

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

Laurent Poirel,¹ Ludovic Cabanne,¹ Haluk Vahaboglu,² and Patrice Nordmann^{1*}

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, 94275 K. Bicêtre, France,¹ and Infectious Diseases & Clinical Microbiology Department, Kocaeli University Medical School, Kocaeli, Turkey²

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, ISPa12 and ISPa13, belonging to the IS4 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 ISPa12 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 ISPa12.

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'Étoile, 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 μ g 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 μ g/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 *rm* 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

* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.ap-hop-paris.fr.

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

Strain	Geographical origin	Genetic support of <i>bla</i> _{PER-1}	IS(s) near <i>bla</i> _{PER-1}	Source or reference
<i>P. aeruginosa</i> RNL-1	Garches, France (Turkish patient)	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	18
<i>P. aeruginosa</i> MUL	Mulhouse, France	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	This study
<i>P. aeruginosa</i> 1	Istanbul, Turkey	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	Gift from C. Bal
<i>P. aeruginosa</i> PER12	Ghent, Belgium	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	6
<i>P. aeruginosa</i> 2622	Warsaw, Poland	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	Gift from M. Gniadkowski
<i>A. baumannii</i> AMA-1	Kremlin-Bicêtre, France	Chromosome	<i>ISPa12</i> , <i>ISPa13</i>	24
<i>A. baumannii</i> C.A.	Ankara, Turkey	Plasmid	<i>ISPa12</i>	24
Serovar Typhimurium TUR	Ankara, Turkey	Plasmid	<i>ISPa12</i>	4
Serovar Typhimurium 147	Istanbul, Turkey	Plasmid	<i>ISPa12</i>	This study
<i>P. stuartii</i> BEN	Kremlin-Bicêtre, France	Chromosome	<i>ISPa12</i> , <i>ISPa13</i> , <i>ISPrst1</i>	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	ATGAATGTCATTATAAAAAGC	<i>bla</i> _{PER-1}	1
PER.D	PCR	AATTTGGGCTTAGGGCAGAA	<i>bla</i> _{PER-1}	2
PER.extA	PCR and cloning	CCTGCAACAGTACCTGCTTG	<i>ISPa12</i>	3
PER.extD	PCR and cloning	AACTGATAAGGTTGCCCTGC	<i>gst</i>	4
PER.5'ext.1	TAIL-PCR and 5'-RACE	ACTGCAACGCCTACAGTGGC	<i>bla</i> _{PER-1}	5
PER.5'ext.2	TAIL-PCR and 5'-RACE	TGACTATGGATTCAATTTGCTC	<i>bla</i> _{PER-1}	6
PER.5'ext.3	TAIL-PCR and 5'-RACE	GGATTGCGCTGAGGTTTCG	<i>bla</i> _{PER-1}	7
ISPa12.B	PCR	GATCTCGCTTTACATTTACC	<i>tnpA</i> of <i>ISPa12</i>	8
ISPa12.A	PCR and TAIL-PCR	ACAATCGCTGATATACATCG	<i>tnpA</i> of <i>ISPa12</i>	9
ISPa13.B	PCR and TAIL-PCR	GGTATCCACCACATATGGGC	<i>tnpA</i> of <i>ISPa13</i>	10
ISPa13.A	PCR	TAACCATATGCACTCAACGG	<i>tnpA</i> of <i>ISPa13</i>	11
ISPa14.A	PCR	AATCAAATGTCCAACCTGCC	<i>tnpA</i> of <i>ISPa14</i>	12
ISPa14.B	PCR	GCCTAATTCGATGCCTTAT	<i>tnpA</i> of <i>ISPa14</i>	13
ISPrst1.A	PCR	ATTTCTGGAACCTTTAACGAC	<i>tnpA</i> of <i>ISPrst1</i>	14
ISPrst1.B	PCR	GACAGTCATTTTTTCAAGGC	<i>tnpA</i> of <i>ISPrst1</i>	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 pST11 from *S. enterica* serovar Typhimurium 147 expressing PER-1; and the *E. coli* DH10B reference strain

β -Lactam(s) ^a	MIC (μ g/ml)											
	<i>P. aeruginosa</i> RNL-1	<i>P. aeruginosa</i> MUL-2	<i>P. aeruginosa</i> 1	<i>P. aeruginosa</i> PER 12	<i>P. aeruginosa</i> 2622	<i>A. baumannii</i> AMA-1	<i>A. baumannii</i> C.A.	<i>P. stuartii</i> BEN	<i>S. enterica</i> serovar Typhimurium TUR	<i>S. enterica</i> serovar Typhimurium 147	<i>E. coli</i> DH10B (pST11)	<i>E. coli</i> 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 μ g/ml; TZB, tazobactam at a fixed concentration of 4 μ g/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, *ISPa12*, was present upstream of *bla*_{PER-1} in all the strains studied. Thirteen base pairs separated the left inverted repeat (IRL) of *ISPa12* 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 *ISPa12* 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 *ISPa12* element. It was also similar to the sequence

located upstream of the *bla*_{PER-2} gene available in the GenBank database, likely indicating that *ISPa12* is associated with this *bla*_{PER}-like gene. In that case, the *ISPa12*-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 Tn1213. 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). *ISPa13* was structurally related to *ISPa12* (see below). The *ISPa13* element was located 462 bp downstream of the *bla*_{PER-1} stop codon. Together with *ISPa12*, *ISPa13* might form a composite transposon, since a duplication of the insertion site was noticed at the left-hand extremity of *ISPa12* and at the right-hand extremity of *ISPa13* (Fig. 1A). This transposon was named Tn1213, and the direct repeat (DR) sequence generated by its insertion was 8 bp (Fig. 1A). The *ISPa12* and *ISPa13* elements were inserted in direct orientations like transposase genes and were transcribed in the same orientation. Thus, the IRL of Tn1213 corresponded to the right inverted repeat (IRR) of *ISPa12*, whereas the IRR of Tn1213 corresponded to the IRL of *ISPa13* (Fig. 1A). Since the sequences of the inverted repeats (IRs) or *ISPa12* and *ISPa13* (see below) were almost identical, Tn1213 possessed almost perfectly complementary IRs. The 462-bp sequence separating the *bla*_{PER-1} gene from the *ISPa13* element contained a gene (*gst*) that had been truncated by the *ISPa13* insertion, and the corresponding protein shared 31 and 30% amino acid identities with the glutathione-S-transferases of *E. coli* and *Ralstonia solanaceae*, respectively.

The Tn1213 sequence in *P. stuartii* BEN was also identified; but it contained an additional element, *ISPrst1*, 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-

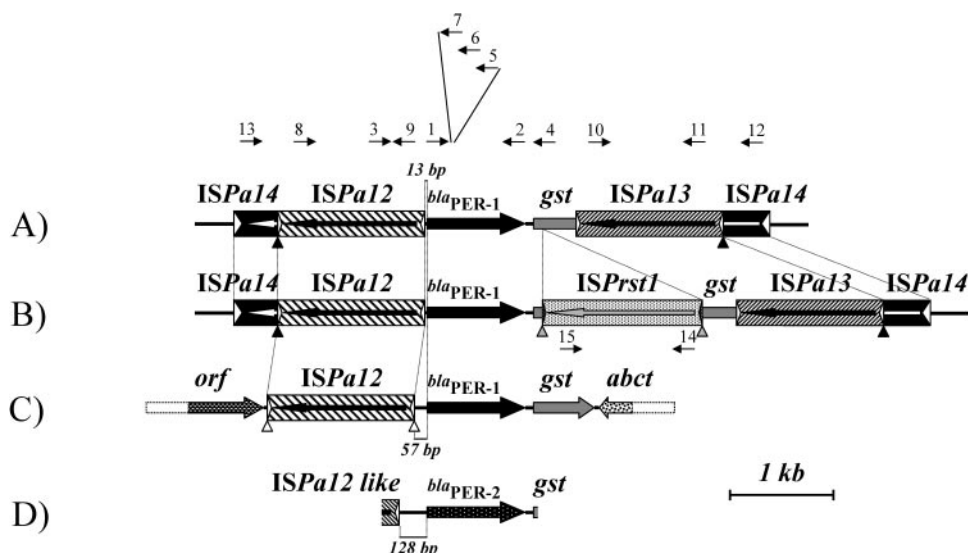


FIG. 1. Schematic map of the *bla*_{PER-1}-containing structures identified in *P. aeruginosa* and *A. baumannii* AMA-1 isolates (A), *P. stuartii* BEN (B), and two *S. enterica* serovar Typhimurium isolates and *A. baumannii* C.A. (C). The in silico-identified structure in a PER-2-producing *S. enterica* 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 ISPa12, grey triangles for ISPrst1, and black triangles for composite transposon Tn1213. 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 ISPrst1 (see above), it is likely that transposition of this element has occurred in the Tn1213 transposon backbone.

Analysis of Tn1213-surrounding sequences in *P. aeruginosa* isolates, *A. baumannii* AMA-1, and *P. stuartii* BEN revealed that the transposition of Tn1213 had occurred inside an IS element named ISPa14 by interrupting its transposase gene (Fig. 1B). This IS element was very similar to ISAb3, which belongs to the IS1 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 ISPa14 was much longer (233 amino acids).

The *bla*_{PER-1} gene is not always part of Tn1213. Analysis of the ISPa12 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 ISPa12 occurs independently of *bla*_{PER-1} gene acquisition. Analysis of the 600-bp ISPa12 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 ISPa13 in these isolates, confirming the absence of a Tn1213 structure. The glutathione-S-transferase-encoding gene identified in part in Tn1213 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).

ISPa12, ISPa13, and ISPrst1 belong to the IS4 family. ISPa12 is 1,387 bp long, and its transposase shares 43% identity with that of ISH8E from a *Halobacterium* sp. (16). The IRs of ISPa12 are likely 11 bp, and it is noteworthy that they are

able to form a hairpin structure (Fig. 2). Transposition of ISPa12 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 ISPa12 as a member of the IS4 family, which is considered a heterogeneous family, since it gathers distantly related IS elements (5).

Similarly, ISPa13 is also 1,387 bp and is a member of the IS4 family. Its transposase shares 63% amino acid identity with that of ISPa12. The IRs of ISPa13 are 12 bp and have a single mismatch. The IRR sequence of ISPa13 was identical to that of ISPa12 when 11 of the 12 bp of their extremities was taken in account. Thus, ISPa12 and ISPa13 are structurally related and are able to form a composite transposon.

ISPrst1 is 1,512 bp, and its transposase shares 54% amino acid identity with that of IS50R, which is also a member of the IS4 family (10). Its IRs are likely 21 bp (with three mismatches in the middle of the sequence). The ISPrst1 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.

ISPa12 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 (TTCAA) 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 ISPa12 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

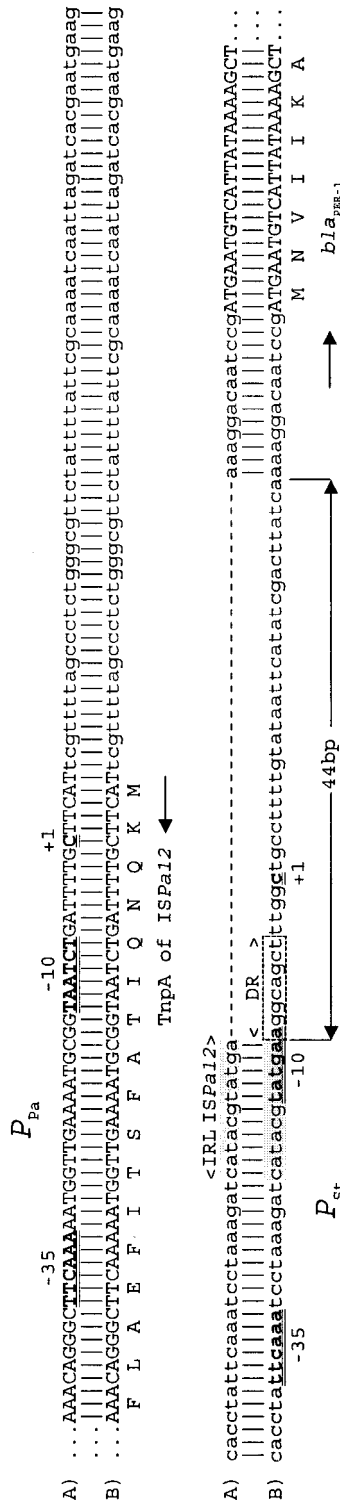


FIG. 2. Nucleotide sequences of the regions identified upstream of the *bla*_{PER-1} gene in *P. aeruginosa* RNL-1 (A) and *S. enterica* serovar Typhimurium 147 (B), with the latter sequence possessing an additional 44-bp fragment. The +1 transcription start site and the -35 and -10 sequences of the *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 ISPa12 are designated in single-letter code below the nucleotide sequence. The IRL of ISPa12 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 ISPa12, the -10 motif overlapped the IRL of ISPa12, 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 Tn1213 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 IS4 family. As is known to be the case for composite transposons, it is possible that only a single transposase (likely that of ISPa12) might be functional in the ISPa12/ISPa13 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 ISPa12 upstream of *bla*_{PER-1} (Fig. 1C); (ii) followed by insertion of ISPa13 downstream of *bla*_{PER-1}, which forms transposon Tn1213 (Fig. 1A); and finally, (iii) insertion of ISPrst1 inside Tn1213 (Fig. 1B).

Since the location of composite transposon Tn1213 was always inside the ISPa14 element, these results suggest that Tn1213 was not acquired separately in the strains studied but was likely associated with a larger structure, such as a transposon. This association with ISPa14, which is very similar to ISAba3 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*.

ISPa12 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 ISPa12 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 ISPa12. 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 *ISEc1* element was identified upstream of different *bla*_{CTX-M} genes originating from distinct *Kluyvera* species (3). Finally, the association between *ISPa12* 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'Éducation Nationale et de la Recherche (grant UPRES-EA3539), Université Paris XI, France, and by the European Community (6th PCRD, grant LSM-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.

REFERENCES

- Bahar, G., B. Erac, A. Mert, and Z. Gulay. 2004. PER-1 production in a urinary isolate of *Providencia rettgeri*. *J. Chemother.* **16**:343–346.
- Bauernfeind, A., I. Stemplinger, R. Jungwirth, P. Mangold, S. Amann, E. Akalin, O. Ang, C. Bal, and J. M. Casellas. 1996. Characterization of β -lactamase gene *bla*_{PER-2}, which encodes an extended-spectrum class A β -lactamase. *Antimicrob. Agents Chemother.* **40**:616–620.
- Bonnet, R. 2004. Growing group of extended-spectrum β -lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother.* **48**:1–14.
- Casin, L., B. Hanau-Berçot, I. Podglajen, H. Vahaboglu, and E. Collatz. 2003. *Salmonella enterica* serovar Typhimurium *bla*_{PER-1}-carrying plasmid pST11 encodes an extended-spectrum aminoglycoside 6'-N-acetyltransferase of type Ib. *Antimicrob. Agents Chemother.* **47**:697–703.
- Chandler, M., and J. Mahillon. 2002. Insertion sequences revisited, p. 305–366. In N. L. Craig et al. (ed.), *Mobile DNA II*. ASM Press, Washington, D.C.
- Claeys, G., G. Verschraegen, T. de Baere, and M. Vaneechoutte. 2000. PER-1 β -lactamase-producing *Pseudomonas aeruginosa* in an intensive care unit. *J. Antimicrob. Chemother.* **45**:924–925.
- Danel, F., L. M. Hall, D. Gür, H. E. Akalin, and D. M. Livermore. 1995. Transferable production of PER-1 β -lactamase in *Pseudomonas aeruginosa*. *J. Antimicrob. Chemother.* **35**:281–294.
- Decusser, J. W., L. Poirel, and P. Nordmann. 2001. Characterization of a chromosomally encoded extended-spectrum class A β -lactamase from *Kluyvera cryocrescens*. *Antimicrob. Agents Chemother.* **45**:3595–3598.
- Girlich, D., L. Poirel, A. Leelaporn, A. Karim, C. Tribuddharat, M. Fennewald, and P. Nordmann. 2001. Molecular epidemiology of the integron-located VEB-1 extended-spectrum β -lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *J. Clin. Microbiol.* **39**:175–182.
- Isberg, R. R., A. L. Lazaar, and M. Syvanen. 1982. Regulation of Tn5 by the right-repeat proteins: control at the level of the transposition reaction? *Cell* **30**:883–892.
- Kieser, T. 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* **12**:19–36.
- Lévesque, C., L. Piché, C. Larose, and P. H. Roy. 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob. Agents Chemother.* **39**:185–191.
- Liu, S. L., A. Hessel, and K. E. Sanderson. 1993. Genomic mapping with I-Ceu I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. *Proc. Natl. Acad. Sci. USA* **90**:6874–6878.
- Liu, Y. G., and R. F. Whittier. 1995. Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. *Genomics* **25**:674–681.
- Melano, R., A. Corso, A. Petroni, D. Centron, B. Orman, A. Pereyra, N. Moreno, and M. Galas. 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum β -lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. *J. Antimicrob. Chemother.* **52**:36–42.
- Ng, W. V., S. A. Ciuffo, T. M. Smith, R. E. Bumgarner, D. Baskin, J. Faust, B. Hall, C. Loretz, J. Seto, J. Slagel, L. Hood, and S. DasSarma. 1998. Snapshot of a large dynamic replicon in a halophilic archaeon: megaplasmid or minichromosome? *Genome Res.* **8**:1131–1141.
- Nordmann, P., and T. Naas. 1994. Sequence analysis of PER-1 extended-spectrum β -lactamase from *Pseudomonas aeruginosa* and comparison with class A β -lactamases. *Antimicrob. Agents Chemother.* **38**:104–114.
- Nordmann, P., E. Ronco, T. Naas, C. Duport, Y. Michel-Briand, and R. Labia. 1993. Characterization of a novel extended-spectrum β -lactamase from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **37**:962–969.
- Pagani, L., E. Mantengoli, R. Migliavacca, E. Nucleo, S. Pollini, M. Spalla, R. Daturi, E. Romero, and G. M. Rossolini. 2004. Multifocal detection of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β -lactamase in northern Italy. *J. Clin. Microbiol.* **42**:2523–2529.
- Pagani, L., R. Migliavacca, L. Pallecchi, C. Matti, E. Giacobone, G. Amicosante, E. Romero, and G. M. Rossolini. 2002. Emerging extended-spectrum β -lactamases in *Proteus mirabilis*. *J. Clin. Microbiol.* **40**:1549–1552.
- Pereira, M., M. Perilli, E. Mantengoli, F. Luzzaro, A. Toniolo, G. M. Rossolini, and G. Amicosante. 2000. PER-1 extended-spectrum β -lactamase production in an *Alcaligenes faecalis* clinical isolate resistant to expanded-spectrum cephalosporins and monobactams from a hospital in northern Italy. *Microb. Drug Resist.* **6**:85–90.
- Petroni, A., A. Corso, R. Melano, M. L. Cacace, A. M. Bru, A. Rossi, and M. Galas. 2002. Plasmidic extended-spectrum β -lactamases in *Vibrio cholerae* O1 El Tor isolates in Argentina. *Antimicrob. Agents Chemother.* **46**:1462–1468.
- Poirel, L., P. Kämpfer, and P. Nordmann. 2002. Chromosome-encoded Ambler class A β -lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **46**:4038–4040.
- Poirel, L., A. Karim, A. Mercat, I. Le Thomas, H. Vahaboglu, C. Richard, and P. Nordmann. 1999. Extended-spectrum β -lactamase-producing strain of *Acinetobacter baumannii* isolated from a patient in France. *J. Antimicrob. Chemother.* **43**:157–158.
- Poirel, L., S. Marqué, C. Héritier, C. Segonds, G. Chabanon, and P. Nordmann. 2005. OXA-58, a novel class D β -lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrob. Agents Chemother.* **49**:202–208.
- Quinteros, M., M. Radice, N. Gardella, M. M. Rodriguez, N. Costa, D. Korbenfeld, E. Couto, G. Gutkind, and the Microbiology Study Group. 2003. Extended-spectrum β -lactamases in *Enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. *Antimicrob. Agents Chemother.* **47**:2864–2867.
- Revathi, G., K. P. Shannon, P. D. Stapleton, B. K. Jain, and G. L. French. 1998. An outbreak of extended-spectrum, β -lactamase-producing *Salmonella senftenberg* in a burns ward. *J. Hosp. Infect.* **40**:295–302.
- Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Vahaboglu, H., S. Dodanlı, C. Eroglu, R. Ozturk, G. Soyletir, I. Yildirim, and V. Avkan. 1996. Characterization of multiple-antibiotic-resistant *Salmonella typhimurium* strains: molecular epidemiology of PER-1-producing isolates and evidence for nosocomial plasmid exchange by a clone. *J. Clin. Microbiol.* **34**:2942–2946.
- Vahaboglu, H., R. Ozturk, H. Akbal, F. Coskuncan, A. Yaman, A. Kaygusuz, H. Lecblebicioglu, I. Balik, K. Aydin, and M. Oktun. 1997. Widespread detection of PER-1-type extended-spectrum β -lactamase among nosocomial *Acinetobacter* and *Pseudomonas aeruginosa* isolates in Turkey: a nationwide multicenter study. *Antimicrob. Agents Chemother.* **41**:2265–2269.
- Yong, D., J. H. Shin, S. Kim, Y. Lim, J. H. Yum, K. Lee, Y. Chong, and A. Bauernfeind. 2003. High prevalence of PER-1 extended-spectrum β -lactamase-producing *Acinetobacter* spp. in Korea. *Antimicrob. Agents Chemother.* **47**:1749–1751.