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Plasmid-Borne Cadmium Resistance Genes in *Listeria monocytogenes* Are Present on Tn*5422*, a Novel Transposon Closely Related to Tn*917*

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The complete (6,449-bp) nucleotide sequence of the first-described natural transposon of *Listeria monocytogenes*, designated Tn*5422*, was determined. Tn*5422* is a transposon of the Tn*3* family delineated by imperfect inverted repeats (IRs) of 40 bp. It contains two genes which confer cadmium resistance (M. Lebrun, A. Audurier, and P. Cossart, J. Bacteriol. 176:3040–3048, 1994) and two open reading frames that encode a transposase (TnpA) and a resolvase (TnpR) of 971 and 184 amino acids, respectively. The cadmium resistance genes and the transposition genes are transcribed in opposite directions and are separated by a putative recombination site (*res*). The structural elements presumed to be involved in transposition of Tn*5422* (IRs, transposase, resolvase, and *res*) are very similar to those of Tn*917*, suggesting a common origin. The transposition genes were not induced by cadmium. Analysis of sequences surrounding Tn*5422* in nine different plasmids of *L. monocytogenes* indicated that Tn*5422* is a functional transposon, capable of intramolecular replicative transposition, generating deletions. This transposition process is probably the reason for the size diversity of the *L. monocytogenes* plasmids. Restriction analysis and Southern hybridization revealed the presence of Tn*5422* in all the plasmid-mediated cadmium-resistant *L. monocytogenes* strains tested but not in strains encoding cadmium resistance on the chromosome.

Cadmium is a heavy metal used extensively in industry for a variety of applications, including electroplating, protection against corrosion, and stabilizing plastic. Over the last century, its increased industrial use has led to cadmium contamination of the environment, plants, animals, food products, and humans. Cadmium is toxic and blocks several metabolic processes in bacteria, including respiration (44). It is not surprising that bacteria have acquired various cadmium resistance mechanisms, recently reviewed (30, 41).

Among *Listeria monocytogenes* strains, 35.8% are cadmium resistant, with a higher incidence among strains isolated from the environment and food. This suggests that cadmium resistance confers a significant selective advantage to *L. monocytogenes* in the environment (27). Of the cadmium-resistant isolates, 87.2% contain plasmids (27). Sequence analysis suggests that the mechanism of plasmid-borne cadmium resistance in *L. monocytogenes* is an energy-dependent cadmium efflux system, involving two proteins, CadA and CadC (26). These proteins are similar to the products of the well-studied cadmium resistance determinants *cadAC* of *Staphylococcus aureus* (41). Cadmium resistance in *L. monocytogenes* is induced by the presence of trace amounts of Cd^{2+} in the culture medium (26).

Various *L. monocytogenes* strains of serogroups 1 and 4 from environmental, clinical, and alimentary sources contain plasmids of between 24 and 106 kb in size. The majority of these plasmids (95%) confer cadmium resistance (27). The distribution of cadmium resistance among *L. monocytogenes* isolates is thus complex: many different strains are resistant; plasmids of different sizes confer resistance; and some plasmid-free strains are resistant and thus presumably carry chromosomal resistance determinants.

Transposons are often implicated in the dissemination of bacterial resistance. Transposons are mobile genetic elements that carry genes for auxiliary traits, such as resistance to antibiotics or heavy metals as well as genes for transposition. Both gram-positive and gram-negative bacterial transposons can be classed into two groups according to their structure (4, 6). The first group contains the composite transposons, such as Tn*5* (7), in which the auxiliary genes are surrounded by insertion sequences in inverted or direct orientations. The second group contains the transposons of the Tn*3* family, whose prototype is Tn*3* (40). These transposons contain transposase, resolvase, and auxiliary genes, bracketed between two inverted repeats (IRs) of approximately 38 bp.

Mercury resistance genes are the only heavy-metal resistance genes to have been shown to be carried by transposons. Two well-characterized transposons are Tn*501* from *Pseudomonas aeruginosa* (5, 10) and Tn*21*, originally isolated from *Shigella flexneri*, which also encodes streptomycin and sulfonamide resistance (13); both belong to the Tn*3* family.

Here, we report that the genes for cadmium resistance in *L. monocytogenes* plasmids are located on a transposable element designated Tn*5422*, closely related to Tn*917*, an *Enterococcus faecalis* transposon of the Tn*3* family conferring erythromycin resistance (2, 39). In addition, we report evidence that Tn*5422* is mobile and capable of intramolecular transposition, generating deletions in plasmids where transposition occurs.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacteria and plasmids used in this study are listed in Table 1.

TABLE 1. Bacterial strains

Strain	Relevant characteristics[a]	Plasmid (size, kb)	Reference or source
E. coli			
MC1061	F⁻ *araD139* Δ(*ara-leu*)*7696* Δ*lacY74 galU galK hsr hsm strA*		12
TG1	K-12, Δ(*lac-pro*) *supE thi hsdD5* F′ *traD36 proAB*⁺ *lacI*^{q} *lacZ*ΔM15		Gibson (Medical Research Council, Cambridge, United Kingdom)
L. monocytogenes			
Lm2	Serogroup 4, Cd^r		27
Lm10	Serogroup 1, Cd^r		27
Lm24	Serogroup 1, Cd^s	pLm24 (14)	27
Lm27	Serogroup 1, Cd^r	pLm27 (25.7)	27
Lm35	Serogroup 1, Cd^r		27
Lm40	Serogroup 1, Cd^r	pLm40 (41)	27
Lm41	Serogroup 1, Cd^r		27
Lm54	Serogroup 1, Cd^r		27
Lm71	Serogroup 1, Cd^r	pLm71 (43.6)	27
Lm74	Serogroup 1, Cd^r	pLm74 (20.5)	27
Lm101	Serogroup 1, Cd^r	pLm101 (53)	27
Lm106	Serogroup 4, Cd^r	pLm106 (61)	27
Lm113	Serogroup 1, Cd^r		27
Lm141	Serogroup 4, Cd^r	pLm141 (87)	27
Lm162	Serogroup 4, Cd^s	pLm162 (53)	27
Lm173	Serogroup 1, Cd^r	pLm173 (106)	27
Lm176	Serogroup 1, Cd^r	pLm176 (81)	27

[a] Cd^s, cadmium sensitive; Cd^r, cadmium resistant.

Strains of *Escherichia coli* were grown in LB medium at 37°C, and *L. monocytogenes* strains were grown in brain heart infusion (BHI) broth or agar (Difco Laboratories, Detroit, Mich.). Media were supplemented with ampicillin at a concentration of 100 μg/ml in agar and 25 μg/ml in liquid medium as appropriate. Ampicillin was purchased from Sigma Chemical Co. Plasmid pUC18 (46) was used to clone DNA fragments in *E. coli* strains.

Molecular cloning and DNA analysis. All cloning procedures were carried out by standard protocols (36) or the reagent manufacturer's instructions. Plasmid DNA and RNA were extracted from *L. monocytogenes* as described in the accompanying article (26), and chromosomal DNA from *L. monocytogenes* was prepared as described previously (29). Probes for Southern or slot blot hybridizations were purified from agarose gels with the GeneClean kit (Bio 101 Inc., La Jolla, Calif.) and labeled with the multiprime system of Amersham. Hybridization experiments were performed under conditions of high stringency with the rapid hybridization system (Amersham, Les Ulis, France) on Hybond N membranes (Amersham) as recommended by the manufacturer. Modifying or restriction enzymes were purchased from Boehringer (Mannheim, Germany), Appligene (Illkirch, France), and Amersham. *Taq* polymerase was obtained from Amersham.

DNA sequencing. To sequence the entire transposon Tn*5422*, plasmids containing various fragments of *L. monocytogenes* plasmid pLm74 were constructed. The recombinant plasmids are shown in Fig. 1. The region corresponding to the cadmium resistance determinants was cloned into pMa4, and its sequence has previously been determined (26). The 377-, 767-, and 1,499-bp *Eco*RI fragments of pLm74, which spanned the entire *tnpR* gene and the 5′ part of the *tnpA* gene, were cloned in pUC18 to generate pMa1, pMa2, and pMa3, respectively. Plasmids pMa40 and pMa41, carrying part of the *tnpA* gene from pLm74, were constructed in two steps. First, the 6.3-kb *Bgl*II-*Bam*HI fragment from pLm74 was cloned into the *Bam*HI site of pUC18 to generate pMa7. Then, the 2.5-kb *Eco*RI fragment of pMa7 was subcloned in both orientations into the *Eco*RI site of pUC18 to generate pMa40 and pMa41.

Sequences were determined by the dideoxynucleotide chain termination method of Sanger et al. (37) by using [^{35}S]dATP and the T7 sequencing kit from Pharmacia. The plasmid DNAs used as templates were purified with the Qiagen kit (Qiagen, Inc.). The sequences of pMa1 and pMa2 were determined on both strands of DNA by using oligonucleotide primers complementary to sequences in the inserts. Plasmids pMa3, pMa40, and pMa41 were digested with exonuclease III (18) with the double-stranded nested deletion kit from Pharmacia. Appropriate clones were sequenced with the universal primer. The sequence of the second strand of DNA of pMa3 was obtained by directly sequencing plasmid pMa3 with oligonucleotide primers derived from the first strand of DNA. Junction sequences between pMa4 and pMa3, pMa3 and pMa2, pMa2 and pMa1, and pMa1 and pMa40 were obtained by sequencing pMa7 with oligonucleotide primers derived from the sequences of the corresponding recombinant plasmids. Oligonucleotides were obtained from the Unité de Chimie Organique, Institut Pasteur, Paris.

Computer analysis of sequences. The translated gene bank (Genpept; release 64.3) and the Swiss-Prot data bank (release 17.0) were screened for sequence similarities with the BLAST program (1).

Inverted PCR. The principle of inverted PCR is given in Fig. 9A. *L. monocytogenes* plasmids were digested with *Alu*I, and the *Alu*I fragments were ligated. A total of 15 ng of the ligated DNA was amplified by using two pairs of oligonucleotides chosen from the sequence of the cadmium resistance transposon: the left junction of the transposon was amplified with oligonucleotides P1 (GTAAGTCGTCTCCCATTAAGG, positions 364 to 384 in Fig. 2) and P2 (TACCGCTATT GTCTAGTTCGT, positions 137 to 155 in Fig. 2), and the right junction was amplified with oligonucleotides P3 (CTTGC CAAAGCATTTACGGC, positions 6103 to 6122 in Fig. 2)

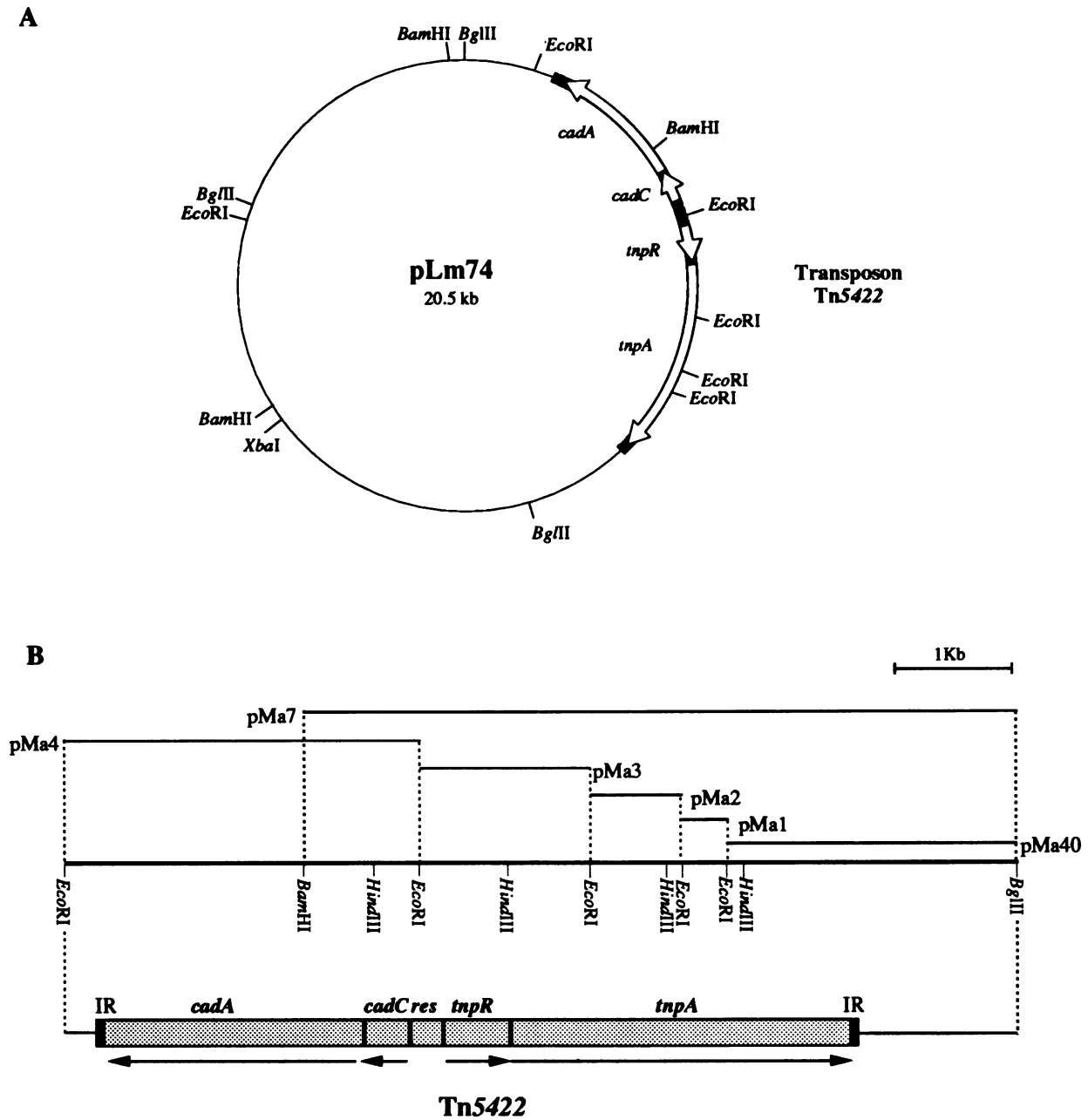

FIG. 1. Physical and genetic maps of pLm74 and Tn*5422*. (A) Plasmid pLm74 from *L. monocytogenes* Lm74. The cadmium resistance genes *cadA* and *cadC* and transposition genes *tnpA* and *tnpR* are indicated by open arrows in transposon Tn*5422*, represented as a thick line. (B) Physical and genetic map of Tn*5422*. The direction of transcription of the *cadA*, *cadC*, *tnpR*, and *tnpA* genes is shown by arrows. *res*, resolution site. Above the transposon, the extents of the various subclones used to generate the sequence of Tn*5422* are shown.

and P4 (GTTATCTATTCCCAAGAGGTTG, positions 6360 to 6381 in Fig. 2). In each PCR, 2.5 pmol of each oligonucleotide was used. Asymmetric PCR was performed on 1% of the amplification product with 0.6 pmol of one oligonucleotide and 12 pmol of the second oligonucleotide. The amplified single-stranded DNA was sequenced directly with the T7 sequencing kit (Pharmacia).

Nucleotide sequence accession number and transposon name. The nucleotide sequence shown in Fig. 2 has been deposited in GenBank under accession number L28104. The transposon name Tn*5422* was officially attributed by the Plasmid Reference Center, Stanford, Calif.

RESULTS AND DISCUSSION

All plasmids from cadmium-resistant *L. monocytogenes* strains contain common sequences in addition to the *cadAC* determinants. The restriction profiles of 11 plasmids from *L. monocytogenes* strains (Table 1) were compared to identify common sequences in plasmids conferring cadmium resis-

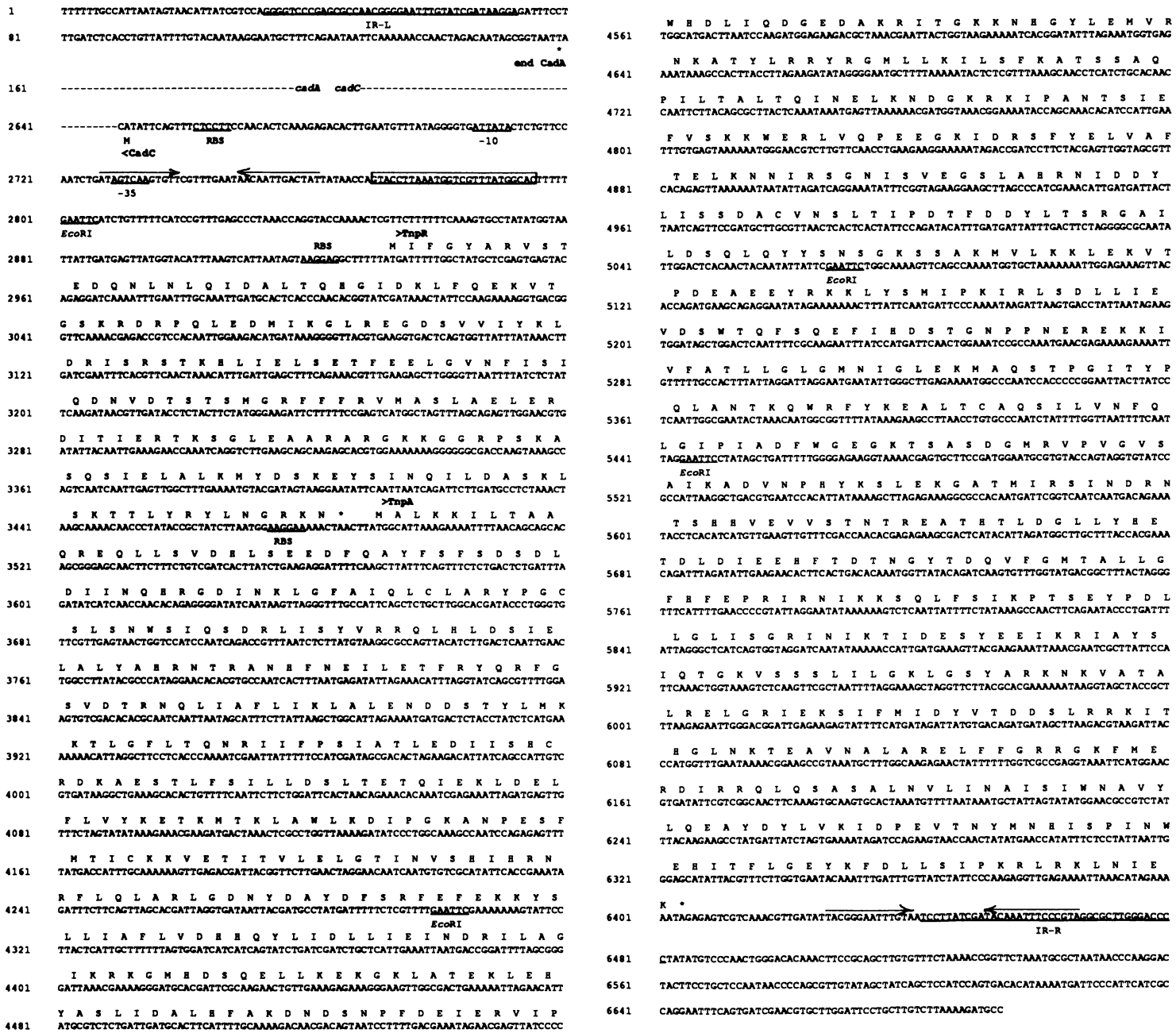
IR-L
end CadA
cadA cadC
M
RBS
-10
<CadC
-35
EcoRI
>TnpR
RBS
M I F G Y A R V S T
RBS
>TnpA
M A L K K I L T A A
RBS
EcoRI
EcoRI
IR-R

FIG. 2. Nucleotide sequence of Tn*5422*. The stop codon of the *cadA* gene and the translation initiation codon of the *cadC* gene are shown. The complete sequence of the *cadA* and *cadC* genes is given in reference 26. The deduced amino acid sequences of the resolvase (*tnpR*) and transposase (*tnpA*) genes are shown above the nucleotide sequence. The left (IR-L) and right (IR-R) terminal IRs of Tn*5422* are underlined. The ribosome-binding site (RBS), −10 and −35 consensus sequences, and *Eco*RI restriction sites are indicated; asterisks indicate stop codons. IR sequences are shown by arrows. Arrowheads indicate the direction of transcription of each gene. The putative resolvase-binding site (see text) is boxed.

tance. The 11 plasmids are listed in Table 1. Nine of the plasmids were from cadmium-resistant strains, and two were from cadmium-sensitive strains. All the plasmids were of different sizes, ranging from 14 to 106 kb. Plasmid pLm74, in which the cadmium resistance genes *cadA* and *cadC* have been located on a 3.1-kb *Eco*RI fragment (26), was included in the study. The plasmids were digested with restriction endonucleases *Bgl*II, *Bam*HI, *Eco*RI, and *Xba*I.

All the plasmids had different restriction profiles (data not shown). However, the nine plasmids from cadmium-resistant strains had three common *Eco*RI fragments of 370, 800, and 1,500 bp, which were absent from the plasmids from sensitive strains. In pLm74, these fragments were contiguous and adjacent to the 3.1-kb *Eco*RI fragment conferring cadmium resistance (Fig. 1A).

Cadmium resistance genes of pLm74 are flanked by sequences similar to transposition genes. The three contiguous *Eco*RI fragments of 370, 800, and 1,500 bp of pLm74 that were common to all the plasmids conferring cadmium resistance were cloned into pUC18, giving pMa1, pMa2, and pMa3, respectively. The complete nucleotide sequences of these three fragments were determined on both strands.

The reconstituted sequence of these contiguous fragments contains two large open reading frames (ORFs). The sequence of the smaller ORF was complete. The complete sequence of the larger ORF was obtained by sequencing pMa40 and pMa41 (Fig. 1B). The two ORFs are transcribed in the opposite orientation from that of *cadC* and *cadA*. A total of 279 bp separate the ATG of *cadC* from that of the first small ORF (Fig. 2). The two consecutive ORFs exhibited sequence similarities with the resolvase (*tnpR*) and transposase (*tnpA*) genes of Tn*3* and related elements. At 36 bp downstream from the stop codon of the putative *tnpA* gene of pLm74, a sequence similar to the terminal IR of transposon Tn*917*, a transposon carrying erythromycin resistance in *E. faecalis* (39), was found. A similar sequence was also present 85 bp downstream from the stop codon of the *cadA* gene of pLm74 (Fig. 2). These results suggest that *L. monocytogenes* plasmid pLm74 harbors a transposon encoding cadmium resistance. This putative transposon was designated Tn*5422*.

The possibility that chromosomal cadmium resistance is also carried by Tn*5422* was investigated by Southern blotting. A *tnpA* probe was hybridized at high stringency to total cellular DNA isolated from six plasmid-free, cadmium-resistant *L. monocytogenes* strains (Lm2, Lm10, Lm35, Lm41, Lm54, and Lm113). No signal was detected. Similarly, in preliminary experiments, a *cadAC* probe did not hybridize with chromosomal DNA even at low stringency (data not shown). Thus, chromosomal cadmium resistance does not result from insertion of Tn*5422*. In all the cadmium-resistant strains tested, transposon Tn*5422* was always found on plasmids, suggesting the preferential localization of Tn*5422* on plasmid DNA sequences.

Terminal IRs. Imperfect 40-bp IRs (positions 33 to 72 and 6442 to 6481 in Fig. 2) delineated the 6,449-bp cadmium resistance transposon Tn*5422*. The two IRs of Tn*5422* differ by only 3 bp (Fig. 3) and are similar to the IRs of the Tn*3* family of transposons (40), especially to the IRs of Tn*917* (Fig. 3) (39). The invariable GGGG sequence found at the extremity of the IRs of transposons of the Tn*3* family is present in the termini of Tn*5422*.

Transposase. The predicted *tnpA* gene of pLm74 encodes a protein of 971 amino acids, with a calculated molecular mass of 111 kDa. The gene is preceded by a ribosome-binding site and is followed by an imperfect 15-bp IR partly included in the right IR (Fig. 2). The same structure has been found in Tn*917* and was postulated to be a putative transcriptional stop signal (39). In Tn*5422*, this stem-loop structure has a ΔG of −18 kcal (9) and, by analogy with Tn*917*, could also act as a *tnpA* transcriptional terminator, although it lacks the run of T's thought to be important for termination.

The deduced polypeptide TnpA shows the greatest similarity to TnpA of Tn*917* (81% identity) (2). The DNA sequences of the *tnpA* gene of Tn*917* and the *tnpA* gene of Tn*5422* are also similar (Fig. 4A). TnpA of Tn*5422* exhibits similarities to transposases from gram-positive and gram-negative bacteria, but a dendrogram of sequence relationships among these transposases (Fig. 5) shows that the transposase of Tn*5422* is most closely related to those of other gram-positive bacteria. Sequence alignments of the transposases show that the amino acid sequence of the C terminus is particularly conserved (data not shown). This observation is in agreement with the presumed function of the C-terminal domain of the transposase, which is predicted to participate in DNA cleavage and ligation, a common feature of all transposases. The N-terminal region has been implicated in recognition of the IR and may thus differ for each transposon (15, 45).

Resolvase. The ORF preceding *tnpA* encodes a protein of 184 amino acids, with a calculated molecular mass of 20.9 kDa, which is similar to the resolvase (TnpR) of Tn*917* (84% identity) (39). This putative *tnpR* gene of Tn*5422* is preceded by a putative ribosome-binding sequence (Fig. 2).

The deduced amino acid sequence of this ORF is similar to those of various site-specific recombinases, including the Pin, Gin, and Cin invertases (Fig. 6). The resolvases of the Tn*3* family have an N-terminal domain containing the putative serine involved in recombination and a C-terminal DNA-binding domain (40). In Tn*5422*, the invariant serine and the helix-turn-helix DNA-binding motif were present (Fig. 6).

The TnpR of Tn*5422* is also closely related to the TnpR of

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IR-L  Tn5422  GGGGTCCCAAGCGCCTACGGGAAATTTGTATCGATAAGGA
IR-R  Tn5422  GGGGTCCCGAGCGCCAACGGGGAATTTGTATCGATAAGGA
IR-R  Tn917   GGGGTCCCGAGCGCTTAGTGGGAATTTGTATCGATAAG
IR-L  Tn917   GGGGTCCCGAGCGCCTACGAGGAATTTGTATCGATAAG
IR-I  Tn917   GAGGTCCCTAGCGCCTACGGGGAATTTGTATCGATAAG
IR    Tn551   GGGGTCC-GAGCGC--ACGAGAAATTTGTATCGATAAG
IR-L  Tn1546  GGGGTAGCGTCAGGAAAATGCGGATTTACAACGCTAAG
IR-R  Tn1546  GGGGTACCGTCAGGAAAATGCGGATTTACAACGTTAAG
              * ***      *  *     **** * ** ****
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FIG. 3. Comparison of the terminal IRs of Tn*5422* with those of Tn*917* from *E. faecalis* (39), Tn*551* from *S. aureus* (22), and Tn*1546* from *Enterococcus faecium* (3). The alignment was produced with the program Clustal (19). IR-I, internal IR.

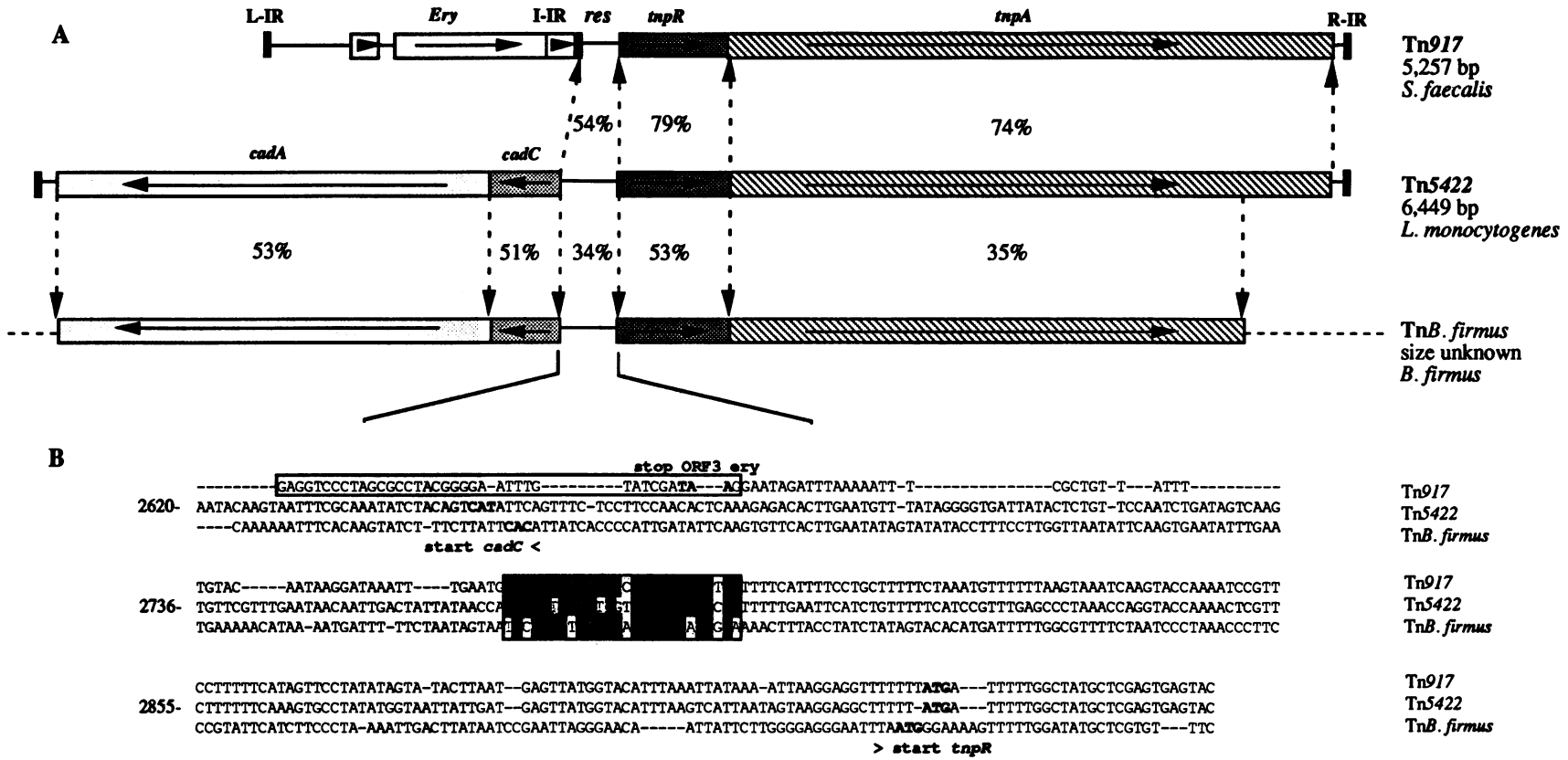


FIG. 4. Comparison of the three closely related transposons Tn*917* from *E. faecalis* (2, 39), Tn*5422* from *L. monocytogenes*, and the putative transposon of *B. firmus* (Tn*B. firmus*) (21). (A) Maps of the transposons. The hatched bars are the inverted repeats (IRs) left IR (L-IR), internal IR (I-IR), and right IR (R-IR). The resistance determinants are indicated by boxes: *Ery*, erythromycin resistance; *cadA* and *cadC*, cadmium resistance. The resolvase (*tnpR*) and transposase (*tnpA*) genes are boxed, and arrows show the direction of transcription. Similarity between DNA sequences of the genes is indicated as percent identity. (B) Multiple alignment of the DNA sequences of the intergenic region between resistance genes and transposition genes of the three transposons Tn*917*, Tn*5422*, and the putative transposon of *B. firmus*. Dashes represent gaps introduced to optimize similarity. The start codons of the resolvases and CadC and the stop codon of ORF3 are in boldface, and arrowheads indicate the direction of transcription. The putative resolvase-binding site is highlighted. The internal IR of Tn*917* is indicated by an open box.

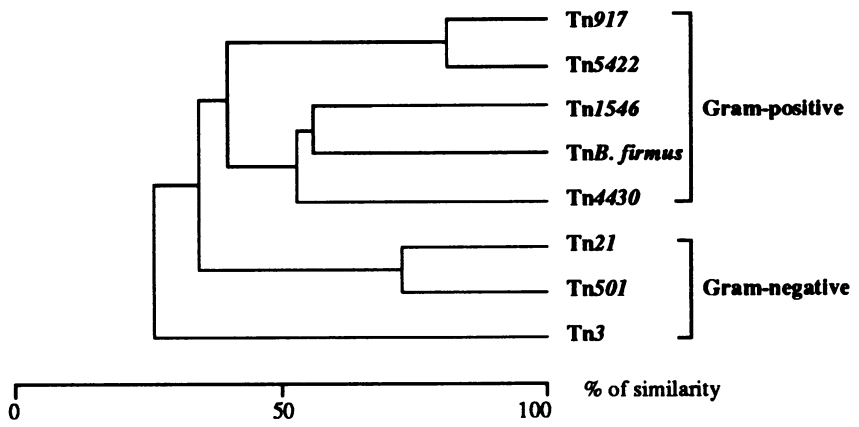


FIG. 5. Tree of sequence relationships among transposases of the Tn*3* family. The lengths of the branches in the tree are measures of the relatedness of the transposases. Primary amino acid sequences compared include the transposases of Tn*917* (2), Tn*5422*, Tn*1546* (3), the putative transposon of *B. firmus* (Tn*B. firmus*) (21), Tn*4430* (28), Tn*21* (45), Tn*501* (11), and Tn*3* (17).

a putative transposon of *Bacillus firmus* OF4 (63% identity) (21). Not all the sequence of this putative transposon has been determined, but it contains genes similar to *tnpA* and *tnpR* of transposons of the Tn*3* family and genes similar to *cadA* and *cadC* of *S. aureus* (32) and *L. monocytogenes* plasmids (26). The *cadC* gene of this putative transposon confers Na^+ resistance on Na^+/H^+-antiporter-deficient *E. coli* strains. The role of this putative transposon in cadmium resistance has not been investigated.

Potential *res* site. The site where cointegrate intermediates resolve through site-specific recombination catalyzed by resolvase is called the *res* site. This site usually spans 120 to 140 bp and contains three resolvase-binding sites of about 30 bp (16). The site of resolution is usually the first resolvase-binding site. By analogy with Tn*917*, the putative *res* site of Tn*5422* lies between the cadmium resistance genes (*cadC* and *cadA*) and the transposition genes (*tnpR* and *tnpA*) (Fig. 2 and Fig. 4). In Tn*917*, one binding site has been described (40). We also found one binding site in the case of Tn*5422*. The Tn*5422* and Tn*917* putative binding sites differ at only five positions. The resolvases of Tn*5422* and Tn*917* are extremely similar, and it is not surprising, therefore, that their *res* sites (the site recognized by the resolvase) are also very similar.

Structural organization of Tn*5422* and comparison with Tn*917* and the putative transposon of *B. firmus*. Tn*5422* is 6,449 bp long, with a G+C content of 37.9%, in agreement with that of the *L. monocytogenes* chromosome, which is between 37 and 39% G+C (38). Tn*5422* contains genes for cadmium resistance functions (*cadC* and *cadA*) as well as genes for transposition (*tnpA* and *tnpR*) and is bracketed by inverted repeats of 40 bp. Therefore, Tn*5422* can be placed in the family of Tn*3* transposons. In Tn*5422*, the transposase and resolvase

FIG. 6. Multiple alignment of site-specific recombinases. The primary amino acid sequences compared include the resolvases of Tn*5422* from *L. monocytogenes*, Tn*917* from *E. faecalis* (39), the putative transposon from *B. firmus* (Tn*B. firmus*) (21), Tn*552* from *S. aureus* (35), Tn*1546* from *E. faecium* (3), Tn*501* from *P. aeruginosa* (14), Tn*3* from *E. coli* (17), and Tn*21* from *S. flexneri* (20). The invertases are Pin from *Shigella boydii* (43), Gin from phage Mu (33), and Cin from phage P1 (24). The presumptive serine involved in recombination is in boldface, and the helix-turn-helix DNA-binding motif is boxed. Asterisks indicate positions where amino acids are identical in all proteins, and dots indicate those where amino acids are similar.

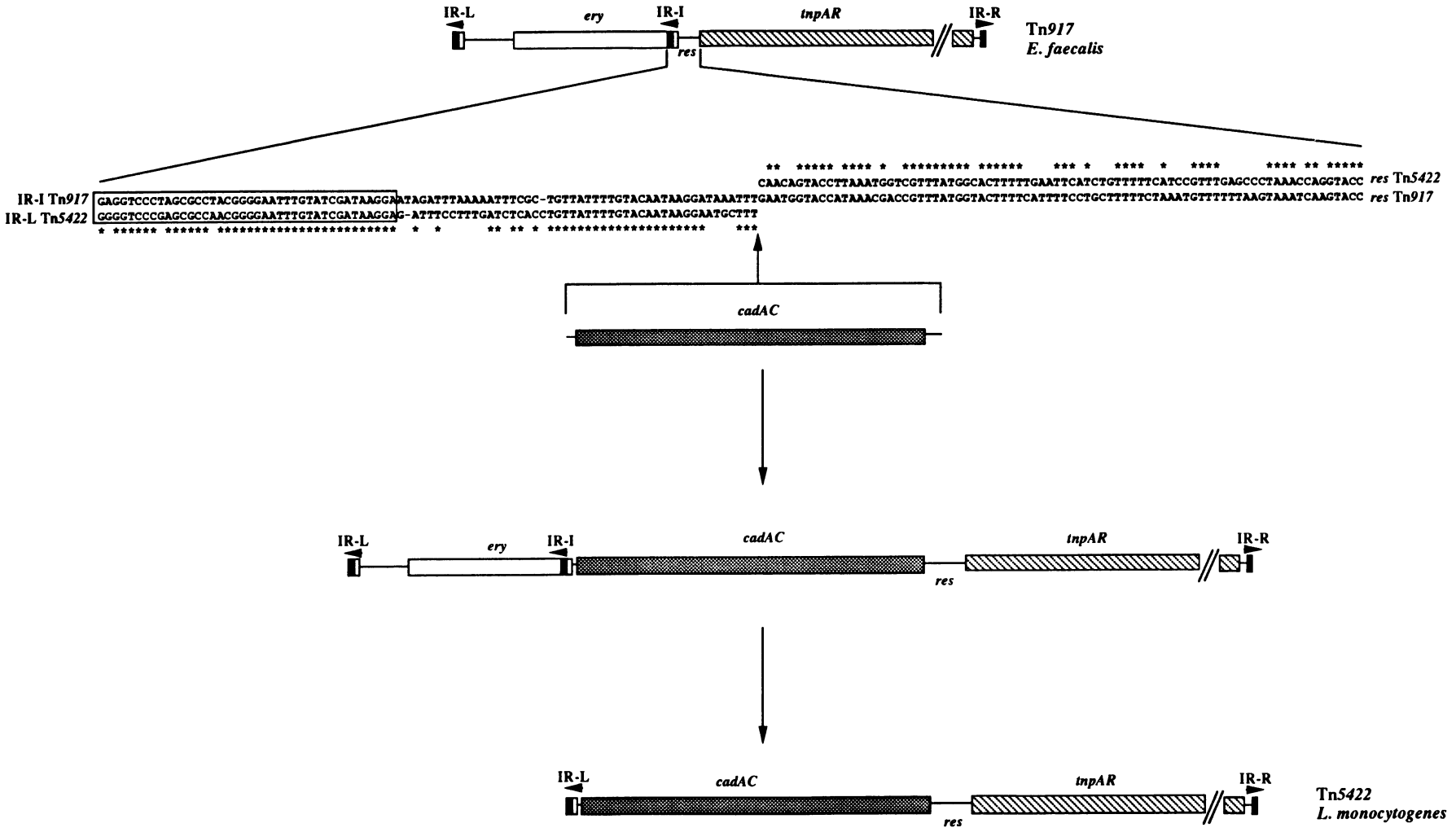


FIG. 7. Schematic representation of the proposed relationship between Tn*917* and Tn*5422*. Solid boxes are the IRs: left IR (IR-L), internal IR (IR-I), and right IR (IR-R). Arrows indicate the orientation of the IRs, and the open boxes are homologous sequences. Resistance determinants, transposition genes, and the resolution site (*res*) are indicated. The DNA sequence of IR-I and its downstream sequence is displayed. The IR-L and *res* sequences of Tn*5422* have been aligned on the contiguous IR-I and *res* sequences of Tn*917*.

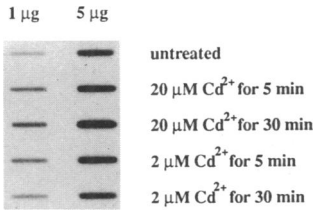


FIG. 8. Effect of the Cd^{2+} on transcription of the *tnpA* and *tnpR* genes. Aliquots of exponentially growing *L. monocytogenes* Lm74 were treated with cadmium-free medium (control), 20 μM Cd^{2+} for 5 min, 20 μM Cd^{2+} for 30 min, 2 μM Cd^{2+} for 5 min, 2 μM Cd^{2+} for 30 min. RNA was then isolated and probed for hybridization with a ^{32}P-labeled *tnpA-tnpR* probe consisting of a 1,499-bp *Eco*RI fragment of pMa3 (Fig. 1) spanning the entire *tnpR* gene and the beginning of *tnpA* and exposed for 2 h at −80°C. The same blot was reprobed with a 16S rRNA probe to assess the total amount of RNA loaded on the membrane (data not shown).

genes are transcribed in the same direction. Hence, Tn*5422* has the organization of the Tn*501* subfamily of the Tn*3* group (40).

Tn*5422* is most similar to Tn*917* and to the putative transposon of *B. firmus*. The sequences of the transposition genes are particularly close between Tn*917* and Tn*5422*, and those of the resistance genes are particularly close between Tn*5422* and the putative transposon of *B. firmus* (Fig. 4A). Tn*5422* and the putative transposon of *B. firmus* have the same organization. In both, the cadmium resistance genes (*cadA* and *cadC*) and transposition genes (*tnpA* and *tnpR*) are transcribed in opposite directions. In Tn*917*, all the genes are transcribed in the same direction (Fig. 4A). In all these transposons, the putative *res* site lies between the resistance and transposition genes (Fig. 4B).

Only the region between the left IR and the distal end of the erythromycin resistance gene is dissimilar in Tn*917* and Tn*5422* (Fig. 4A). Tn*917* contains an internal IR upstream of the *res* site. Shaw and Clewell (39) proposed that the segment comprising the internal IR, *res* site, *tnpR*, *tnpA*, and the right IR might be capable of independent transposition. Such independent transposition by a common ancestor may have led to the divergence of Tn*917* and Tn*5422*.

The length of the intergenic region between the transposition genes and resistance genes in Tn*917*, Tn*5422*, and the putative transposon of *B. firmus* is 216, 279, and 270 bp, respectively. The internal IR of Tn*917* is not present in Tn*5422* or in the putative transposon of *B. firmus*. The similarity between Tn*917* and Tn*5422* starts at the *res* site. These observations suggest that Tn*5422* is not derived from transposition of a Tn*917* ancestor which contained only the internal and right IRs, *res* site, and *tnpA* and *tnpR* genes. However, it is possible that Tn*917* and Tn*5422* have a common origin. Examination of the sequences of Tn*917* and Tn*5422* reveals that Tn*5422* may have derived from Tn*917*, according to the model shown in Fig. 7. Insertion of a *cadAC* "cassette" downstream from the internal IR of Tn*917* may have resulted in the generation of a structure which gave rise to Tn*5422* by internal deletion of erythromycin genes.

Transcription of *tnpA* and *tnpR* genes is not induced by cadmium. Transposition of the erythromycin resistance transposon Tn*917* and the mercury resistance transposon Tn*501* is induced by erythromycin and mercury, respectively (23, 39, 42). We tested whether the transposition genes *tnpA* and *tnpR* are induced by the presence of cadmium by slot blot hybridization with a probe spanning the *tnpA* and *tnpR* genes. The conditions used to test induction were the same as those used for cadmium resistance gene induction (26). RNA from cadmium-treated and untreated cells was hybridized with a *tnpA/tnpR*-specific probe: there was no significant difference in hybridization signal intensity (Fig. 8). Thus, the transposition genes of Tn*5422* are not induced by cadmium under the conditions which induced the *cadA* and *cadC* cadmium resistance genes (26).

In Tn*917* and Tn*501*, all the genes are transcribed in the same direction and a transcript corresponding to the full length of Tn*917* appears after induction with eythromycin, presumably leading to increased transposition frequency (39). The cadmium resistance genes in Tn*5422* are transcribed in the opposite direction from the *tnpA* and *tnpR* genes, and it is therefore not surprising that the transposition genes are not induced by cadmium.

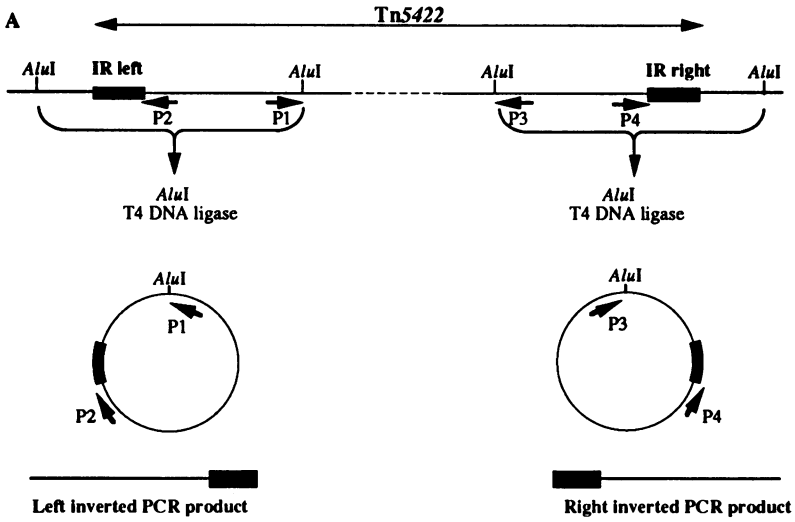
Evidence for intramolecular transposition of Tn*5422*. We tried to demonstrate intermolecular transposition of Tn*5422* by various different methods without success (data not shown). However, evidence for intramolecular transposition was obtained.

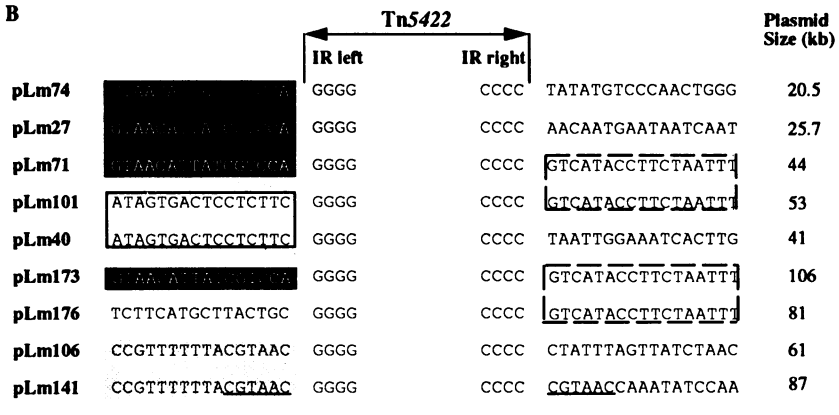
The sequence of Tn*5422* from pLm74 does not contain small direct repeats at each end of the transposon (Fig. 2). These short sequences are normally the result of transposon insertion and are usually found in transposons of the Tn*3* family. Whether the absence of duplication of short DNA sequences was a characteristic of Tn*5422* was tested by sequencing the two transposon-plasmid junctions in eight different plasmids derived from eight independent cadmium-resistant *L. monocytogenes* strains. The *Eco*RI profiles of each of the plasmids display the common transposon-internal *Eco*RI fragments (377, 767, and 1,499 bp) but different transposon-host plasmid junction fragments (data not shown). Junction fragments were amplified by inverted PCR, and PCR products were sequenced with oligonucleotides derived from the cadmium resistance transposon sequence (Fig. 9A). The sequences surrounding Tn*5422* from pLm74, pLm27, pLm71, pLm101, pLm106, pLm141, pLm173, and pLm176 are given in Fig. 9B.

All the transposons found in these plasmids have IR sequences identical to the IR sequences of pLm74 (data not shown). A duplication of the target site was found in only one plasmid (pLm141), in which a 6-bp direct repeat is present (Fig. 9B). The sequences flanking the left IR were identical in pLm27, pLm74, pLm71, and pLm173 (for at least 50 bp; data not shown) but were different outside the right IR, except for pLm71 and pLm173. Identity on one side and difference on the other were also shown for the other plasmids studied (Fig. 9B).

To explain these results, since pLm74, pLm27, and pLm71 have the same sequence on the left side of the transposon but different ones on the right side, we compared their maps (Fig. 9C). The three plasmids had the same restriction map on the left side but not on the right side of the transposon, in agreement with the sequence data. This indicated that deletions of 23.5 and 18.3 kb of pLm71 adjacent to the right end of the transposon could have generated pLm74 and pLm27, respectively. Plasmid pLm74 could also be derived from pLm27 by a deletion of 5.2 kb.

Similar deletions adjacent to one of the extremities of a transposon have been described previously for Tn*3* and Tn*1*: these deletions were shown to be the result of intramolecular replicative transposition (8, 31). Intramolecular replicative transposition can yield different rearrangements, depending upon the relative orientation of the element and the target site (7). Two daughter circles may be produced, each with a copy of the transposon and a portion of the parental replicon. Since only one daughter molecule contains a replication origin, the





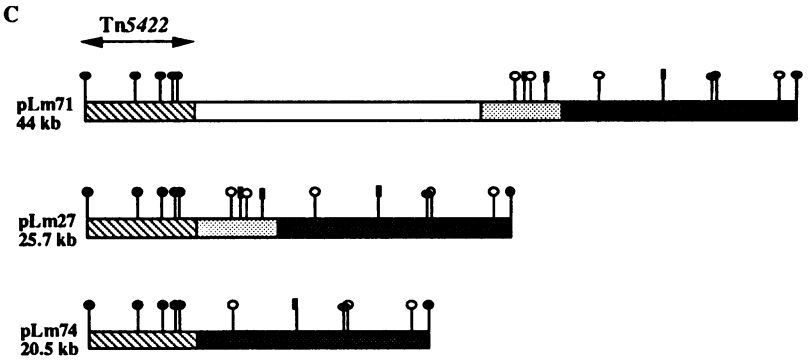


FIG. 9. Characterization of Tn*5422*-host plasmid junctions. (A) Amplification of left and right IRs of Tn*5422* and flanking regions. Inverted PCR was performed with primers P1 and P2 for the left side of the transposon and with primers P3 and P4 for the right side of the transposon. Left and right inverted PCR products were sequenced with the P2 or P4 primer, respectively. (B) Junction sequences of terminal left and right IRs of plasmids pLm74, pLm27, pLm71, pLm101, pLm40, pLm173, pLm176, pLm106, and pLm141. Identical sequences are indicated by boxes of the same type. The 6-bp direct repeat of pLm141 is underlined. (C) Restriction map of the related plasmids pLm74, pLm27, and pLm71. Regions that are conserved among these plasmids are indicated by identical boxes. Restriction sites: ○, *Eco*RI; ●, *Bgl*II; ▮, *Xba*I. Arrows indicate the location of Tn*5422*. The open box contains six *Eco*RI, seven *Xba*I, and four *Bgl*II restriction endonuclease sites (not shown).

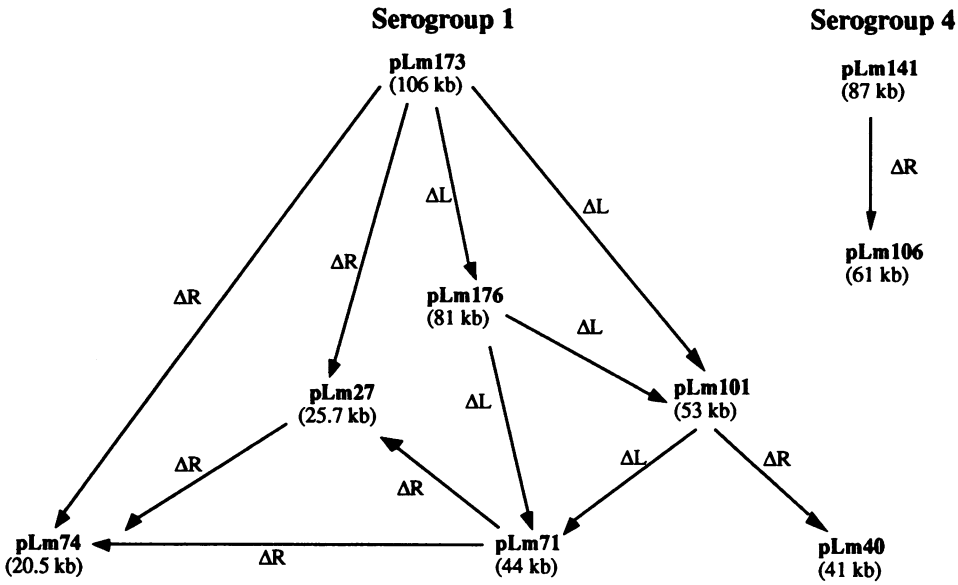


FIG. 10. Phylogeny of *L. monocytogenes* plasmids after putative intramolecular replicative transposition of Tn*5422*. Two independent groups are shown: one group includes pLm74, pLm27, pLm40, pLm71, pLm101, pLm176, and pLm173 (listed in order by increasing size), and the second group includes plasmids pLm106 and pLm141. Deletions at the left and right side of the end of the transposon are represented by ΔL and ΔR, respectively.

second molecule will be lost. The remaining plasmid appears to have suffered a deletion adjacent to the transposon. Intramolecular transposition does result in target duplication. However, the two copies of the target segregate with different copies of the transposon (each transposon copy keeps one parental junction and gets one of the new target junctions). Intramolecular replicative transposition is the best explanation for the absence of direct repeats at the extremity of Tn*5422* in the majority of *L. monocytogenes* plasmids.

Successive intramolecular transpositions of Tn*5422* would generate plasmids of decreasing size with different restriction maps, depending on the site of insertion. The size diversity of *L. monocytogenes* cadmium resistance plasmids (27) is consistent with intramolecular replicative transposition of Tn*5422*. Phylogenetic trees of *L. monocytogenes* cadmium resistance plasmids can be constructed (Fig. 10). Two distinct groups of plasmids emerge. The first includes plasmids pLm74, pLm27, pLm40, pLm71, pLm101, pLm173, and pLm176, all extracted from *L. monocytogenes* strains of serotype 1. The second comprises pLm106 and pLm141 from serotype 4 strains. Plasmid pLm141 may be the ancestral plasmid of this second group of related plasmids, because it has a duplication of the target sequence, whereas pLm176 was presumably derived by intramolecular transposition from an ancestral plasmid already containing the transposon, since its IRs are not flanked by direct repeats. There is no evidence for a common ancestor for the two groups. This classification is consistent with a study of the diversity of *L. monocytogenes* plasmids (27), which showed that plasmids from serogroup 1 and serogroup 4 *L. monocytogenes* strains were different.

Target site of the cadmium resistance transposon. The target sequence is that recognized by the transposon for its insertion. The majority of transposons generate characteristic short direct repeats of DNA sequence where they insert (6).

Only one Tn*5422*-containing plasmid, pLm141, was flanked by a direct repeat. The length of the duplication was 6 bp, which is unusual since all other transposons of the Tn*3* family create 5-bp duplications of DNA at their insertion sites. However, since this 6-bp duplication was observed only once, further analysis is required to determine whether the target duplication is indeed 6 bp.

Transposons show variable specificities of insertion. Some members of the Tn*3* family appear to prefer A+T-rich sequences or sites similar to the ends of the transposon (6). Others, like Tn*554*, are site specific (25). The target specificity of Tn*5422* was evaluated by comparing the sequences flanking the transposon. The nine different plasmids had nine different sequences adjacent to the transposon, each corresponding to an insertion site (Fig. 9B). Tn*5422* therefore does not appear to have any target specificity.

In conclusion, this study is the first detailed report of a natural transposon in *L. monocytogenes*. Tn*5422* is closely related to Tn*917*, a transposon of *E. faecalis* that confers erythromycin resistance (39). A previous study of *L. monocytogenes* reported that the tetracycline resistance gene *tet* (M) is probably carried by mobile genetic elements similar to Tn*1545*, a conjugative transposon of *E. faecalis* (34). Thus, natural isolates of *L. monocytogenes* may contain various different types of transposon.

Cadmium is used extensively for a variety of industrial applications, which has led to contamination of soil, water, plants, the food chain, animals, and humans. In *L. monocytogenes*, the existence of Tn*5422*, a transposon conferring cadmium resistance, is favorable for the dissemination of cadmium resistance among the *L. monocytogenes* population and presumably provides a relative advantage for survival in the environment.

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