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Distribution of Class II Transposase and Resolvase Genes in Soil Bacteria and Their Association with *mer* Genes

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Southern hybridization was performed on 30 gram-negative, mercury-resistant soil bacteria isolated from three terrestrial sites in Great Britain; two of these sites were mercury polluted (SO and SE), and one was pristine (SB). Most of the isolates (20 of 30) hybridized to probes encoding regions of the transposase (*tnpA*) and resolvase (*tnpR*) genes from Tn*501* and Tn*21*. Isolates SE9 and SB3 hybridized to the Tn*21* but not the Tn*501 tnpA* probe; however, they differed in that SB3 hybridized to both Tn*501* and Tn*21 tnpR* probes while SE9 did not hybridize to either *tnpR* probe. The remaining isolates (7 of 30) did not hybridize to any of the transposon gene probes under the conditions used. *tnpA* and *tnpR* regions were PCR amplified from most of the hybridizing isolates and from Tn*501* and Tn*21*, and variation was assessed by restriction fragment length polymorphism analysis. On the basis of these data, *tnpA* regions were divided into eight restriction fragment length polymorphism classes and *tnpR* regions were divided into five classes. Similarity coefficients were calculated between classes and used to construct dendrograms showing percent similarity. A compilation of the data from this study on *tnpA* and *tnpR* regions and a previous study on *merRTΔP* regions (A. M. Osborn, K. D. Bruce, P. Strike, and D. A. Ritchie, Appl. Environ. Microbiol. 59:4024–4030, 1993) indicates the presence of hybrid transposons and provides evidence for extensive recombination, both between transposon genes and between transposon and *mer* genes, within these natural populations of bacteria.

Resistance to mercury (Hg^r) is found in many genera of gram-negative and gram-positive bacteria isolated from both natural and clinical environments (20, 27). Mercury is present in the environment as a result of natural causes such as leaching of ores and from anthropogenic sources such as pollution from industrial processes and the burning of fossil fuels. A number of Hg^r operon structures have been characterized; they typically consist of regions coding for transport proteins (MerT, MerP, and MerC), regulatory proteins (MerR and MerD), mercuric reductase enzyme (MerA), and in some cases the organomercurial lyase (MerB) (15, 25). Hg^r determinants are often located on plasmids, e.g., pKLH2 (12), and transposons, e.g., Tn*501* (4) and Tn*21* (1), enabling their rapid dissemination throughout bacterial communities, whereas others are chromosomally located, e.g., the *mer* operon of *Thiobacillus ferrooxidans* (24).

The majority of characterized mercury resistance transposons belong to the same family of class II transposable elements as the Ap^r transposon Tn*3*. Class II transposons are characterized by the presence of 35- to 48-bp terminal inverted repeats, transposase (*tnpA*), resolvase (*tnpR*) genes, a *res*- internal resolution site, and genes other than those required for transposition (10).

The Tn*3* family has been subdivided, with a major subgroup represented by Tn*21*. The Tn*21* subgroup is further divided into the Tn*21* and Tn*1722* branches (22). The Tn*21* branch is represented by Tn*21* (Hg^r Su^r Sm^r) and Tn3926 (Hg^r) (14), and the Tn*1722* branch is represented by Tn*501* (Hg^r) and Tn*1721* (Tc^r) (23). The classification of transposable elements is based on structural and functional homology of the *tnpA* and *tnpR* genes (9, 10). The Tn*21* subgroup comprises a diverse group of transposable elements with differing combinations of antibiotic and heavy metal resistance, together with transposition functions (3, 21). It is believed that the basis of their evolution stems from the insertion and deletion of DNA sequences into an ancestral transposon. Tanaka et al. (27) suggested that Tn*2613* may be the ancestral *mer* transposon of the Tn*21* branch, since it consists solely of the *mer* operon, *tnpA*, *tnpR*, *res*, and inverted repeat regions. The evolution of these elements within the Tn*21* subgroup is facilitated by their possession of integrons, mobile elements which are usually derived from transposons and contain a gene for the Int1 integrase and cassette integration site *att1* (26). Gene cassettes, e.g., *aadA* in Tn*21*, which encodes an adenylation gene for the modification of aminoglycoside antibiotics, insert into integrons (11, 21).

In this study, the distribution of sequences homologous to the *tnpA* and *tnpR* genes of Tn*501* and Tn*21* was investigated in natural populations of soil bacteria isolated from three previously characterized sites in Great Britain. These included two mercury-polluted sites (SE and SO) at Fiddlers Ferry and one pristine site (SB) at Salter Brook Bridge. The diversity of *tnpA* and *tnpR* regions was investigated by PCR-restriction fragment length polymorphism (RFLP) analysis. These results were related to those obtained in an earlier study (16), in which bacteria were isolated from the same three sites and *merRTΔP* regions were characterized by PCR-RFLP analysis. The data provide evidence for extensive recombination between related transposable elements, both between the transposon genes (*tnpA* and *tnpR*) and between the transposon genes and the *mer* determinant, of relevance to the study of transposon evolution.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial host strain for plasmids used in this study was *Escherichia coli* AB1157 (Sm^r). The transposons used were carried on plasmids pACYC184::Tn*501* (Hg^r Cm^r) (4) and pACYC184::Tn*21* (Hg^r Cm^r) (1). Environmental isolates used are shown in Table 1, where the prefixes to the environmental isolates refer to their sites of isolation. The sites are as follows: SO

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TABLE 1. Environmental isolates used in this study

Soil site (Hg concn)[a]	Isolate	Species[b]
Fiddlers Ferry sediment		
(0.161 ± 0.029)	SE3	*Pseudomonas testosteroni*
	SE6	*Acinetobacter calcoaceticus*
	SE9	*Alcaligenes faecalis*
	SE11	*Acinetobacter calcoaceticus*
	SE12	*Acinetobacter calcoaceticus*
	SE18	*Pseudomonas testosteroni*
	SE20	*Alcaligenes faecalis*
	SE23	*Pseudomonas testosteroni*
	SE31	*Klebsiella oxytoca*
	SE35	*Alcaligenes faecalis*
Salter Brook Bridge		
(<0.12)	SB2	*Pseudomonas* sp.
	SB3	*Pseudomonas* sp.
	SB4	*Pseudomonas fluorescens*
	SB5	*Pseudomonas* sp.
	SB8	*Pseudomonas* sp.
	SB12	*Pseudomonas* sp.
	SB13	*Pseudomonas* sp.
	SB22	*Pseudomonas* sp.
	SB24	*Pseudomonas* sp.
	SB29	*Pseudomonas* sp.
Fiddlers Ferry Soil		
(0.441 ± 0.039)	SO1	*Enterobacter cloacae*
	SO2	*Enterobacter cloacae*
	SO3	*Enterobacter cloacae*
	SO5	*Enterobacter cloacae*
	SO6	*Enterobacter cloacae*
	SO7	*Enterobacter cloacae*
	SO8	*Enterobacter cloacae*
	SO9	*Enterobacter cloacae*
	SO12	*Pseudomonas* sp.
	SO13	*Enterobacter cloacae*

[a] The mercury concentration (ppm) was determined by neutron activation analysis at the Centre for Analytical Research in the Environment, Imperial College, Ascot, England. (The detection limit for total mercury was <0.12 ppm.)
[b] Species identification by API; for further details, see reference 16.

and SE, soil and sediment, respectively, from Fiddlers Ferry on the banks of the River Mersey, representing mercury-polluted sites; and SB, a pristine site situated on the bank of the River Etherow at Salter Brook Bridge in the Peak District National Park.

DNA preparation and Southern hybridization. Plasmid DNA was prepared by the alkali lysis method of Birnboim and Doly (2) followed by cesium chloride density gradient ultracentrifugation. Total genomic DNA was prepared by the guanidine thiocyanate lysis method described by Pitcher et al. (19).

Total genomic DNA was digested with *Pvu*II and electrophoresed on 0.7% agarose Tris-borate-EDTA (TBE) gels. DNA was depurinated in 0.25 M HCl for 15 min, denatured in 0.4 M NaOH–0.6 M NaCl for 30 min, and neutralized with 1.5 M NaCl–0.5 M Tris HCl (pH 7.5) for 30 min. The DNA was transferred onto Genescreen Plus hybridization membranes by capillary action overnight with 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as a transfer buffer. Membranes were prehybridized and hybridized at 65°C to detect ≥70% homology, and washes were performed as specified by the manufacturer (Du Pont). Membranes were exposed to Fuji-RX film at −70°C for 2 to 48 h. The probes were removed by boiling membranes in 0.1× SSC–1% sodium dodecyl sulfate for 15 to 30 min. DNA probes prepared by restriction enzyme digestion or PCR amplification were excised from low-melting-point 1% agarose Tris-acetate-EDTA gels and labelled by random priming with [α-^{32}P]dCTP with a random-primed labelling kit (Boehringer Mannheim).

The 515-bp *tnpR*, 2,884-bp *tnpA*, and 1,000-bp *merRTΔP* probes were amplified by PCR from pACYC184::Tn*501* with the primer pairs 501 R1/501 R2, 501 A1/501 A2, and RX/PX, respectively (Table 2). The other probes used were prepared by restriction enzyme digestion as follows: pMJ400 *merD* URF1 URF2, 2,200-bp *Hin*cII fragment; Tn*21 tnpA*, 1,300-bp *Eco*RI fragment; and Tn*21 tnpR*, 350-bp *Hin*dIII fragment (13).

PCR amplification of *tnpR* and *tnpA* regions and restriction analysis of PCR products. *tnpR* regions of ~500 bp were amplified with primers 501 R1/C and 501 R2/C (Table 2), designed to consensus sequences of Tn*501* and Tn*21*. PCR was performed for 30 cycles consisting of 1 min at 94°C, 1 min at 62°C, and 2 min at 72°C, followed by 10 min at 72°C. *tnpA* regions of ~1,200 bp were amplified with primers 1406 and 2638 (Table 2). PCR was performed as above but with an annealing temperature of 61°C. PCR was performed in a 100-μl reaction volume consisting of ~0.05 μg of target DNA, 20 pmol of each primer, 50 μM each dATP, dCTP, dGTP, and dTTP, 2.5 U of *Taq* DNA polymerase (Gibco BRL), and 10 μl of 10× *Taq* polymerase buffer. The reaction mixture was overlaid with 100 μl of mineral oil. A negative control containing no target DNA was used for each PCR. A 15-μl sample of each PCR product was digested under the reaction conditions recommended by the manufacturer (Gibco BRL). *tnpA* regions were digested with *Sty*I, *Rsa*I, *Hae*II, and *Hin*cII, and *tnpR* regions were digested with *Hin*fI, *Sau*3A, and *Sty*I. Digested DNA was electrophoresed on 3% agarose TBE gels containing 0.5 μg of ethidium bromide ml^{-1}.

Data analysis. RFLP patterns were used to determine different classes with similarity coefficients calculated between pairs of isolates by using the following formula: $S = 100\ [n_{xy}/(n_x + n_y)]$, where n_{xy} is the number of bands shared between pairs of isolates (x and y) and n_x and n_y are the number of bands of isolates x and y, respectively (7). Bands which were smaller than 45 bp were ignored for the purpose of this study because of difficulties in visualizing the smaller fragments or accurately inferring their size. Dendrograms were constructed from the similarity coefficients by the unweighted pair group method with arithmetic mean (UPGMA) in the program NEIGHBOR on PHYLIP 3.5 C (8), using the Seqnet computing facility at Daresbury.

RESULTS AND DISCUSSION

This study presents data on the distribution, variation, and association of Tn*501*/Tn*21*-related *tnpA* and *tnpR* sequences in mercury-resistant gram-negative soil bacteria previously isolated from two mercury-polluted sites, SO and SE, and one pristine site, SB. These 30 isolates form part of a group which has been characterized on the basis of PCR-RFLP variation in *merRTΔP* regions (16) and sequence variation in the *merR* gene (17). The results of the hybridization analysis are shown in Table 3. Most of the isolates hybridized to the four transposon gene probes, hybridizing to fragments ranging from an estimated 0.65 to 15 kb (data not shown). Hybridization was also undertaken with probes derived from the *merRTΔP* region from Tn*501* and the *merD* URFI URF2 region from pMJ400 (a close relative of pKLH2) (12). With the majority of isolates (18 of 23), hybridization occurred to common fragments with both the transposon gene probes and the *mer* gene probes (data not shown). It can therefore be concluded that the transposon genes and *mer* genes were adjacent. Twenty of the isolates hybridized to both the Tn*21* and Tn*501 tnpR* and *tnpA* probes. SE9 and SB3 hybridized to the Tn*21* but not the Tn*501 tnpA* probe. Since the level of homology between the *tnpA* genes of Tn*501* and Tn*21* is 75% and hybridization was carried out at 65°C to detect levels of homology of ≥70%, this suggests that the transposase genes of SE9 and SB3 contain regions homologous to Tn*21* but may differ in the region where homology usually exists between Tn*501* and Tn*21*. Two isolates, SE9 and SO12, did not hybridize to either *tnpR* probe but differed in that SE9 hybridized only to the Tn*21 tnpA* probe

TABLE 2. Oligonucleotides used in this study

Primer	Sequence (5′→3′)[a]
501 R1	GTC AGC AGC TTC GAC CAG AA
501 R2	GAG GTA CTG GTA GAG GGT TT
501 A1	ATG CCG CGT CGC TTG ATC CT
501 A2	AGG TTG ATG TGC TCC CAG CC
501 R1/C	GTT CAG CA[CG] CTT CGA CCA G
501 R2/C	TA[CG] AGG GTT TC[GC] CG[AG] CTG AT
1406	TGC GCT CCG GCG ACA TCT GG
2638	TCA GCC CGG CAT GCA CGC G
RX	ATA AAG CAC GCT AAG GC[GA] TA
PX	TTC TTG AC[TA] GTG ATC GGG CA

[a] Bases in brackets refer to degeneracy in sequences.

TABLE 3. Results of hybridization analysis with Tn*501* and Tn*21* *tnpA* and *tnpR* probes

Isolate	Hybridization of isolate with[a]:			
	tnpR		*tnpA*	
	Tn*501*	Tn*21*	Tn*501*	Tn*21*
SE3	+	+	+	+
SE6	−	−	−	−
SE9	−	−	−	+
SE11	−	−	−	−
SE12	−	−	−	−
SE18	+	+	+	+
SE20	+	+	+	+
SE23	+	+	+	+
SE31	+	+	+	+
SE35	+	+	+	+
SB2	+	+	+	+
SB3	+	+	−	+
SB4	+	+	+	+
SB5	−	−	−	−
SB8	−	−	−	−
SB12	−	−	−	−
SB13	+	+	+	+
SB22	+	+	+	+
SB24	−	−	−	−
SB29	+	+	+	+
SO1	+	+	+	+
SO2	+	+	+	+
SO3	+	+	+	+
SO5	+	+	+	+
SO6	+	+	+	+
SO7	+	+	+	+
SO8	+	+	+	+
SO9	+	+	+	+
SO12	−	−	+	+
SO13	+	+	+	+

[a] +, positive hybridization; −, no hybridization.

while SO12 hybridized to both the Tn*501* and Tn*21* *tnpA* probes.

Previously, Zühlsdorf and Wiedemann (29) had found, using hybridization analysis with Tn*21* *tnpA* and *tnpR* probes, that 6% of gram-negative clinical isolates had deleted or disrupted *tnpA* or *tnpR* genes. Transposons in which one of the main transposition genes are missing or disrupted are no longer able to transpose. Such defective transposons are believed to be responsible for ensuring that genes which confer advantage upon the host become fixed (22). Either SE9 and SO12 contain defective transposons in which the *tnpR* genes are missing or disrupted or, alternatively, these isolates may represent hybrid transposon structures such as that identified in Tn*5431* (28). Tn*5431* was found on an *E. coli* ColV plasmid which encodes aerobactin synthesis and is composed of Tn*1721*- and Tn*3*-like genes. This is believed to have occurred as a result of transposition of Tn*3* into Tn*1721*. This type of transpositional event could result in a *tnpR* gene of one type and a *tnpA* gene of another type. A number of isolates did not hybridize to any of the four transposon probes. These included three isolates from SE (SE6, SE11, and SE12) and four from SB (SB5, SB8, SB12, and SB24). PCR was tried on all of the nonhybridizing isolates with the primer pairs 501 R1/C and 501 R2/C and 1406 and 2638, which amplify the *tnpR* and *tnpA* regions, respectively. However, no amplification products were obtained. This may be due to *mer* genes being present on an unrelated transposon or to deletion of the transposon genes, as identified in the plasmid pKLH2 (12).

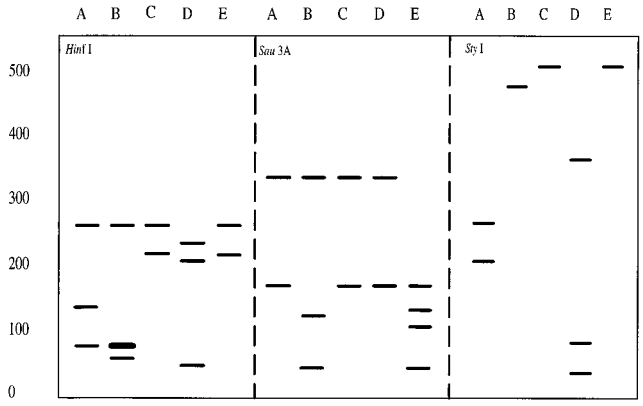

FIG. 1. Schematic representation of RFLP patterns of amplified *tnpR* PCR products with the enzymes *Hin*fI, *Sau*3A, and *Sty*I represented by RFLP groups A to E. Doublets are represented by bands of double thickness.

The use of probes for single genes in hybridization analysis indicates the distribution and association of these genes in environmental isolates. However, little knowledge of the variation that exists between genes can be gained; consequently, previous studies have used PCR-RFLP analysis to assess such variation (5, 16). To assess variation in the *tnpR* region of the transposition genes, *tnpR* regions of ~500 bp were amplified from Tn*501*, Tn*21*, and 16 of the hybridizing isolates. The PCR products all hybridized to the Tn*501* *tnpR* probe. The banding patterns produced by restriction enzyme digestion (Fig. 1) were used to calculate similarity coefficients. This resulted in the division of amplified *tnpR* regions into three classes, $tnpR_A$ through $tnpR_C$, with two further classes represented by Tn*501* ($tnpR_D$) and Tn*21* ($tnpR_E$). $tnpR_A$ was the most abundant class, consisting of 12 of the 16 isolates and containing isolates from all three sites. On the dendrogram (Fig. 2), $tnpR_C$ and $tnpR_E$ were the most closely related classes and $tnpR_A$ and $tnpR_B$ also showed a high degree of similarity. No relationship was apparent between the *tnpR* class and the type of host species, and it was concluded that the class of *tnpR* region is not species specific.

A similar analysis was undertaken with the transposase genes, in which *tnpA* regions were amplified from Tn*501*, Tn*21*, and 18 of the hybridizing isolates from the three sites. PCR products showed little size variation, being ~1,200 bp. All PCR products hybridized to the 2,884-bp *tnpA* probe of Tn*501*. On the basis of restriction data, the *tnpA* regions were divided into six classes, $tnpA_A$ through $tnpA_F$ and, as with *tnpR* regions, two further classes represented by Tn*501* ($tnpA_G$) and Tn*21*

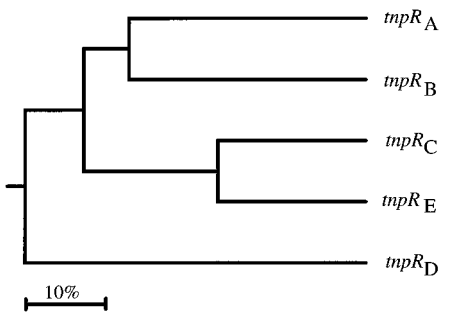

FIG. 2. UPGMA dendrogram showing the relationship between the different amplified *tnpR* RFLP class types from isolates from the three sites and Tn*501* and Tn*21*. Divergence between classes A to E is expressed as percent similarity.

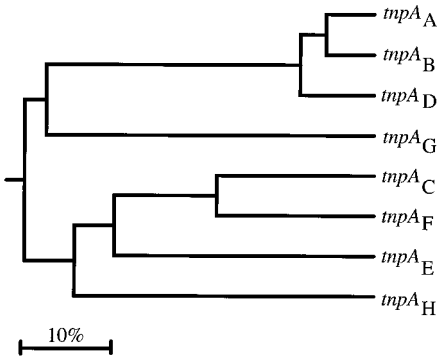

FIG. 3. UPGMA dendrogram showing the relationship between the different amplified *tnpA* RFLP class types from isolates from the three sites and Tn*501* and Tn*21*. Divergence between classes A to H is expressed as percent similarity.

($tnpA_H$). $tnpA_F$ was the most abundant class, containing nine isolates. All nine isolates were from SO and were *Enterobacter* sp. These nine SO isolates were always in the same class and appear to be identical, suggesting that they may represent a clonal population. This was also observed in the previous study by Osborn et al. on the *merRTΔP* regions (16).

On the dendrogram (Fig. 3), there are two major groupings. The first group contains $tnpA_A$, $tnpA_B$, $tnpA_D$, and $tnpA_G$, of which Tn*501* ($tnpA_G$) is the most divergent member within this group and the least divergence is shown by classes $tnpA_A$ and $tnpA_B$, which on RFLP patterns differ only by a *Hae*II restriction site. In the second group, containing $tnpA_C$, $tnpA_F$, $tnpA_E$, and $tnpA_H$, Tn*21* ($tnpA_H$) is the most divergent sequence. The highest degree of similarity is shown by classes $tnpA_C$ and $tnpA_F$, which differ only by a *Hinc*II restriction site. As with *tnpR* regions, there is no strict relationship between species and class. Greater diversity is apparent among the *tnpA* regions than the *tnpR* regions; however, one common feature is that the genes from environmental isolates are distinct from those carried by Tn*501* and Tn*21*.

Analysis of the linkage between *tnpA* and *tnpR* regions revealed a number of different relationships (Table 4). However, this analysis was somewhat limited because not all hybridizing isolates yielded amplification products, and in some cases only the *tnpA* or *tnpR* region could be amplified. Lack of amplification products from some isolates may have been due to sequence variation in the primer regions. The isolate SE31 was always in its own distinct class ($tnpR_B$ and $tnpA_C$). SE3, SB22, SO1, SO2, SO3, SO5, SO6, SO7, SO8, and SO9 were all in class $tnpR_A$ but were divided among classes $tnpA_A$, $tnpA_E$, and $tnpA_F$. These data suggest that these isolates may contain transposons consisting of hybrid transposon gene structures. In some transposons, e.g., Tn*3*, the *tnpA* and *tnpR* genes are divergently transcribed and the *res* site is between them. In this situation, it is anticipated that *tnpR* genes may be exchanged by recombination. With the Tn*21* group of transposable elements, the *res* site is situated 5′ of the transposition genes, and it is therefore assumed that the transposition genes evolve as a discrete unit (10).

The association between *mer* and transposon regions has also been investigated (Table 4). The isolates investigated in this study form part of a group of isolates which had previously been characterized on the basis of PCR-RFLP variation in the *merRTΔP* region (16). Class A of the *merRTΔP* region consisted of the isolates SB2, SB4, SB8, SB22, and SB29, which were like Tn*501*. In this study, SB8 did not hybridize to any of the four transposon gene probes and the other isolates were not like Tn*501* with respect to amplified *tnpA* and *tnpR* regions. This suggests recombinational events within the transposon or evolution away from Tn*501*-like transposition genes.

A comparison can also be made between the isolates in *merRTΔP* classes C, D, F, and G, which were identified as being closely related to the *mer* determinant of pKLH2 upon sequence analysis of their *merR* genes (17). Three of the isolates in these four *merRTΔP* classes (SE6, SE11, and SE12) did not hybridize to any of the *tnpA* or *tnpR* probes. This suggests that these isolates may represent aberrant transposons as identified in pKLH2, an aberrant *mer* transposon isolated from a strain of *Acinetobacter* from a Russian mercury mine (12). Vestigial sequences were present at the ends of the pKLH2 operon, suggesting that pKLH2 once possessed Tn*21* transposition genes. Alternatively, the nonhybridizing isolates may be due to genetic drift. The isolates SE31, SO1, SO2, SO3, SO5, SO6, SO7, SO8, SO9, and SO13 were also identified to possess pKLH2-like *mer* genes. However, these isolates hybridized to the four transposon probes. PCR-RFLP analysis of the *tnpR* regions revealed that SE31 and SO1, SO2, SO3, SO5, SO6, SO7, SO8, SO9, and SO13 were closely related, belonging to the $tnpR_B$ and $tnpR_A$ classes, respectively, which may differ by only a single base pair. The *tnpA* regions of SE31 and SO1, SO2, SO3, SO5, SO6, SO7, SO8, SO9, and SO13 were also closely related (classes $tnpA_C$ and $tnpA_F$, respectively). They may therefore be environmental derivatives of the transposon Tn*3926*, which is believed to possess pKLH2-like *mer* genes. A recent study found regions of homology to pKLH2 in Tn*3926*, suggesting that a transposon closely related to Tn*3926* may have transposed into pKLH2 and that this may have been followed by a resolution event, resulting in the loss of the transposon. A 500-bp region of the *mer* operon of Tn*3926* shows 97% sequence homology to that of pKLH2 (18). These data present evidence for the widespread distribution of Tn*501*- and Tn*21*-related *tnpA* and *tnpR* sequences in both

TABLE 4. Classification of Hg^r isolates on the basis of PCR-RFLP analysis of *tnpR*, *tnpA*, and *merRTΔP* regions

Isolate	Class for:		
	tnpR	*tnpA*	*merRTΔP*[a]
SE3	$tnpR_A$	$tnpA_A$	B
SE18	$tnpR_A$	—[b]	B
SE20	—	$tnpA_B$	E
SE23	—	$tnpA_A$	B
SE31	$tnpR_B$	$tnpA_C$	F
SE35	—	$tnpA_D$	B
SB2	$tnpR_C$	$tnpA_E$	A
SB3	$tnpR_C$	—	H
SB4	—	$tnpA_E$	A
SB22	$tnpR_A$	$tnpA_E$	A
SB29	$tnpR_C$	$tnpA_E$	A
SO1	$tnpR_A$	$tnpA_F$	G
SO2	$tnpR_A$	$tnpA_F$	G
SO3	$tnpR_A$	$tnpA_F$	G
SO5	$tnpR_A$	$tnpA_F$	G
SO6	$tnpR_A$	$tnpA_F$	G
SO7	$tnpR_A$	$tnpA_F$	G
SO8	$tnpR_A$	$tnpA_F$	G
SO9	$tnpR_A$	$tnpA_F$	G
SO13	$tnpR_A$	$tnpA_F$	G
Tn*501*	$tnpR_D$	$tnpA_G$	A
Tn*21*	$tnpR_E$	$tnpA_H$	ND[c]

[a] PCR-RFLP class derived in data from reference 16.
[b] —, no amplification product.
[c] ND, not determined.

polluted and pristine soil environments. Furthermore, such widespread distribution is supported by a recent report of the occurrence of Tn*3*-, Tn*21*-, and Tn*501*-related *tnpA* sequences in total bacterial DNA from marine environments (6). In addition, the data described in this paper provide evidence that extensive recombinational events have occurred both between different transposon genes and between *mer* and transposon genes in natural environments. The presence of hybrid transposons in natural populations has been clearly shown. We have also provided evidence that as yet uncharacterized Hg^r-associated transposable elements also exist. Further work on these isolates is necessary to confirm whether they contain transposon genes and to investigate any relationship to the *mer* genes.

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