ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2005, p. 1708–1713 Vol. 49, No. 5
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.5.1708–1713.2005

Genetic Environment and Expression of the Extended-Spectrum β-Lactamase bla_{PER-1} Gene in Gram-Negative Bacteria

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Received 22 October 2004/Returned for modification 6 January 2005/Accepted 3 February 2005

The genetic location of the gene coding for the expanded-spectrum β-lactamase PER-1 was analyzed in a series of gram-negative isolates. It was identified as part of a composite transposon bracketed by two novel insertion elements, IS*Pa12* and IS*Pa13*, belonging to the IS*4* family that possess transposases that share 63% amino acid identity and that are chromosomally located in *Pseudomonas aeruginosa*, *Providencia stuartii*, and *Acinetobacter baumannii*. On the contrary, the bla_{PER-1} gene was identified just downstream of an IS*Pa12* element but not within a composite transposon when it was located on a plasmid in *Salmonella enterica* serovar Typhimurium and *A. baumannii* isolates. In both cases, expression of the bla_{PER-1} gene was driven by promoter sequences located in IS*Pa12*.

The extended-spectrum β-lactamase (ESBL) PER-1 was first detected in 1993 in a *Pseudomonas aeruginosa* isolate from a Turkish patient in France (18). This enzyme is weakly related to the other ESBLs and confers resistance to penicillins, cefotaxime, ceftibuten, ceftazidime, and the monobactam aztreonam but spares resistance to carbapenems and cephamycins. Its activity is inhibited by clavulanic acid (17, 18). The bla_{PER-1} gene is widespread in *Acinetobacter* spp., *P. aeruginosa*, and *Salmonella enterica* serovar Typhimurium in Turkey (29, 30) and has also been detected in *Providencia rettgeri* in that country (1). In addition, PER-1 has been identified in *Acinetobacter* sp. isolates in Korea (31) but has rarely been identified in France (24). In Italy, PER-1 was detected in *P. aeruginosa*, *Alcaligenes faecalis*, and *Proteus mirabilis* isolates (19–21).

The β-lactamase PER-2, which shares 86% amino acid identity with PER-1, was first identified from an *S. enterica* serovar Typhimurium isolate from Argentina in 1996 (2) and then in other gram-negative bacteria, including *S. enterica* serovar Senftenberg (27), *Klebsiella pneumoniae* (15, 26), *Enterobacter aerogenes* (26), *Enterobacter cloacae* (26), and *Vibrio cholerae* (22), in that country.

Whereas the biochemical properties and clinical implications of PER-1-expressing strains have been detailed, the genetic background of the bla_{PER-1} gene remains unknown. Its location either on the chromosome or on the plasmid has been reported (7, 18). The aim of the present study was to characterize the genetic structures that may explain bla_{PER-1} gene acquisition in distantly related gram-negative species.

MATERIALS AND METHODS

Bacterial isolates. Five *P. aeruginosa*, two *Acinetobacter baumannii*, two *S. enterica* serovar Typhimurium, and one *P. stuartii* isolates were included in this study; all of them produced PER-1 (Table 1). They had been identified by using the API 20E and API 32GN systems (bioMérieux, Marcy-l'Etoile, France). Electrocompetent *Escherichia coli* DH10B (GIBCO BRL, Life Technologies, Cergy Pontoise, France) was used as the recipient strain in transformation experiments.

PCR amplification for detection of PER-1-encoding gene, class 1 integrons, and sequencing. Under standard PCR conditions (28), primers PER-A and PER-D (Table 2) were used for detection of the bla_{PER-1} gene. For each reaction, 0.5 μg of whole-cell DNA of the clinical isolates was used. The primers used to detect class 1 integrons were located in the 5′ conserved sequence (5′-CS) and in the 3′-CS regions (primers 5′-CS and 3′-CS [Table 2] [12]). A combination of primer 5′-CS or 3′-CS and a primer specific for the bla_{PER-1} gene was also used to identify the location of bla_{PER-1} in the integron. The extension step was increased to 6 min, and the amount of *Taq* polymerase was increased to 6 units per reaction mixture to amplify large DNA fragments. Sequencing of both strands was performed with an automated sequencer (ABI 3100; Applied Biosystems, Foster City, Calif.). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

PFGE. Pulsed-field gel electrophoresis (PFGE) analysis was done according to the instructions of the manufacturer (Bio-Rad, Ivry-sur-Seine, France). The XbaI, ApaI, and SpeI restriction enzymes (Amersham Pharmacia Biotech, Orsay, France) were used to genotype the *S. enterica* serovar Typhimurium, *A. baumannii*, and *P. aeruginosa* isolates, respectively, as described previously (19, 24).

Hybridizations. DNA-DNA hybridizations of the PFGE gels were performed by the Southern technique, as described previously (28). The probe consisted of a 927-bp PCR fragment internal to bla_{PER-1} generated from *P. aeruginosa* RNL-1 DNA (17). Labeling of the probe and signal detection were carried out with a nonradioactive labeling and detection kit, according to the instructions of the manufacturer (Amersham Pharmacia Biotech).

Electroporation and plasmid DNA content analysis. Plasmid DNA of the bacterial isolates was extracted by the method of Kieser (11). They were electroporated into *E. coli* DH10B, and recombinant strains were selected on ceftazidime-containing (2 μg/ml) Trypticase soy agar plates.

To search for a chromosomal location of the β-lactamase gene, we used the endonuclease I-CeuI (New England Biolabs, Ozyme), which digests a 26-bp sequence in the *rrn* genes for the 23S large-subunit rRNA (13), and separated the fragments by PFGE. Hybridization was performed with two different probes: a 1,504-bp PCR-generated probe specific for the 16S and 23S rRNA genes (9) and a 927-bp probe specific for the bla_{PER-1} gene, as indicated above.

TAIL-PCR. Three successive amplification reactions were performed with nested primers complementary to known sequences and arbitrary degenerated primers that hybridize to adjacent sequences, as described previously (14). The primers used to obtain the sequences upstream of the bla_{PER-1} gene were

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TABLE 1. PER-1-positive bacterial strains used in this study

Strain	Geographical origin	Genetic support of bla_{PER-1}	IS(s) near bla_{PER-1}	Source or reference
P. aeruginosa RNL-1	Garches, France (Turkish patient)	Chromosome	IS*Pa12*, IS*Pa13*	18
P. aeruginosa MUL	Mulhouse, France	Chromosome	IS*Pa12*, IS*Pa13*	This study
P. aeruginosa 1	Istanbul, Turkey	Chromosome	IS*Pa12*, IS*Pa13*	Gift from C. Bal
P. aeruginosa PER12	Ghent, Belgium	Chromosome	IS*Pa12*, IS*Pa13*	6
P. aeruginosa 2622	Warsaw, Poland	Chromosome	IS*Pa12*, IS*Pa13*	Gift from M. Gniadkowski
A. baumannii AMA-1	Kremlin-Bicêtre, France	Chromosome	IS*Pa12*, IS*Pa13*	24
A. baumannii C.A.	Ankara, Turkey	Plasmid	IS*Pa12*	24
Serovar Typhimurium TUR	Ankara, Turkey	Plasmid	IS*Pa12*	4
Serovar Typhimurium 147	Istanbul, Turkey	Plasmid	IS*Pa12*	This study
P. stuartii BEN	Kremlin-Bicêtre, France	Chromosome	IS*Pa12*, IS*Pa13*, IS*Prst1*	This study

TP.5′ext.1, TP.5′ext.2, and TP.5′ext.3 (Table 2). The products obtained by thermal asymmetric interlaced PCR (TAIL-PCR) were sequenced and analyzed as described above.

Determination of the transcription initiation sites by 5′-RACE. Total RNA was isolated from *P. aeruginosa* RNL-1 and *S. enterica* serovar Typhimurium 147 isolates with an Rneasy Midi kit (QIAGEN, Courtaboeuf, France), using the recommendations of the manufacturer. The 5′ rapid amplification of cDNA ends (5′-RACE) reactions were performed with 5 μg of total RNA of *P. aeruginosa* RNL-1 and *S. enterica* serovar Typhimurium TUR and a 5′-RACE system kit (Version 2.0; Invitrogen), following the recommendations of the manufacturer. The first-strand synthesis was primed with the specific primer TP.5′ext.1, and amplification of the target cDNA was performed with the dC-tailed cDNAs as templates by using the newly described primers TP.5′ext.2 and TP.5′ext.3 (Table 2).

Nucleotide sequence accession number. The nucleotide sequences of the insertion sequences (ISs) reported in this paper have been submitted to the IS Finder Website (http://www-is.biotoul.fr). They have been also deposited in the EMBL/GenBank nucleotide sequence database and have been assigned accession no. AY779042.

RESULTS

Preliminary PCR detection of the bla_{PER-1} β-lactamase gene, MICs, and genotyping. The 10 bla_{PER-1}-positive isolates screened by PCR and included in this study were of different geographical origins (Table 1). They belonged to four different gram-negative species (Table 1). The MICs of β-lactams for the isolates are indicated in Table 3. PFGE analysis with whole-cell DNA of *S. enterica* serovar Typhimurium, *P. aeruginosa*, and *A. baumannii* isolates showed that isolates of a given species were not clonally related (data not shown).

Chromosomal or plasmid location of the bla_{PER-1} gene. Transfer of the ceftazidime resistance marker by transformation and extraction of plasmid DNAs suggested that the bla_{PER-1} gene was chromosomally located in *P. aeruginosa* and *P. stuartii.* On the other hand, isolates *S. enterica* serovar Typhimurium 147

TABLE 2. Primers used in this study

Primer name	Experiment	Nucleotide sequence (5′–3′)	Location	Position[a]
PER.A	PCR	ATGAATGTCATTATAAAAGC	bla_{PER-1}	1
PER.D	PCR	AATTTGGGCTTAGGGCAGAA	bla_{PER-1}	2
PER.extA	PCR and cloning	CCTGCAACAGTACCTGCTTG	IS*Pa*12	3
PER.extD	PCR and cloning	AACTGATAAGGTTGCCCTGC	*gst*	4
PER.5′ext.1	TAIL-PCR and 5′-RACE	ACTGCAACGCCTACAGTGGC	bla_{PER-1}	5
PER.5′ext.2	TAIL-PCR and 5′-RACE	TGACTATGGATTCAATTTGCTC	bla_{PER-1}	6
PER.5′ext.3	TAIL-PCR and 5′-RACE	GGATTGCGCTGAGGTTTCG	bla_{PER-1}	7
ISPa12.B	PCR	GATCTCGCTTTACATTTACC	*tnpA* of IS*Pa*12	8
ISPa12.A	PCR and TAIL-PCR	ACAATCGCTGATATACATCG	*tnpA* of IS*Pa*12	9
ISPa13.B	PCR and TAIL-PCR	GGTATCCACCACATATGGGC	*tnpA* of IS*Pa*13	10
ISPa13.A	PCR	TAACCATATGCACTCAACGG	*tnpA* of IS*Pa*13	11
ISPa14.A	PCR	AATCAAATGTCCAACCTGCC	*tnpA* of IS*Pa*14	12
ISPa14.B	PCR	GCCTAATTCGATGCCTTAT	*tnpA* of IS*Pa*14	13
ISPrst1.A	PCR	ATTTCTGGAACTTTAACGAC	*tnpA* of IS*Prst*1	14
ISPrst1.B	PCR	GACAGTCATTTTTTCAAGGC	*tnpA* of IS*Prst*1	15
5′-CS	PCR	GGCATCCAAGCAGCAAG	5′-CS, class 1 integron	
3′-CS	PCR	AAGCAGACTTGACCTGA	3′-CS, class 1 integron	
AAP	5′-RACE	GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG[b]		
AUAP	5′-RACE	GGCCACGCGTCGACTAGTAC		

[a] Position of the primers indicated in Fig. 1.
[b] I, deoxyinosine.

TABLE 3. MICs of β-lactams for the PER-1-producing *P. aeruginosa* RNL-1, MUL-2, 1, PER12, and 2622 clinical isolates; the PER-1-producing *A. baumannii* AMA-1 and C.A. clinical isolates; the *S. enterica* serovar Typhimurium TUR and 147 clinical isolates; the *E. coli* DH10B strain harboring natural plasmid pSTI1 from *S. enterica* serovar Typhimurium 147 expressing PER-1; and the *E. coli* DH10B reference strain

β-Lactam(s)[a]	MIC (μg/ml)											
	P. aeruginosa RNL-1	*P. aeruginosa* MUL-2	*P. aeruginosa* 1	*P. aeruginosa* PER 12	*P. aeruginosa* 2622	*A. baumannii* AMA-1	*A. baumannii* C.A.	*P. stuartii* BEN	*S. enterica* serovar Typhimurium TUR	*S. enterica* serovar Typhimurium 147	*E. coli* DH10B (pST11)	*E. coli* DH10B
Amoxicillin	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	4
Amoxicillin + CLA	>512	>512	>512	>512	>512	32	8	512	32	32	2	4
Ticarcillin	512	>512	512	>512	512	>512	>512	512	>512	>512	512	4
Ticarcillin + CLA	256	128	512	>512	128	128	256	16	128	64	1	4
Piperacillin	8	16	16	64	16	64	512	16	256	256	16	1
Piperacillin + TZB	8	16	16	64	16	64	256	16	64	64	2	1
Cefuroxime	>512	>512	>512	>512	>512	>512	512	256	512	512	512	2
Ceftazidime	256	512	128	512	512	>512	512	512	512	512	512	0.06
Cefotaxime	256	>512	256	>512	>512	>512	>512	32	256	512	128	0.12
Cefepime	32	64	16	512	32	512	512	2	128	256	8	0.06
Aztreonam	256	512	256	512	512	512	>512	16	128	256	512	0.12
Imipenem	1	0.5	32	64	32	0.5	0.25	2	0.12	0.25	0.12	0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

and TUR harbored bla_{PER-1} on different plasmids (100 and 120 kb, respectively). The chromosomal location of bla_{PER-1} in isolate *P. aeruginosa* RNL-1 was confirmed by using the endonuclease I-CeuI technique, which gave a hybridization signal corresponding to a fragment of 900 kb (data not shown). Transformation and plasmid extraction failed to provide evidence of any plasmid in isolate *A. baumannii* AMA-1, whereas a slight plasmid band (>250 kb) was detected in *A. baumannii* C.A. Further analysis by the I-CeuI technique revealed that bla_{PER-1} was chromosomally encoded in isolate *A. baumannii* AMA-1 (size of the hybridized band, 500 kb) and was plasmid borne in isolate *A. baumannii* C.A. (data not shown). PCR experiments failed to identify a class 1 integron location of the bla_{PER-1} gene in these isolates.

Identification of sequences surrounding bla_{PER-1}. By using whole-cell DNAs of *P. aeruginosa* RNL-1 and *S. enterica* serovar Typhimurium TUR as templates, TAIL-PCR experiments allowed the identification of the upstream and the downstream regions flanking the bla_{PER-1} gene. Sequence analysis of the regions flanking the 5′ end of the bla_{PER-1} gene revealed that a novel IS element, IS*Pa12*, was present upstream of bla_{PER-1} in all the strains studied. Thirteen base pairs separated the left inverted repeat (IRL) of IS*Pa12* from the bla_{PER-1} start codon in the five *P. aeruginosa* isolates, *A. baumannii* AMA-1, and *P. stuartii* BEN. This distance was longer in *A. baumannii* C.A. and in the two *S. enterica* serovar Typhimurium isolates, since an additional 44-bp fragment was present, indicating that the IS*Pa12* insertion occurred at different sites upstream of bla_{PER-1}. The latter insertion site was, in fact, detected only in isolates in which bla_{PER-1} was plasmid borne. The nucleotide sequence identified upstream of the bla_{PER-1} gene in the *P. aeruginosa* isolates was identical to that found in a fragment recently characterized in bla_{PER-1}-positive isolate *P. aeruginosa* Ps101PAT from Italy (19), which also contained at least part of an identical IS*Pa12* element. It was also similar to the sequence located upstream of the bla_{PER-2} gene available in the GenBank database, likely indicating that IS*Pa12* is associated with this bla_{PER}-like gene. In that case, the IS*Pa12*-like element was located 128 bp upstream of the bla_{PER-2} gene.

The chromosomally encoded bla_{PER-1} gene is part of composite transposon Tn*1213*. Analysis of the regions located downstream revealed that bla_{PER-1} is bracketed by another IS element in the *P. aeruginosa* isolates and in *A. baumannii* AMA-1 (Fig. 1). IS*Pa13* was structurally related to IS*Pa12* (see below). The IS*Pa13* element was located 462 bp downstream of the bla_{PER-1} stop codon. Together with IS*Pa12*, IS*Pa13* might form a composite transposon, since a duplication of the insertion site was noticed at the left-hand extremity of IS*Pa12* and at the right-hand extremity of IS*Pa13* (Fig. 1A). This transposon was named Tn*1213*, and the direct repeat (DR) sequence generated by its insertion was 8 bp (Fig. 1A). The IS*Pa12* and IS*Pa13* elements were inserted in direct orientations like transposase genes and were transcribed in the same orientation. Thus, the IRL of Tn*1213* corresponded to the right inverted repeat (IRR) of IS*Pa12*, whereas the IRR of Tn*1213* corresponded to the IRL of IS*Pa13* (Fig. 1A). Since the sequences of the inverted repeats (IRs) or IS*Pa12* and IS*Pa13* (see below) were almost identical, Tn*1213* possessed almost perfectly complementary IRs. The 462-bp sequence separating the bla_{PER-1} gene from the IS*Pa13* element contained a gene (*gst*) that had been truncated by the IS*Pa13* insertion, and the corresponding protein shared 31 and 30% amino acid identities with the glutathione-*S*-transferases of *E. coli* and *Ralstonia solanacearum*, respectively.

The Tn*1213* sequence in *P. stuartii* BEN was also identified; but it contained an additional element, IS*Prst1*, a novel IS that had been inserted just downstream of the bla_{PER-1} gene, which truncated the glutathione-*S*-transferase gene (Fig. 1B). Since a target site sequence duplication was evidenced in both extrem-

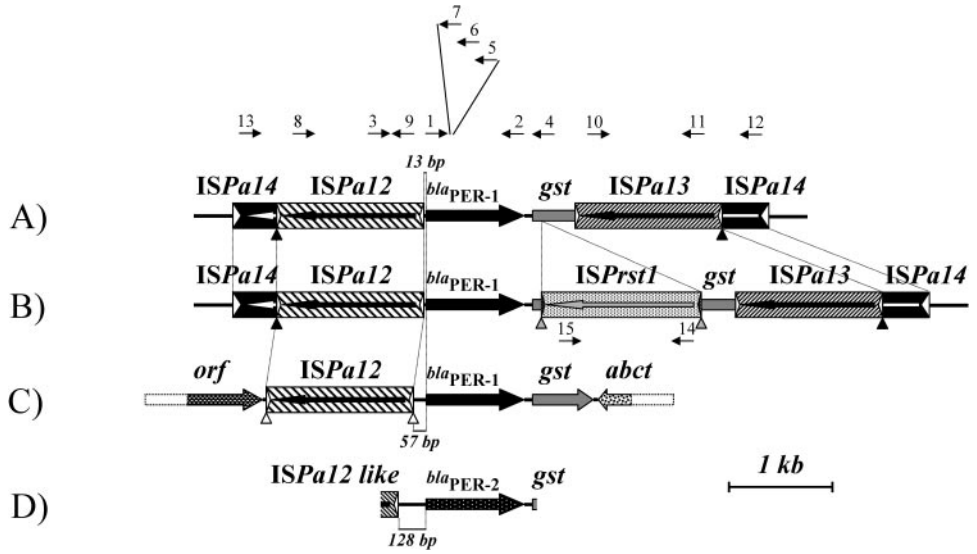

FIG. 1. Schematic map of the bla_{PER-1}-containing structures identified in *P. aeruginosa* and *A. baumannii* AMA-1 isolates (A), *P. stuartii* BEN (B), and two *S. enterica* serovar Typhimurium isolates and *A. baumannii* C.A. (C). The in silico-identified structure in a PER-2-producing *S. enterica* serovar Typhimurium isolate (GenBank accession no. X93314) is indicated for comparison (D). The genes and their corresponding transcriptional orientations are indicated by horizontal arrows. The DR sequences generated by transposition events are indicated by white triangles for IS*Pa12*, grey triangles for IS*Prst1*, and black triangles for composite transposon Tn*1213*. The glutathione-*S*-transferase gene (*gst*) and the ABC-type multidrug transporter gene (*abct*) are also indicated. Arrows indicate the primers used in this study, as indicated in Table 2.

ities of IS*Prst1* (see above), it is likely that transposition of this element has occurred in the Tn*1213* transposon backbone.

Analysis of Tn*1213*-surrounding sequences in *P. aeruginosa* isolates, *A. baumannii* AMA-1, and *P. stuartii* BEN revealed that the transposition of Tn*1213* had occurred inside an IS element named IS*Pa14* by interrupting its transposase gene (Fig. 1B). This IS element was very similar to IS*Aba3*, which belongs to the IS*1* family and which was recently identified in an *A. baumannii* clinical isolate (25). Their transposases shared 95% amino acid identity over 145 amino acids, but the transposase of IS*Pa14* was much longer (233 amino acids).

The bla_{PER-1} gene is not always part of Tn*1213*. Analysis of the IS*Pa12* insertion sites revealed a target site duplication (see above) in the two *S. enterica* serovar Typhimurium isolates and in isolate *A. baumannii* C.A., in which the bla_{PER-1} gene was plasmid located (Fig. 1C). This suggests that transposition of IS*Pa12* occurs independently of bla_{PER-1} gene acquisition. Analysis of the 600-bp IS*Pa12* sequence located upstream revealed an open reading frame encoding a putative RNase E that shared 30% identity with that of *P. aeruginosa* PAO1. PCRs failed to identify IS*Pa13* in these isolates, confirming the absence of a Tn*1213* structure. The glutathione-*S*-transferase-encoding gene identified in part in Tn*1213* was also present and was not truncated; it encoded a putative protein 191 amino acids long. Another gene (*abct*) encoding a 105-amino-acid protein was identified downstream of this gene. It shared 90 and 87% identities with the ABC-type proteins for multidrug transporters of *Shewanella oneidensis* and *Vibrio cholerae*, respectively (Fig. 1).

IS*Pa12*, IS*Pa13*, and IS*Prst1* belong to the IS*4* family. IS*Pa12* is 1,387 bp long, and its transposase shares 43% identity with that of IS*H8E* from a *Halobacterium* sp. (16). The IRs of IS*Pa12* are likely 11 bp, and it is noteworthy that they are able to form a hairpin structure (Fig. 2). Transposition of IS*Pa12* generated an 8-bp duplication at its insertion site in *S. enterica* serovar Typhimurium isolates, whereas no DR sequence bracketing this element was evidenced in *P. aeruginosa*. These structural features defined IS*Pa12* as a member of the IS*4* family, which is considered a heterogeneous family, since it gathers distantly related IS elements (5).

Similarly, IS*Pa13* is also 1,387 bp and is a member of the IS*4* family. Its transposase shares 63% amino acid identity with that of IS*Pa12*. The IRs of IS*Pa13* are 12 bp and have a single mismatch. The IRR sequence of IS*Pa13* was identical to that of IS*Pa12* when 11 of the 12 bp of their extremities was taken in account. Thus, IS*Pa12* and IS*Pa13* are structurally related and are able to form a composite transposon.

IS*Prst1* is 1,512 bp, and its transposase shares 54% amino acid identity with that of IS*50R*, which is also a member of the IS*4* family (10). Its IRs are likely 21 bp (with three mismatches in the middle of the sequence). The IS*Prst1* insertion generated a 9-bp duplication inside the glutathione-*S*-transferase-like enzyme-encoding gene located just downstream of bla_{PER-1} in the *P. stuartii* isolate.

IS*Pa12* enhances bla_{PER-1} expression. By using the 5′-RACE PCR technique, the sites of the initiation of transcription of the bla_{PER-1} gene were mapped in isolates *P. aeruginosa* RNL-1 and *S. enterica* serovar Typhimurium 147. The +1 transcription start was located 112 bp upstream of the start codon of the bla_{PER-1} gene in *P. aeruginosa* (Fig. 2). Upstream of this transcriptional start site, a −35 sequence (TTCAAA) separated by 17 bp from a −10 sequence (TAATCT) constituted a P_{Pa} promoter (Fig. 2). These promoter sequences as well as the +1 signal were part of the IS*Pa12* element. In *S. enterica* serovar Typhimurium, the nucleotide sequence of the 5′ RACE PCR product showed that the +1 transcription start site was different, since it was located 45 bp upstream of the

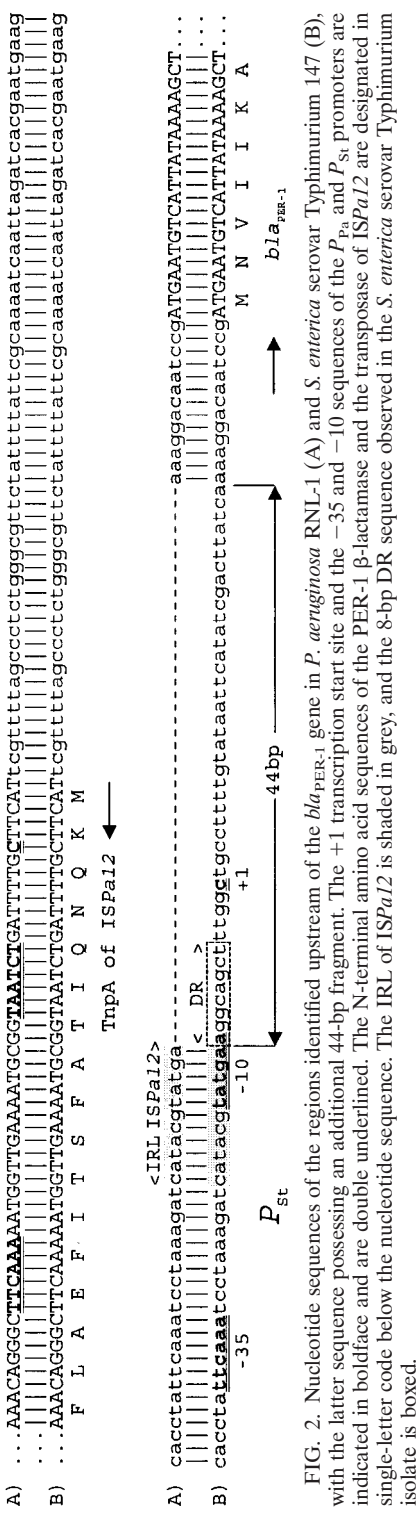

FIG. 2. Nucleotide sequences of the regions identified upstream of the *bla*$_{PER-1}$ gene in *P. aeruginosa* RNL-1 (A) and *S. enterica* serovar Typhimurium 147 (B), with the latter sequence possessing an additional 44-bp fragment. The +1 transcription start site and the −35 and −10 sequences of the P_{Pa} and P_{St} promoters are indicated in boldface and are double underlined. The N-terminal amino acid sequences of the PER-1 β-lactamase and the transposase of IS*Pa12* are designated in single-letter code below the nucleotide sequence. The IRL of IS*Pa12* is shaded in grey, and the 8-bp DR sequence observed in the *S. enterica* serovar Typhimurium isolate is boxed.

start codon of *bla*$_{PER-1}$ (Fig. 2). Upstream of this transcriptional start site, a −35 sequence (TTCAAA) separated by 16 bp from a −10 sequence (TAAGAA) formed another P_{St} promoter (Fig. 2). In that case, the −35 promoter sequence belonged to IS*Pa12*, the −10 motif overlapped the IRL of IS*Pa12*, and the +1 signal was part of the sequence flanking the 5′ end of the *bla*$_{PER-1}$ gene, which was absent in *P. aeruginosa*.

DISCUSSION

This work characterized the genetic bases of the acquisition of the β-lactamase PER-1-encoding gene and its expression. Unlike other non-TEM, non-SHV ESBL genes, such as those that code for VEB- or GES-type β-lactamases, the *bla*$_{PER-1}$ gene was not identified as a gene cassette and was not located inside a class 1 integron. However, it was part of a transposon-related structure. *bla*$_{PER-1}$-containing transposon Tn*1213* was detected in several species from distinct geographical origins.

This report is the first description of a β-lactamase-mediated composite transposon composed of two different IS elements, both of which belong to the IS*4* family. As is known to be the case for composite transposons, it is possible that only a single transposase (likely that of IS*Pa12*) might be functional in the IS*Pa12*/IS*Pa13* transposon, thus preventing overproduction of the transposase. Further in vitro mobilization experiments will be necessary to assess this hypothesis.

Interestingly, the sequences surrounding the *bla*$_{PER-1}$ gene may be different from one species to another in a given country. In addition, comparison of the structures of the sequences surrounding *bla*$_{PER-1}$ in the variety of these isolates may indicate the subsequent events: (i) insertion of IS*Pa12* upstream of *bla*$_{PER-1}$ (Fig. 1C); (ii) followed by insertion of IS*Pa13* downstream of *bla*$_{PER-1}$, which forms transposon Tn*1213* (Fig. 1A); and finally, (iii) insertion of IS*Prst1* inside Tn*1213* (Fig. 1B).

Since the location of composite transposon Tn*1213* was always inside the IS*Pa14* element, these results suggest that Tn*1213* was not acquired separately in the strains studied but was likely associated with a larger structure, such as a transposon. This association with IS*Pa14*, which is very similar to IS*Aba3* identified in *A. baumannii* isolates recovered from several countries (personal data), might suggest that the entire structure may have been acquired first in *A. baumannii* and then in *P. aeruginosa*.

IS*Pa12* drove the expression of the *bla*$_{PER-1}$ gene in *S. enterica* serovar Typhimurium and *P. aeruginosa* by bringing promoter sequences. Interestingly, these promoter sequences are different in these two species; and the +1 transcription initiation start site was located 45 bp upstream of the start codon of *bla*$_{PER-1}$ in *S. enterica* serovar Typhimurium, whereas it was located 112 bp upstream of the start codon of *bla*$_{PER-1}$ in *P. aeruginosa*. Although the −35 promoter sequences were identical in both isolates, the sequences of their −10 regions differed.

Since recombinant plasmids expressing the *bla*$_{PER-1}$ gene in *E. coli* were difficult to obtain, it is likely that the hairpin structure provided by the IRL of IS*Pa12* upstream of *bla*$_{PER-1}$ may limit the cloning efficiency. However, it is likely that high-level expression of the *bla*$_{PER-1}$ gene in vivo may necessitate the presence of IS*Pa12*. Identification of a progenitor that

weakly expresses bla_{PER-1} may be difficult, as has been observed for bla_{CTX-M} genes originating from β-lactam-susceptible *Kluyvera* species (8, 23). Identification of similar mobile structures upstream of the bla_{PER-1} and the bla_{PER-2} genes would indicate their common origin. This result was observed with CTX-M-type β-lactamase genes, in which an identical IS*Ecp1* element was identified upstream of different bla_{CTX-M} genes originating from distinct *Kluyvera* species (3). Finally, the association between IS*Pa12* and bla_{PER-1} adds to the diversity of IS elements that are sources of β-lactamase expression.

ACKNOWLEDGMENTS

This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (grant UPRES-EA3539), Université Paris XI, France, and by the European Community (6th PCRD, grant LSHM-CT-2003-503-335). L.P. is a researcher from INSERM, France.

We thank C. Bal and Z. Aktas for the gift of *P. aeruginosa* 1, G. Claeys for the gift of *P. aeruginosa* PER12, and M. Gniadkowski for the gift of *P. aeruginosa* 2622. We are indebted to M. F. Lartigue, who isolated the *P. stuartii* BEN strain. We also thank P. Séguier and M. Chandler for helpful discussions.

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