



# Transposition of Tn1213 Encoding the PER-1 Extended-Spectrum $\beta$ -Lactamase

Stefano Mancini,<sup>a</sup> Laurent Poirel,<sup>a,b,c</sup> Nicolas Kieffer,<sup>a,b</sup> Patrice Nordmann<sup>a,b,c,d</sup>

<sup>a</sup>Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland

<sup>b</sup>INSERM European Unit (IAME, France), University of Fribourg, Fribourg, Switzerland

<sup>c</sup>Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland

<sup>d</sup>Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

**ABSTRACT** PER-1 is an extended-spectrum  $\beta$ -lactamase that is encoded by a gene located in composite transposon Tn1213 made by two distinct insertion sequences, namely, ISPa12 and ISPa13. *In vitro* mobilization performed in *Escherichia coli* shows that Tn1213 is functional and is able to mobilize the *bla*<sub>PER-1</sub> gene, although at a very low frequency (ca.  $1 \times 10^{-9}$ ).

**KEYWORDS** PER-1, extended-spectrum beta-lactamase, transposition

The PER-like enzymes are class A extended-spectrum  $\beta$ -lactamases (ESBL) that confer resistance to penicillins, oxyimino-cephalosporins, and aztreonam but not resistance to cephamycins and carbapenems. Their activity is inhibited *in vitro* by clavulanic acid and tazobactam (1). Since the first identification in 1993 of the *bla*<sub>PER-1</sub> gene on a plasmid in *Pseudomonas aeruginosa* (2), eight PER-like variants have been reported. Based on their sequence similarity to PER-1, they have been classified into two groups. The first group comprises PER-1 and derivatives (PER-3, PER-4, PER-5, PER-7, and PER-8) (3, 4), whereas the other is composed of PER-2 and PER-6, sharing 85% amino acid identity with PER-1 (5, 6). PER-coding genes have been identified in glucose-nonfermenting Gram-negative bacilli (2, 3, 7), in *Enterobacteriaceae* (5, 8–14), in *Aeromonas* spp. (6, 15, 16), in *Shewanella* spp. (17), and in *Vibrio cholerae* (18). While PER-1 is prevalent, in particular, in *P. aeruginosa*, in Asia and Europe, reports of PER-2 have so far been confined to South America (19). PER-3, PER-6, PER-7, and PER-8 have been identified only in sporadic cases (3, 4, 6, 20).

Previous studies have reported that the *bla*<sub>PER-1</sub> gene is carried on either a chromosome or a plasmid, being part of a composite transposon (Tn1213) bracketed by insertion sequences ISPa12 and ISPa13, which are two members of the IS4 insertion sequence family (21). Of note, the *bla*<sub>PER-2</sub> gene had been identified in *Citrobacter freundii* on a self-conjugative plasmid close to an ISPa12 element (14), suggesting a mobilization mechanism similar to that of the *bla*<sub>PER-1</sub> gene. Other studies recently reported the presence of the *bla*<sub>PER-1</sub>, *bla*<sub>PER-3</sub>, *bla*<sub>PER-4</sub>, *bla*<sub>PER-7</sub>, and *bla*<sub>PER-8</sub> genes in association with the ISCR1 element located inside a *sul1*-type integron structure (4, 22). While the role of ISCR1 in the mobilization of the *bla*<sub>PER</sub> genes has been studied (18, 22), the functionality of the composite transposon Tn1213 has not been hitherto investigated. The aim of this study was to investigate experimentally the mobility of composite transposon Tn1213 in *Escherichia coli* and elucidate its mode of action.

ISPa12 and ISPa13 elements are 1,265 and 1,271 bp long, respectively, and code for proteins that belong to the ISH8 group of the heterogeneous family of IS4 transposases. ISPa12 and ISPa13 exhibit 61% and 84% amino acid identity with a predicted transposase of the IS4 family of *Alishewanella* sp. strain WH16-1 (GenBank accession no.

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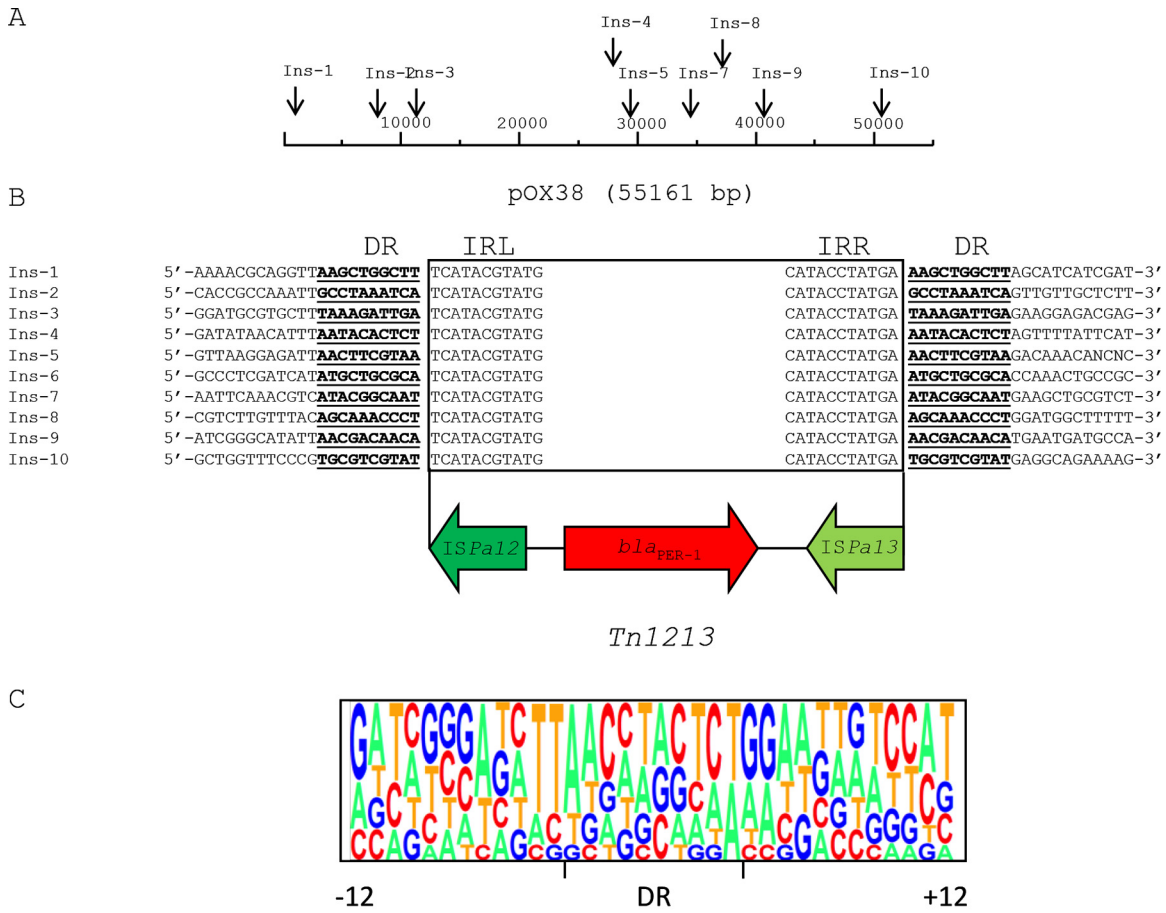
Address correspondence to Laurent Poirel, laurent.poirel@unifr.ch.

**TABLE 1** Strains and plasmids used in this study

Strain or plasmid	Specific feature(s)	Source or reference
Strains		
<i>E. coli</i> TOP10	F <sup>-</sup> <i>mcrA</i> Δ( <i>mrr hsdRMS mcrBC</i> ) φ80 <i>lacZ</i> Δ <i>M15</i> Δ <i>lacX74 nupG recA1 araD139</i> Δ( <i>ara-leu</i> )7697 <i>galE15 galK16 rpsL</i> (Str <sup>r</sup> ) <i>endA1</i> λ <sup>-α</sup>	Invitrogen
<i>E. coli</i> RZ211	Harboring pOX38	24
<i>E. coli</i> J53	Azide resistant; used for mating-out assays	24
<i>P. aeruginosa</i> MUL	IS <i>Pa12 bla</i> <sub>PER-1</sub> IS <i>Pa13</i>	21
Plasmids		
pACYC184	Chloramphenicol resistant, low-copy-no. plasmid	New England Biolabs
pOX38	F derivative, 55 kb, self-conjugative	25
pSM01	pACYC184 derivative containing IS <i>Pa12 bla</i> <sub>PER-1</sub> IS <i>Pa13</i>	This study

<sup>α</sup>Str<sup>r</sup>, streptomycin resistance.

WP\_057788381), respectively. Since their right inverted repeats (IRRs) are almost identical, IS*Pa12* and IS*Pa13* are structurally related and may conceivably form a composite transposon (21). The analysis of the Tn1213 regions of five *P. aeruginosa* and two *Acinetobacter baumannii* clinical isolates revealed the presence of an 8-bp direct repeat (DR) sequence flanking this putative composite transposon, which is likely the signature of a transposition process. Our aim was therefore to experimentally demonstrate the functionality of transposon Tn1213. For that purpose, the whole Tn1213 was PCR amplified from *P. aeruginosa* MUL (21) genomic DNA using primers PER-1-BamHI-fw (5'-ATATTAGGATCCGGCGTAAATCATACGTATGTC-3') and PER-1-Sall-rv (5'-ATATATGTCGACTTTACGCCTCATAGGTATGAT-3') (the underlined sequences represent the BamHI and Sall restriction sites, respectively). Then, the PCR product of 4,216 bp was cloned into low-copy-number plasmid pACYC184 using the BamHI and Sall restriction enzymes. (Strains and plasmids used in this study are listed in Table 1.) The resulting pSM01 recombinant plasmid was transformed into *E. coli* TOP10 (Invitrogen, Thermo Fisher Scientific, Écublens, Switzerland). Transformants were selected on Luria-Bertani (LB) agar plates containing 30 μg/ml of chloramphenicol and 100 μg/ml of ampicillin. Plasmid pSM01 was then transformed into the *E. coli* RZ211 strain carrying the self-conjugative, IS-free pOX38 plasmid that harbors the gentamicin resistance gene, which is used as a target for putative transposition events. Transformants were selected on LB agar plates containing 30 μg/ml of chloramphenicol, 100 μg/ml of ampicillin, and 10 μg/ml of gentamicin. Mating-out assays were performed to select events of Tn1213 transposition from the recombinant to the transfer-proficient pOX38 plasmid. To this end, *E. coli* strain RZ211 was used as a donor strain and azide-resistant *E. coli* strain J53 as a recipient strain. Briefly, overnight cultures of the donor and recipient strains were diluted 1:100 in LB and cultured at 37°C until the exponential phase was reached. Suspensions containing donor and recipient cells at a 1:10 ratio were inoculated on a sterile disk previously deposited on LB agar plates. After overnight incubation at 37°C, transconjugants were selected on plates containing 8 μg/ml of gentamicin, 100 μg/ml of ampicillin, and 100 μg/ml of sodium azide. Colonies were screened for chloramphenicol resistance to rule out the presence of spontaneous azide-resistant donor cells. The transposition frequency was assessed by dividing the number of transconjugants by the number of donor cells. The transposition assays were successful even though the transpositions occurred at low frequency ( $1.1 \times 10^{-9}$ ). Prior exposure of the donor cells to subinhibitory concentrations of ampicillin (100 μg/ml) or ciprofloxacin (0.005 μg/ml) or incubation at different temperatures (30°C and 42°C) had no effect on the transposition rates, which were 1.15, 1.3, 1.4, and  $1.15 \times 10^{-9}$ , respectively. The insertion sites of 10 transposition events were analyzed by using an inverse PCR strategy. Briefly, DNA extracted from 10 transconjugants using a GenElute Bacterial genomic kit (Sigma-Aldrich) was digested with the Sall restriction enzyme (Invitrogen). The digestion products were subsequently self-circularized by ligation and then used as the template for the inverse PCR using the IS*Pa12*-inv (5'-TAAATTGCCGGTGCACATCG-3') and IS*Pa13*-inv (5'-AGCCGAAACGTTGATTTGGG-3') outward primers. Sequencing of the PCR prod-



**FIG 1** Target site analysis of Tn1213. (A) Schematic representation of the positions of the integration sites of Tn1213 on the pOX38 plasmid. (B) Sequence alignment of 10 transposition events identified in pOX38. The right inverted repeat (IRR) and left inverted repeat (IRL) regions of Tn1213 are boxed. The 10-bp duplicated target site sequences are highlighted in bold. (C) Pictogram showing the relative frequencies of A, T, G, and C residues at the target site, deduced from 10 transposition events analyzed here and shown in panel B.

ucts showed that transposition had occurred in 10 different sites on the pOX38 recipient plasmid (Fig. 1). In contrast to what was previously observed with the putative Tn1213 of *P. aeruginosa* (2) and *Acinetobacter baumannii* (CP024576.1) or with individual insertion sequences of Tn1213 (KP054476.2 [CP017671.1] and MF150123.1), which were surrounded by an 8-bp DR, a 10-bp DR was systematically detected at each extremity of each transposed fragment. It is noteworthy that, consistent with the data obtained in this study, IS elements of the IS4 family group ISH8 have been reported to produce 10-bp duplicated regions upon insertion (23). Despite the fact that variability of the DR size may be expected for IS elements, the investigation of the mode of action of other members of this IS family may help to clarify these contrasting observations. It is noteworthy that *in silico* analysis of the insertion sites revealed that they were mostly preceded by TT nucleotide tandems and followed by GA nucleotide tandems, suggesting a target site-specific preference for the Tn1213 composite transposon.

Here we present evidence of the mobilization of the *bla*<sub>PER-1</sub> gene located in the previously described Tn1213 composite transposon. This work showed that Tn1213 is functional and therefore can still actively contribute to the further dissemination of the *bla*<sub>PER-1</sub> gene among clinically relevant Gram-negative species.

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