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# Transposition of Tn*1213* encoding the PER-1 Extended Spectrum β-lactamase

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Running title: In vitro mobilization of $bla_{PER-1}$

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ABSTRACT

PER-1 is an extended-spectrum β-lactamase that is encoded by a gene located into the composite transposon Tn*1213* made by two distinct insertion sequences, namely IS*Pa12* and IS*Pa13*. In vitro mobilization performed in *Escherichia coli* shows that Tn*1213* is functional and is able to mobilize the $bla_{PER-1}$ gene although at a very low frequency (ca $1x10^{-9}$).

PER-like enzymes are class A extended spectrum ß-lactamases (ESBL) that confer resistance to penicillins, oxymino-cephalosporins and aztreonam, but spare cephamycins and carbapenems. Their activity is inhibited in vitro by clavulanic acid and tazobactam (1). Since the first identification in 1993 of the *bla*$_{PER-1}$ 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 and 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 cholera* (18). While PER-1 is prevalent in Asia and Europe in particular in *P. aeruginosa*, reports of PER-2 have been so far confined in 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*$_{PER-1}$ gene is either chromosome or plasmid encoded being part of a composite transposon (Tn*1213*) bracketed by insertion sequences IS*Pa12* and IS*Pa13*, being two members of the IS*4* insertion sequence family (21). Of note, the *bla*$_{PER-2}$ gene had been identified in *C. freundii* on a self-conjugative plasmid close to an IS*Pa12* element (14), suggesting a similar mobilization mechanism of that of the *bla*$_{PER-1}$ gene. Other studies recently reported the *bla*$_{PER-1}$, *bla*$_{PER-3}$, *bla*$_{PER-4}$, *bla*$_{PER-7}$ and *bla*$_{PER-8}$ genes in association with the IS*CR1* element located inside a *sul1*-type integron structure (4, 22). While the role of IS*CR1* in the mobilization of the *bla*$_{PER}$ genes

has been studied (18, 22), the functionality of the composite transposon Tn*1213* has not been hitherto investigated. The aim of this study was to investigate experimentally the mobility of the composite transposon Tn*1213* in *E. coli* and elucidate its mode of action.

IS*Pa12* and IS*Pa13* elements are 1265- and 1271-bp long, respectively, and code for proteins that belong to the group IS*H8* of the heterogeneous family of IS*4* transposases. IS*Pa12* and IS*Pa13* exhibit 61% and 84% amino acid identity with a predicted transposase of the IS*4* family of *Alishewanella* sp . WH16-1 (ref. WP_057788381), respectively. Since their 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 Tn*1213* regions of five *P. aeruginosa* and two *A. baumannii* clinical isolates revealed the presence of an eight-bp direct repeat (DR) sequence flanking this putative composite transposon, which might likely be the signature of a transposition process. Our aim was therefore to experimentally demonstrate the functionality of transposon Tn*1213*. For that purpose, the whole Tn*1213* was PCR amplified from *P. aeruginosa* MUL (21) genomic DNA using primers PER-1-BamHI-fw (5'-ATATTAGGATCCGGCGTAAATCATACGTATGTC-3') and PER-1-SalI-rv (5'-ATATATGTCGACTTTACGCCTCATAGGTATGAT-3'). Then, the PCR product of 4,216 bp was cloned into the low-copy plasmid pACYC184 using the BamHI and SalI restriction enzymes. The resulting recombinant plasmid pSM01 was transformed into *E. coli* TOP10 (Invitrogen, Thermo Fisher Scientific, Ecublens, 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, was used as a target for putative transpositions 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 transposition events of Tn*1213* from the recombinant to the transfer proficient plasmid pOX38 plasmid. To this end, the *E. coli* strain RZ211 was used as a donor and the 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. 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 spontaneous azide-resistant donor cells. The transposition frequency was assessed by dividing the number of the transconjugants by the number of the donor cells. Transposition assays were successful even though they occurred at low frequency ($1.1 \times 10^{-9}$). Prior exposition of the donor cells to sub-inhibitory concentrations of ampicillin (100 µg/ml) or ciprofloxacin (0.005 µg/ml) or incubation at different temperatures (30°C or 42°C) had no effect on the transposition rate, which were of 1.15, 1.3, 1.4 and $1.15 \times 10^{-9}$, respectively. The insertion sites of ten transposition events were analyzed by using an inverse PCR strategy. Briefly, the DNA extracted from ten transconjugants using the GenElute Bacterial genomic kit (Sigma Aldrich) was digested with

the SalI restriction enzyme (Invitrogen). The digestion products were subsequently self-circularized by ligation and then used as 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 products showed that transposition had occurred in ten different sites on the recipient plasmid pOX38 (Figure 1). By contrast to what was previously observed with the putative Tn*1213* of *P. aeruginosa* (2), *Acinetobacter baumanni* (CP024576.1) or 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. Noteworthy, and consistent with the data obtained in this study, IS elements of the IS*4* family group IS*H8* have been reported to produce 10-bp duplicated regions upon insertion (23). Despite 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. Noteworthy, in-silico analysis of the insertion sites revealed that they were mostly preceded by TT and followed by GA nucleotide tandems, suggesting a target site specific preference for the composite transposon Tn*1213*.

Here we evidenced the mobilization of the $bla_{PER-1}$ gene located in the previously described composite transposon Tn*1213.* This work showed that Tn*1213* is functional and therefore can still actively contribute to the further dissemination of the $bla_{PER-1}$ gene among clinically relevant Gram-negative species.

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Table 1. Strains and plasmids used in this study

| Strains | Specific features | Origin |
|---|---|---|
| *E. coli* TOP10 | F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZ*ΔM15 Δ*lacX*74 *nup*G *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*E15 *gal*K16 *rps*L(Str$^R$) *end*A1 λ$^-$ | Invitrogen |
| *E. coli* RZ211 | Harboring pOX38 | (24) |
| *E. coli* J53 | Azide-resistant used for mating-out assays | (24) |
| *P. aeruginosa* MUL | IS*Pa12*-*bla*$_{PER-1}$-IS*Pa13* | (21) |
| **Plasmids** | | |
| pACYC184 | Chloramphenicol resistant, low-copy plasmid | New England Biolabs |
| pOX38 | F derivative, 55 kb, self-conjugative | (25) |
| pSM01 | pACYC184 derivative, containing IS*Pa12*-*bla*$_{PER-1}$-IS*Pa13* | This study |

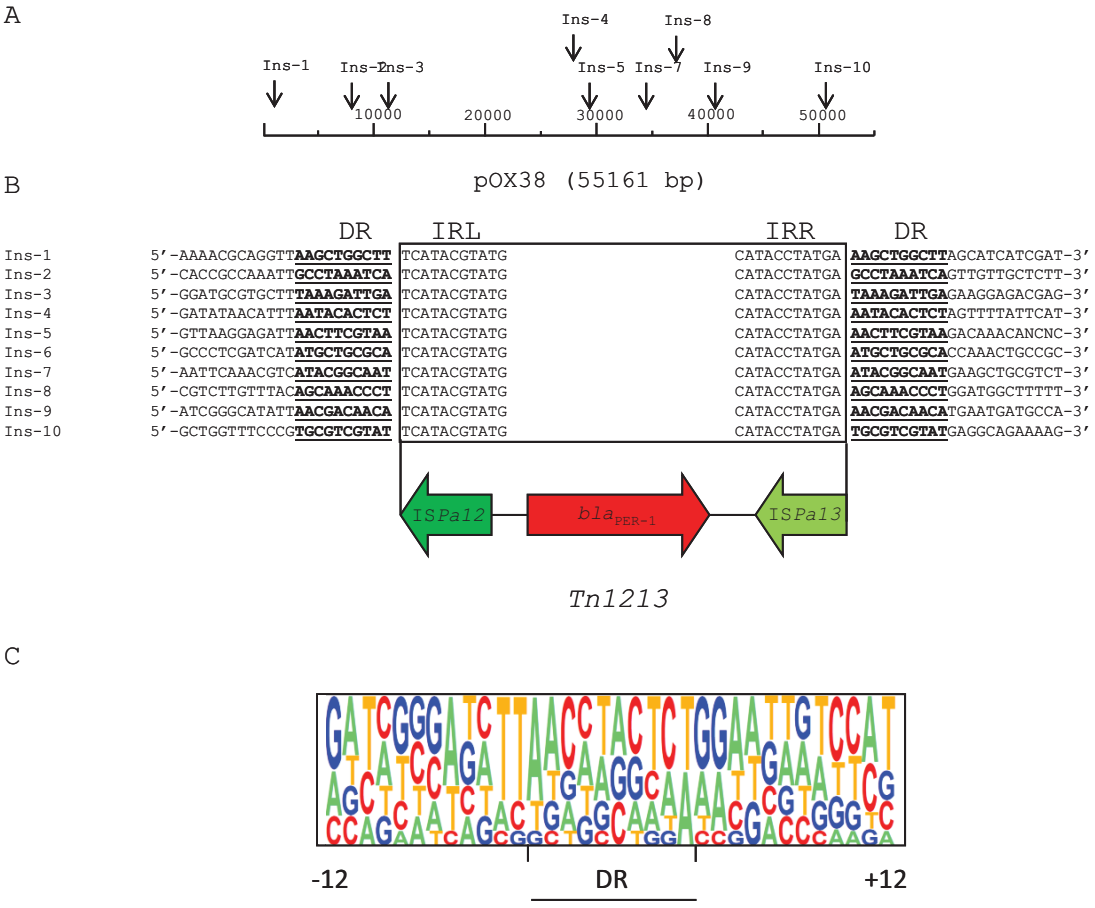


**Figure.** Target site analysis of Tn*1213*. (A) Schematic representation of the positions of the integration sites of Tn*1213* on the plasmid pOX38. (B) Sequence alignment of ten transposition events identified into pOX38. The IRR and IRL regions of Tn*1213* are boxed. The 10-bp duplicated target site sequences are highlighted in bold. (C) Pictogram showing the realtive frequency of A, T, G and C residues at the target site, deduced from ten transposition events analysed here and shown in panel B.