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Structures of Homologous Composite Transposons Carrying *cbaABC* Genes from Europe and North America

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IS*1071* is a class II transposable element carrying a *tnpA* gene related to the transposase genes of the Tn*3* family. Copies of IS*1071* that are conserved with more than 99% nucleotide sequence identity have been found as direct repeats flanking a remarkable variety of catabolic gene sequences worldwide. The sequences of chlorobenzoate catabolic transposons found on pBRC60 (Tn*5271*) in Niagara Falls, N.Y., and on pCPE3 in Bologna, Italy, show that these transposons were formed from highly homologous IS*1071* and *cbaABC* components (levels of identity, >99.5 and >99.3%, respectively). Nevertheless, the junction sequences between the IS*1071L* and IS*1071R* elements and the internal DNA differ by 41 and 927 bp, respectively, suggesting that these transposons were assembled independently on the two plasmids. The formation of the right junction in both transposons truncated an open reading frame for a putative aryl-coenzyme A ligase with sequence similarity to benzoate- and *p*-hydroxybenzoate-coenzyme A ligases of *Rhodopseudomonas palustris*.

Rearrangements in bacterial genomes occur frequently, even in resting cells (3, 25). These changes often confer a powerful selective advantage to a particular population under defined conditions (for instance, a population in a diseased host or under environmental stress). The genotypes that have been rearranged include genotypes that confer virulence and toxigenicity to pathogenic bacteria (27, 36), antibiotic and heavy metal resistance (2, 34), and catabolic pathways for the degradation of organic pollutants (46, 48, 51). One of the important processes that create variability in bacterial genomes is the recruitment of chromosomal genes onto conjugative plasmids and the transfer of these genes to new hosts. The results of recent studies performed in independent laboratories have implicated the insertion element IS*1071* in the mobilization of a remarkable variety of catabolic genes and operons into composite transposon structures on plasmids (Table 1). We first described IS*1071* as a direct repeat flanking the *cbaABC* operon for chlorobenzoate degradation (28–30, 32). The composite transposon designated Tn*5271* is located on plasmid pBRC60 in *Alcaligenes* sp. strain BR60, a strain isolated from a contaminated tributary of the Niagara River at the Hyde Park chemical landfill in Lewiston, Niagara Falls, N.Y. The other IS*1071*-containing composite transposons and IS*1071*-associated catabolic operons listed in Table 1 were isolated in Japan and Europe. Where the sequences are known, the flanking elements are more than 99% identical to the sequence of IS*1071* from *Alcaligenes* sp. strain BR60. This level of sequence conservation suggests that IS*1071* has been distributed globally in a number of host genera in the recent past. An unresolved question is whether gene or operon mobilization events mediated by elements like IS*1071* occur rarely and are followed by selection and global distribution of the rare genotype or occur frequently in different locations, drawing on a common, globally distributed pool of genetic resources. To study this, we investigated the structure and environmental distribution of Tn*5271*-like elements in bacteria from the Niagara River watershed and elsewhere (31, 35).

In an independent study, Fava et al. (11) described a natural polychlorinated biphenyl (PCB)-degrading mixed culture isolated from PCB-contaminated soils that had been collected throughout Italy. From this mixed culture Fava et al. isolated a number of chlorobenzoate-degrading pure cultures. One of these cultures was an *Alcaligenes* sp. strain CPE3 culture that could grow on 3-chloro-, 4-chloro-, and 3,4-dichlorobenzoates. Resting cell metabolism studies showed that isolate CPE3 metabolized chlorobenzoates through protocatechuate. This pattern of substrate utilization was identical to the pattern found for *Alcaligenes* sp. strain BR60. Therefore, a collaborative investigation of the similarity of the genetic determinants for chlorobenzoate degradation and the flanking DNA in these isolates was performed.

Alcaligenes sp. strains BR60 and CPE3 were grown on minimal media A and MM, respectively (11, 50), supplemented with 4 and 3.2 mM chlorobenzoates, respectively. The structure of pBRC60 and Tn*5271* and cloning and sequencing of IS*1071* and the *cbaABC* genes have been described elsewhere (28–30, 32, 51). Plasmid DNAs were extracted from *Alcaligenes* sp. strain CPE3 and deletion derivatives CPE3-I and CPE3-II (see below) by the method of Casse et al. (5) and were purified by CsCl equilibrium density gradient centrifugation as described previously (40). Plasmids were digested with restriction enzymes *Eco*RI, *Hin*dIII, *Pst*I, *Not*I, and *Nhe*I (New England Biolabs Inc., Beverly, Mass.) and were resolved by agarose gel electrophoresis. *Alcaligenes* sp. strains BR60 and CPE3 each contained plasmids that were approximately 85 kb in size (pBRC60 and pCPE3, respectively). The *Hin*dIII, *Eco*RI, *Nhe*I, *Not*I, and *Pst*I restriction enzyme digestion patterns of these plasmids differed in almost all respects except for the occurrence of fragment sizes that corresponded to fragment sizes on the known Tn*5271* map (28, 32). A restriction digestion map of pCPE3 in the region containing the *cba* genes (Fig. 1) was constructed with the enzymes mentioned above by transferring fragments to nylon membranes and hybridizing them with digoxigenin-labelled heterologous probes derived from Tn*5271*, as recommended by the manufacturer (Boehringer Mannheim Canada, Montreal, Quebec, Canada). The

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TABLE 1. Association of IS*1071* with catabolic genes or operons in independent isolates from various sources[a]

Genetic element(s)[b]	Catabolic genotype[c]	Compound(s) degraded	Size (kb)	No. of IS*1071* copies[d]	Host(s)	Source	Reference(s)
Tn*5271*, pBRC60	*cbaABC*	Chlorobenzoates	17.1	2[e]	*Alcaligenes* sp. strain BR60	Hyde Park landfill, Lewiston, N.Y.	28, 30, 32
pUO1	*dehH2*	Chloroacetate	8.6	2[e]	*Moraxella* sp. strain B	Industrial wastewater, Japan	23, 24, 51
pTDN1	*tdnQTA$_1$A$_2$BR*	Aromatic amines	30	2[e]	*Pseudomonas putida* UCC22, a strain mt-2 (TOL) derivative	Wales	14
pOPH1	*pht*	*o*-Phthalate	70	2	*Comamonas acidovorans* UCC61	Sewage sludge, Wales	9
IS*1071*::IS*1471*, pIJB1	*tfd*	2,4-Dichlorophenoxyacetate	4.3	1[e,f]	*Burkholderia cepacia* 2a	Soil, United Kingdom	54
pPOB	*pobAB*	Carboxydiphenyl ethers	ND[g]	1[e,h]	*Pseudomonas pseudoalcaligenes* POB310	Soil and activated sludge, Germany	7
pPSB, pTSA	*psbAC*	*p*-Sulfobenzoate	ND	2[e,i]	*Comamonas testosteroni* T-2 and PSB-4	Soils, Switzerland	21, 22
pCPE3	*cbaABC*	Chlorobenzoates	16.2	2[e]	*Alcaligenes* sp. strain CPE3	PCB-contaminated soils, Italy	11; this study

[a] The data have been summarized previously (53).
[b] A transposon designation is given where known; otherwise the plasmid designation is given.
[c] *cbaABC*, chlorobenzoate 3,4-(4,5)-dioxygenase, reductase, and dehydrogenase genes; *dehH2*, chloroacetate dehalogenase gene; *tdnQTA$_1$A$_2$BR*, aniline and toluidine catabolic genes; *pht*, phthalate catabolic genes; *tfd*, 2,4-dichlorophenoxyacetate catabolic genes; *pobAB*, *p*-phenoxybenzoate catabolic genes; *psbAC*, *p*-sulfobenzoate catabolic genes.
[d] Elements with two copies have direct repeats of IS*1071* flanking the catabolic genes.
[e] Complete or partial IS*1071* sequences are known.
[f] The IS*1071 tnpA* gene on pIJB1 is interrupted by a class I insertion sequence, IS*1471* (1.1 kb), which is inserted at nucleotide 864 of IS*1071*.
[g] ND, not determined.
[h] The one copy of IS*1071* is located 356 bp 5′ to the start codon of *pobA*.
[i] Two copies of IS*1071* are linked to mobilization of the *psbAC* genes from pPSB to the chromosome of strain PSB-4.

mapping analysis showed that pCPE3 contains a composite transposon similar to Tn*5271*, which contains *cbaABC* genes and is flanked by directly repeated IS*1071* copies, but that the pCPE3 element is approximately 0.9 kb smaller than Tn*5271*.

Escherichia coli XL-1 Blue (Stratagene Cloning Systems, La Jolla, Calif.) was used as the host when we cloned pCPE3 restriction fragments spanning the two IS*1071* junctions with the internal DNA of the composite transposon, designated junction L (the *Eco*RI 2.9-kb fragment) and junction R (the *Hin*dIII 3.4-kb fragment), as well as the *Hin*dIII 8.4-kb fragment containing the *cbaABC* genes, into the vector pCRScript (Stratagene). The nucleotide sequences of junctions L and R of pCPE3, as well as sequences in the *cbaA* gene of pCPE3, were determined by double-stranded plasmid sequencing performed with fluorescent dideoxy chain termination inhibitors and a model ABI 373 Stretch automated sequencer (Perkin-Elmer–Applied Biosystems Inc., Foster City, Calif.) at the University of Ottawa Biotechnology Research Institute, Ottawa, Ontario, Canada. The oligonucleotides used for sequencing were prepared with a PCR-Mate oligonucleotide synthesizer (Perkin-Elmer–Applied Biosystems).

Alignment of the junction L and R sequences of pCPE3 with the corresponding sequences of Tn*5271* showed that the junctions between IS*1071* and the internal DNA of the transposons differed (Fig. 2 and 3). The transposon on pCPE3 contains an additional 41 bp of DNA sequence at junction L immediately adjacent to the inverted repeat of IS*1071L*, which is missing from the Tn*5271* sequence (Fig. 2). At junction R the pCPE3 transposon is missing 927 bp of DNA immediately adjacent to the inverted repeat of IS*1071R* that is present in Tn*5271* (Fig. 3). Aside from the 41- and 927-bp blocks of DNA that differed at junctions L and R, respectively, the alignments revealed that the level of identity between the pCPE3 transposon and Tn*5271* was more than 99.5%. There was complete conservation of the distal 38 bp of the 110-bp inverted repeat sequences of IS*1071*, which are known to be the recognition sequences of the class II TnpA transposases (16, 43). The nucleotides immediately adjacent to the IS*1071* junctions showed no sequence conservation when they were compared to 10 other known junction sequences from the strains listed in Table 1. Therefore, IS*1071* shows little if any target site specificity, which is in agreement with the results of previous studies of the class II or Tn*3* family transposons (16, 43). The 800-bp sequence that was determined for the *cbaA* [3-chlorobenzoate 3,4-(4,5)-dioxygenase] gene of pCPE3, corresponding to nucleotides 4840 to 5240 and 5280 to 5680 in the Tn*5271* numbering system (30, 32), was 99.3% identical to the Tn*5271 cbaA* gene sequence (data not shown). The *cbaA* reading frame was conserved in the region sequenced.

An open reading frame, ORF8, was found at junction R (reading from right to left in Fig. 1). ORF8 begins at the third nucleotide inside junction R of Tn*5271* and continues for 1,146 nucleotides into the internal DNA of the transposon (Fig. 3). Only the last 221 nucleotides of ORF8 are found in the pCPE3 transposon. A search of the nucleic acid sequence databases performed with the NCBI BLAST search algorithm and alignments prepared with CLUSTAL V (1, 20) and the EMBL (Heidelberg, Germany) PredictProtein server (39) revealed similarities between the putative translation product of ORF8 (382 amino acids) and aryl- and acyl-coenzyme A ligase se-

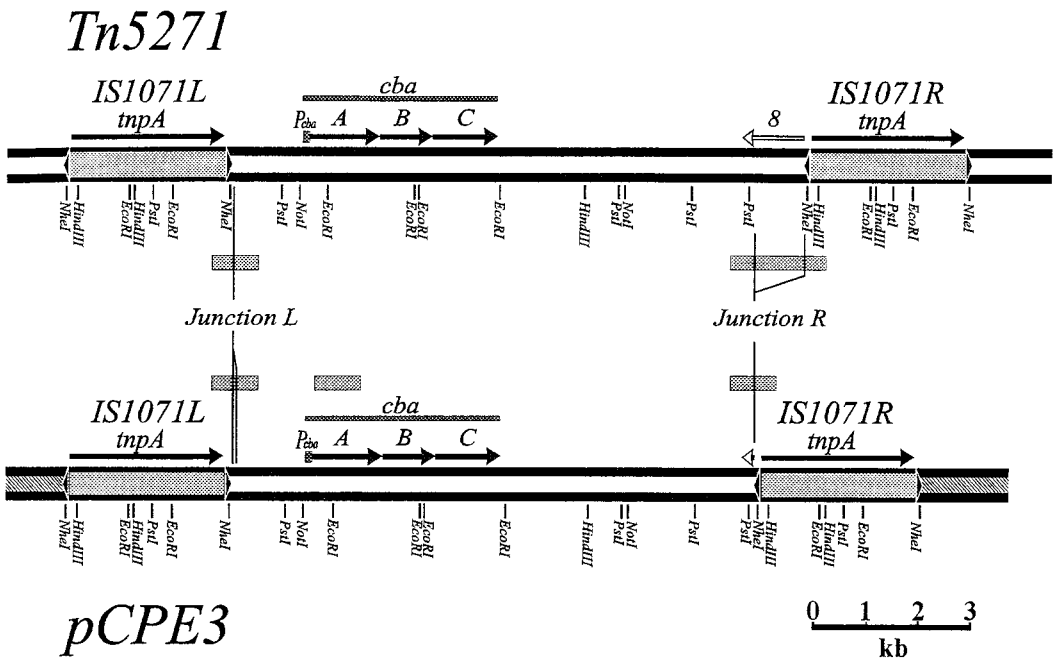

FIG. 1. Comparison of the structures of composite transposons in the Niagara isolate *Alcaligenes* sp. strain BR60(pBRC60, Tn*5271*) and the Italian isolate *Alcaligenes* sp. strain CPE3(pCPE3). The *cbaABC* genes encode chlorobenzoate 3,4-(4,5)-dioxygenase, reductase, and dehydrogenase under the control of the P_{cba} promoter. IS*1071L* and IS*1071R* flank both transposons. Junction L and ORF8 at Junction R were sequenced, as were two regions within the *cbaA* gene (shaded boxes between the maps). The restriction maps within the transposons corresponded exactly except for the two junction regions. Restriction sites on the plasmids outside the transposon regions did not correspond (data not shown).

quences. The aligned sequences included a conserved acyl-adenylate binding site consensus sequence (4). The ORF8 amino acid sequence was most similar to the amino acid sequences of the benzoic acid- and 4-hydroxybenzoic acid-coenzyme A ligases of *Rhodopseudomonas palustris* (levels of similarity, 67 and 49%, respectively) (10, 15). The sizes of all of the similar sequences except the *Sulfolobus* open reading frame ranged from 476 to 578 amino acids. The ORF8 sequence could be aligned with approximately 70% of the carboxy termini of these sequences, indicating that approximately 30% of the original ORF8 gene was truncated when Tn*5271* was formed. More than 85% of this putative gene was truncated during mobilization to pCPE3. The *Sulfolobus* open reading frame codes for only 369 amino acids, and, like the ORF8 amino acid sequence, the sequence of this open reading frame aligned with approximately 70% of the C termini of the coenzyme A ligases. An unrooted phylogenetic tree (Fig. 4) based on the distances between the aligned sequences illustrates the

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pCPE3                          NheI                                    Junction L
   AACACCACCTCCGCGCCAGTGCTAGCTTTTCGTACCGTCACTTATTGCACTGAAAACGAGGAGACCCC
   AACACCACCTCCGCGCCAGTGCTAGCTTTTCGTACCGTCACTTATTGCACTGAAAACGAGGAGACCCC
Tn5271                                                                3201

pCPE3  Junction L
CAAGATCGGGCCCCTGTGCGCCGACACGCCGGCCATCGCCG [41 bp]

pCPE3                                    ♦
ATGCCCTGCTCGACGCCTTGCTGGGCTGCGTGCTGCTGCGTGA-GCCGGTGTACCTCGATGTCCCCGAGCC
ATGCCCTGCTCGACGCCTTGCTGGGCTGCGTGCTGCTGCGTGAGGCCGGTGTACCTCGATGTCCCCGAGCC
Tn5271 Junction L

pCPE3                          ♦
CAACCGCGAGGCCATCGCGATGGCCGAGC-GCCAGGGCATGGTGCAGGGCTTTCCGACCGCGCGCATGTAC
CAACCGCGAGGCCATCGCGATGGCCGAGCGGCCAGGGCATGGTGCAGGGCTTTCCGACCGCGCGCATGTAC
Tn5271

pCPE3                                 ♦
CGCGGGCCGGCGCCGCGGCTGGACCTGGGGCGTTTGTTATGCCTTGACGGCCTTGGAGGTCGGCTGAGCCT
CGCGGGCCGGCGCCGCGGCTGGACCTGGGGCGTT-GTTATGCCTTGACGGCCTTGGAGGTCGGCTGAGCCT
Tn5271                                                                3413
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FIG. 2. Junction L DNA sequences of pCPE3 and Tn*5271*. The pCPE3 sequence (underlined) contains an additional 41 bp of DNA at junction L compared to the Tn*5271* sequence. The first comparison line shows the sequence for 68 bp of DNA from the right inverted repeat of IS*1071L*; the *Nhe*I restriction enzyme site and the CCCC border sequence (nucleotide 3201 of Tn*5271*) are shown. Sites at which the sequences of pCPE3 and Tn*5271* were dissimilar (aside from the junction sequence) are indicated by solid diamonds. A dash indicates a gap.

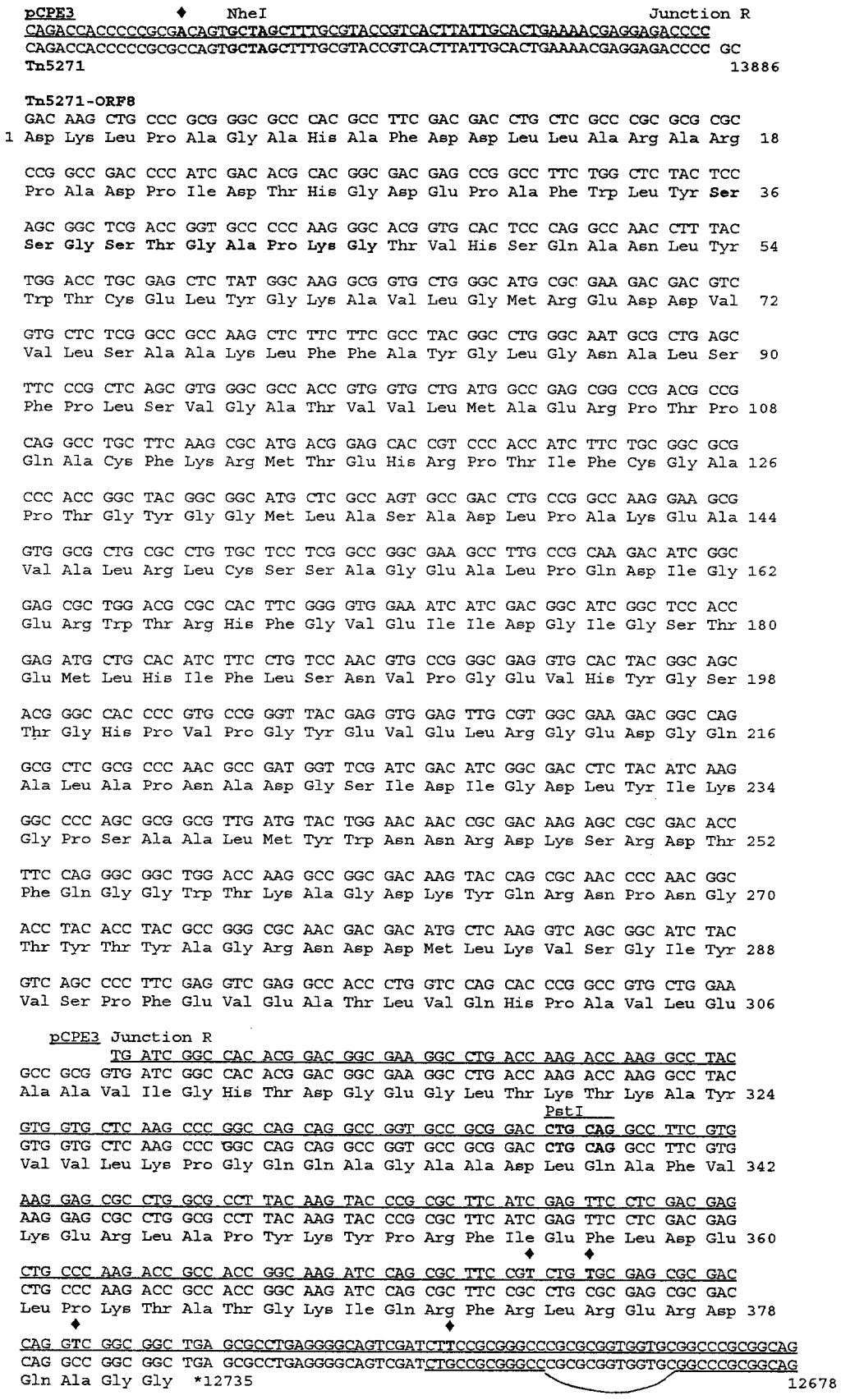

FIG. 3. Junction R DNA sequences of pCPE3 and Tn*5271*. The pCPE3 sequence (underlined) contains 927 bp less DNA at junction R than the Tn*5271* sequence contains. The first comparison line shows 68 bp of DNA from the left inverted repeat of IS*1071R*; the *Nhe*I restriction enzyme site and the CCCC border sequence (nucleotide 13886 of Tn*5271*) are shown. Note that the nucleotide numbering system refers to Tn*5271*, and therefore the numbers decrease in the inverse complement sequences. The translation product of ORF8 is shown below the Tn*5271* sequence and is numbered. The sites at which the sequences of pCPE3 and Tn*5271* were dissimilar (aside from the junction sequence) are indicated by solid diamonds. A stem-loop putative transcription termination structure is shown at the end of the sequence.

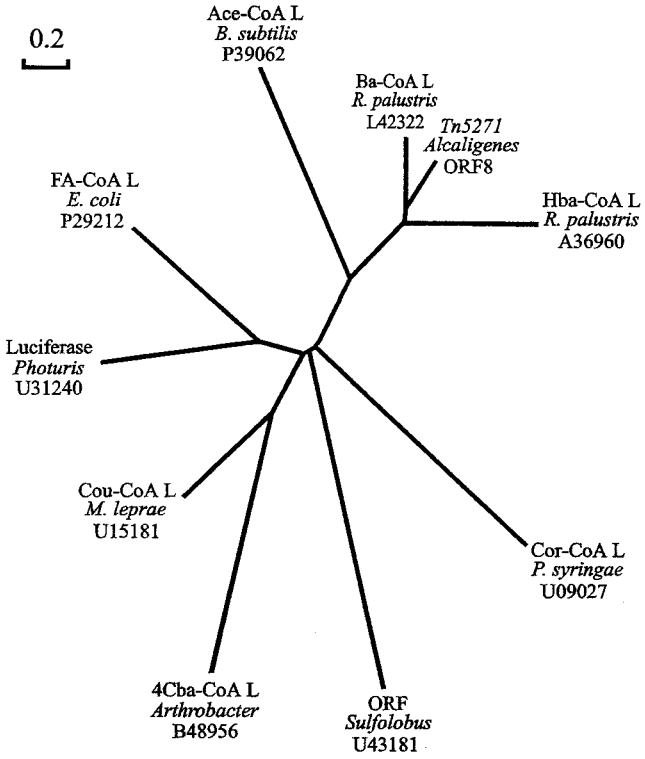

FIG. 4. Unrooted phylogenetic tree based on distances among 11 protein domains that aligned with the putative 382-amino-acid translation product of ORF8. The tree was created with the PROTDIST program of PHYLIP 3.5 (12) by using the Kimura two-parameter model with 100 bootstrapped data sets and by the Fitch-Margoliash least-squares method with the FITCH program of PHYLIP 3.5. The *Sulfolobus acidocaldarius* sequence (accession no. U43181) was used as an outgroup. The accession numbers of the sequences used are as follows: benzoate-coenzyme A ligase (Ba-CoA L) of *Rhodopseudomonas palustris* (10), L42322(gi); 4-hydroxybenzoate-coenzyme A ligase (Hba-CoA L) of *Rhodopseudomonas palustris* CGA009 (15), A36960(pir); acetyl-coenzyme A ligase (Ace-CoA L) of *Bacillus subtilis*, P39062(sp); long-chain fatty acid-coenzyme A ligase (FA-CoA L) of *E. coli*, P29212(sp); 4-coumarate-coenzyme A ligase (Cou-CoA L) of *Mycobacterium leprae*, U15181(gi); luciferase of *Photuris pennsylvanica* (firefly), U31240(gi); 4-chlorobenzoate-coenzyme A ligase (4Cba-CoA L) of *Arthrobacter* sp. strain SU, B48956(pir); coronafacate-coenzyme A ligase (Cor-CoA L) of *Pseudomonas syringae* pv. glycinea, U09027(gi); and *Sulfolobus acidocaldarius* open reading frame (ORF), U43181(gi).

divergence between ORF8 and the 4-chlorobenzoate-coenzyme A ligases represented by the sequence from *Arthrobacter* sp. strain SU (accession no. B48956) (41). ORF8 may once have been part of a functional gene or operon expressing a coenzyme A-mediated aromatic ring degradation pathway, but it is unlikely to have been involved in hydrolytic dechlorination of chlorobenzoates (38, 41, 42). There is no similarity between the *cbaABC*-determined pathway for chlorobenzoate degradation and the hydrolytic dechlorination pathway.

Spontaneous mutants of strain CPE3 that were not able to grow on chlorobenzoates were isolated at a frequency of approximately 10^{-3} cell^{-1} generation^{-1} on nonselective media. Reversion of these mutants was tested by plating 10^{10} cells on agar containing 3-chlorobenzoate, and under these conditions reversion could not be detected, indicating that the *cbaABC* genes had been deleted. The frequency of the deletions corresponded to the frequency of loss of Tn*5271* from pBRC60, which was previously determined to be the result of homologous recombination between the direct repeats of IS*1071* (28). Similar deletions have been described for composite transposon structures involving IS*1071* on plasmids pUO1, pTDN1, pOPH1, and pPSB listed in Table 1 (9, 14, 22, 24). The pCPE3 plasmid had a deletion, designated pCPE3-I, consisting of 13 kb of DNA corresponding to the internal DNA of the transposon plus one copy of IS*1071*; this deletion was similar to the deletions caused by homologous recombination in the other transposons. In addition, pCPE3 had a larger deletion, designated pCPE3-II, corresponding to the pCPE3-I deletion plus 14 kb of flanking DNA outside the region of the transposon (data not shown). The nature of the latter deletion is not known; however, the high deletion frequency suggests it was also due to homologous recombination. These deletions left a single copy of IS*1071* on the plasmid (data not shown). They were irreversible and therefore cannot account for the observed differences in transposon structure between Tn*5271* and the element on pCPE3.

The components of class I and II transposons have been detected by enrichment and PCR-based methods in soil, freshwater, and marine environments, indicating that they are widely available for genome rearrangement (6, 26). Chromosome mobilization mediated by class I insertion sequences like IS*3* on F and IS*21* on R68 can transfer large segments of chromosomes into compatible recipients in laboratory matings (13, 17, 37). Class II elements are also important agents for gene mobilization (16, 18, 55), and both types of elements have been implicated in the mobilization, amplification, and recombination of catabolic genes and operons (14, 28, 33, 45–47, 49, 51). Nevertheless, the formation of new transposon structures is almost never observed directly in clinical or environmental settings, presumably because of the low frequency with which the events occur. The exceptions to this include the observation by Hawkey et al. (19) of the probable path of evolution of an R plasmid from a cryptic plasmid in clinical isolates of *Providencia stuartii* collected over an 18-month period from a single chronic-care patient in the Bristol Royal Infirmary. van der Ploeg et al. (49) observed transposition of the class I insertion sequence IS*1247* from an unlinked site on the chromosome of *Xanthobacter autotrophicus* GJ10 to a chromosomal location upstream of the haloacetate dehalogenase gene *dhlB*. This led to overexpression of *dhlB* and mobilization of this gene by IS*1247*. We have proposed that IS*1071* mobilized the *cbaABC* genes onto plasmid pBRC60 by a two-step process involving intermolecular and intramolecular transpositions, which is consistent with the class II transposition mechanism (51) (Fig. 5). This mechanism of mobilization explains the lack of 5-bp direct repeats of DNA flanking Tn*5271* on pBRC60, which would be expected if transposition of Tn*5271* to the plasmid was the result of a single step involving the IS*1071* transposase (28). If the two transposition steps shown in Fig. 5 are not sequence specific for targets 1 and 2, then different lengths of internal DNA would be observed in composite elements formed following independent mobilization events, as is the case for Tn*5271* and the pCPE3 transposon. Other factors that support the hypothesis that these elements originated independently at PCB-contaminated sites in North America and Europe include the geographic distance between the locations from which the two hosts were isolated, the observed differences in restriction patterns for the two plasmids (with the exception of the transposon regions), and the involvement of IS*1071* in the mobilization of a diverse set of catabolic genes and operons at other locations (Table 1).

An alternative hypothesis to explain the formation of these homologous transposons is that they shared a common ancestor and that internal junctions L and R were altered by the activities of IS*1071*. Class II elements transpose almost exclusively by a replicative mechanism that results in deletions or inversions following intramolecular transposition (16, 43). We

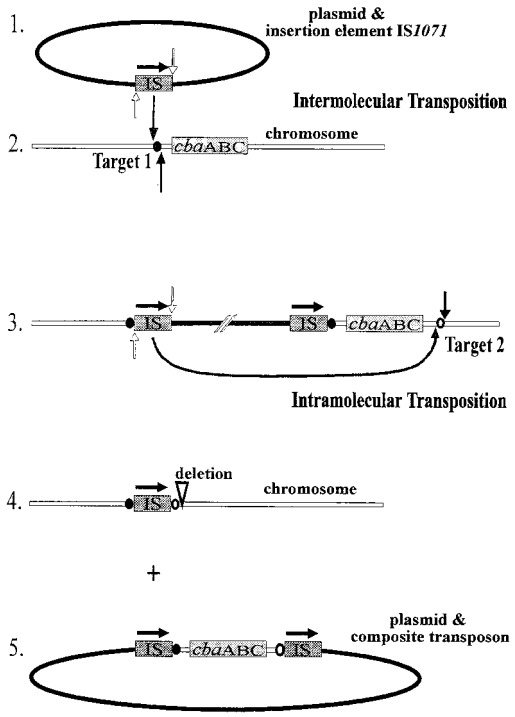

FIG. 5. Proposed steps in the acquisition of *cbaABC* catabolic genes by plasmids carrying IS*1071*. Sequential inter- and intramolecular transpositions into locations on the chromosome designated Target 1 (●) and Target 2 (○) generate a composite transposon with direct repeats of IS*1071*. (Step 1) Plasmid carrying a single copy of IS*1071* (IS). The horizontal arrow indicates the direction of transcription of the *tnpA* gene, and the vertical arrows indicate sites nicked by TnpA at the 3′ ends of IS*1071*. (Step 2) Vertical arrows indicate the Target 1 (●) site nicked by TnpA, adjacent to the *cbaABC* operon. (Step 3) The integrated plasmid (solid line) flanked by direct repeats of IS*1071* and the repeated target site sequence (●). Intramolecular transposition of the left IS*1071* element to Target 2 (○) (curved arrow) generates product 4, a chromosomal copy of IS*1071* next to a *cbaABC* deletion site (▽), and product 5, a plasmid carrying the composite transposon. Note that the 5-bp duplications of target site DNA created by the two transpositions (● and ○) are separated on the plasmid and chromosome.

have observed IS*1071*-mediated inversions of flanking DNA in pBRC60 derivatives in the laboratory and in industrial wastewaters (52). In order to have additional DNA at junction R in Tn*5271* and at junction L in pCPE3, a common ancestral element that underwent independent IS*1071*-mediated deletions at opposite ends, followed by intramolecular replicative transpositions of the remaining IS*1071* copy, would have to be invoked. The expected frequency of this series of events would be about 10^{-20} (or four transposition steps involving the presumptive ancestral element). Even at that, the resulting elements would have inverted copies of IS*1071* flanking the *cba* genes, which have not been observed. Independent mobilization events involving two transposition steps, as shown in Fig. 5, seem to be a more likely mechanism for the formation of these transposons.

The origin of the DNA within Tn*5271* and within the pCPE3 transposon is not known. The similarity of the restriction maps (Fig. 1) and the level of identity of the *cbaA* regions sequenced (99.3%) suggest that these DNA segments were mobilized from virtually identical loci. Other examples of strong conservation of nucleotide sequences in pollutant-degrading bacteria from different sources are known; for example, naphthalene catabolic operons exhibiting more than 93% sequence conservation were found in isolates from three continents (8, 44). The results of our study indicate that virtually identical operons can be distributed worldwide, either by physical movement of host strains or by horizontal transfer, and that mobilization of these operons by independent transposition events onto plasmids may occur frequently.

Nucleotide sequence accession number. The nucleotide sequences determined in this study have been deposited in the GenBank database under accession no. AF041042.

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