)	AL513383	24
	res tnpA tnpR	>99.8 Tn*21* (*res tnpA tnpR*)	AF071412	18
	urf2M	>97.1 hypothetical *urf2m* from Tn*21*Δ	AF071413	18
	IS*5075*L	>99.5 IS*5075*R	AF457211	This work
		>99.6 Tn*21*-like transposon from pHCM1 (IS elements)	AL513383	24
		>91.0 pDU1358 5′ to *mer* operon	M24940	6
		>91.0 R831b 5′ to *mer* operon	U77087	23
		>91.0 IS*4321*L, IS*4321*R, from R751	U60777	30

cell. We found no transposition of Tn*5075* in our assays, which could detect frequencies of $>10^{-7}$.

In conclusion, the internal genetic structure of Tn*5075* is consistent with the recently proposed structure of Tn*21*Δ (Fig. 1B) (2, 18), and Tn*5073* is closely related to Tn*5036* and Tn*1696* (25). The sequence data from Tn*5075* support the hypothesis that Tn*21* evolved from an Hg^r transposon similar to Tn*5075*, rather than from Tn*2613* (29). Tn*1696* and Tn*21* represent independent lineages of *mer* transposons that have acquired integrons (25). The dates of isolation of Tn*5073* (1940) and Tn*5075* (1931) and the close relationship of these transposons to other lineages (Tn*5073 merAD* genes are >99.9% identical to those of Tn*1696*; Tn*5075* is >99.6% identical to Tn*21* except where In2 is not present) are consistent with the idea that integrons transposed into preexisting clinical Hg^r transposons. The Tn*5074 mer* operon, isolated from a clinical source, has the greatest DNA identity to the Tn*5053* and pMER327/419 *mer* operons, which have been isolated from both environmental and nonclinical (16, 20) sources.

The DNA sequence data suggest that Tn*5075*, the Tn*21*-like *mer* transposon in pHCM1, and Tn*21* had a common ancestor and may have evolved as shown in Fig. 1B; i.e., an ancestral *mer* transposon acquired IS*5075*L and IS*5075*R, leading to the formation of Tn*5075*. Alternatively, an integron related to In2 inserted into the ancestral *mer* transposon, leading to the formation of Tn*21*. Gene insertions and deletions within the integron in Tn*21* could lead to the formation of Tn*21* variants (7). Acquisition of IS*5075*-like elements by Tn*21* could have led to the formation of a hypothetical *mer* transposon. Deletion of *res* and *tnpR* and partial deletion of In2 and *tnpA* from this transposon followed by insertion of antibiotic resistance-carrying IS elements, could have led to the formation of the Tn*21*-like transposon in pHCM1 (24). Although the Tn*21*-like transposon in pHCM1 is flanked by IS elements which are 99.6% identical to IS*5075*L and IS*5075*R from Tn*5075*, it is more closely related to Tn*21* than to Tn*5075* because it contains a vestige of the In2 sequence, which Tn*5075* does not and is 100% identical to Tn*21* across the *mer* genes and *tnpA*.

Nucleotide sequence accession numbers. The GenBank accession numbers of the *mer* sequences determined in this study are as follows: Tn*5073* (strain M426), AF461013; Tn*5074* (strain M567), AF461012; and Tn*5075* (strain M634), AF457211. The 16S rRNA gene sequence accession number for strain M567 is AF461011.

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