AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

MECHANISMS OF RESISTANCE

Transposition of Tn*1213* Encoding the PER-1 Extended-Spectrum β-Lactamase

Stefano Mancini,[a] Laurent Poirel,[a,b,c] Nicolas Kieffer,[a,b] Patrice Nordmann[a,b,c,d]

[a]Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland

[b]INSERM European Unit (IAME, France), University of Fribourg, Fribourg, Switzerland

[c]Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland

[d]Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

ABSTRACT PER-1 is an extended-spectrum β-lactamase that is encoded by a gene located in 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. 1×10^{-9}).

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

The PER-like enzymes are class A extended-spectrum β-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_{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, 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_{PER-1} gene is carried on either a chromosome or a plasmid, being part of a composite transposon (Tn*1213*) bracketed by insertion sequences IS*Pa12* and IS*Pa13*, which are two members of the IS*4* insertion sequence family (21). Of note, the bla_{PER-2} gene had been identified in *Citrobacter freundii* on a self-conjugative plasmid close to an IS*Pa12* element (14), suggesting a mobilization mechanism similar to that of the bla_{PER-1} gene. Other studies recently reported the presence of 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 composite transposon Tn*1213* in *Escherichia coli* and elucidate its mode of action.

IS*Pa12* and IS*Pa13* elements are 1,265 and 1,271 bp long, respectively, and code for proteins that belong to the IS*H8* group 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. strain WH16-1 (GenBank accession no.

Received 30 November 2017 **Returned for modification** 16 December 2017 **Accepted** 22 December 2017

Accepted manuscript posted online 8 January 2018

Citation Mancini S, Poirel L, Kieffer N, Nordmann P. 2018. Transposition of Tn*1213* encoding the PER-1 extended-spectrum β-lactamase. Antimicrob Agents Chemother 62:e02453-17. https://doi.org/10.1128/AAC.02453-17.

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		
E. coli TOP10	F$^-$ *mcrA* Δ(*mrr hsdRMS mcrBC*) ϕ80*lacZ*ΔM15 Δ*lacX74 nupG recA1 araD139* Δ(*ara-leu*)*7697 galE15 galK16 rpsL*(Str^r) *endA1* λ^{-a}	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-no. 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

[a]Str^r, 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 Tn*1213* 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 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′) (the underlined sequences represent the BamHI and SalI restriction sites, respectively). Then, the PCR product of 4,216 bp was cloned into low-copy-number plasmid pACYC184 using the BamHI and SalI 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 Tn*1213* 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 (1.1×10^{-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 1.15×10^{-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 SalI 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-

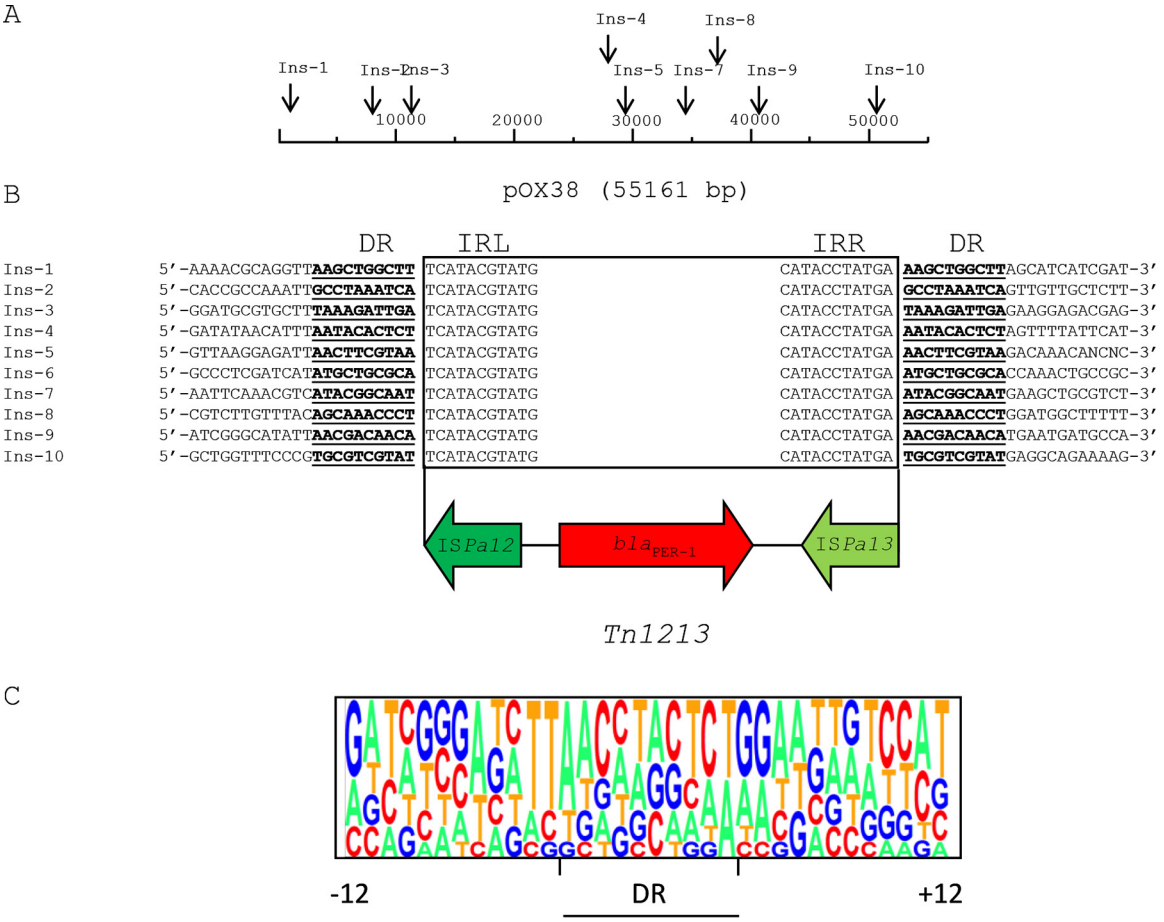

FIG 1 Target site analysis of Tn*1213*. (A) Schematic representation of the positions of the integration sites of Tn*1213* on the pOX38 plasmid. (B) Sequence alignment of 10 transposition events identified in pOX38. The right inverted repeat (IRR) and left inverted repeat (IRL) regions of Tn*1213* are 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 Tn*1213* 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 IS*4* family group IS*H8* 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 Tn*1213* composite transposon.

Here we present evidence of the mobilization of the bla_{PER-1} gene located in the previously described Tn*1213* composite transposon. 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.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation (project FNS-31003A_163432) and by the University of Fribourg.

REFERENCES

1. Potron A, Poirel L, Nordmann P. 2015. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. Int J Antimicrob Agents 45:568–585. https://doi.org/10.1016/j.ijantimicag.2015.03.001.
2. Nordmann P, Ronco E, Naas T, Duport C, Michel-Briand Y, Labia R. 1993. Characterization of a novel extended-spectrum ß-lactamase from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 37:962–969. https://doi.org/10.1128/AAC.37.5.962.
3. Bonnin RA, Potron A, Poirel L, Lecuyer H, Neri R, Nordmann P. 2011. PER-7, an extended-spectrum beta-lactamase with increased activity toward broad-spectrum cephalosporins in *Acinetobacter baumannii*. Antimicrob Agents Chemother 55:2424–2427. https://doi.org/10.1128/AAC.01795-10.
4. Tada T, Shrestha S, Shimada K, Ohara H, Sherchand JB, Pokhrel BM, Kirikae T. 2017. PER-8, a novel extended-spectrum-ß-lactamase PER variant, from an *Acinetobacter baumannii* clinical isolate in Nepal. Antimicrob Agents Chemother 61:e02300-16. https://doi.org/10.1128/AAC.02300-16.
5. Bauernfeind A, Stemplinger I, Jungwirth R, Mangold P, Amann S, Akalin E, Ang O, Bal C, Casellas JM. 1996. Characterization of ß-lactamase gene bla_{PER-2}, which encodes an extended-spectrum class A ß-lactamase. Antimicrob Agents Chemother 40:616–620.
6. Girlich D, Poirel L, Nordmann P. 2010. PER-6, an extended-spectrum ß-lactamase from *Aeromonas allosaccharophila*. Antimicrob Agents Chemother 54:1619–1622. https://doi.org/10.1128/AAC.01585-09.
7. Pereira M, Perilli M, Mantengoli E, Luzzaro F, Toniolo A, Rossolini GM, Amicosante G. 2000. PER-1 extended-spectrum ß-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. https://doi.org/10.1089/mdr.2000.6.85.
8. Paul D, Ingti B, Bhattacharjee D, Maurya AP, Dhar D, Chakravarty A, Bhattacharjee A. 2017. An unusual occurrence of plasmid-mediated bla_{OXA-23} carbapenemase in clinical isolates of *Escherichia coli* from India. Int J Antimicrob Agents 49:642–645. https://doi.org/10.1016/j.ijantimicag.2017.01.012.
9. Melano R, Corso A, Petroni A, Centron D, Orman B, Pereyra A, Moreno N, Galas M. 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum ß-lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. J Antimicrob Chemother 52:36–42. https://doi.org/10.1093/jac/dkg281.
10. Quinteros M, Radice M, Gardella N, Rodriguez MM, Costa N, Korbenfeld D, Couto E, Gutkind G; Microbiology Study Group. 2003. Extended-spectrum ß-lactamases in Enterobacteriaceae in Buenos Aires, Argentina, public hospitals. Antimicrob Agents Chemother 47:2864–2867. https://doi.org/10.1128/AAC.47.9.2864-2867.2003.
11. Pagani L, Migliavacca R, Pallecchi L, Matti C, Giacobone E, Amicosante G, Romero E, Rossolini GM. 2002. Emerging extended-spectrum ß-lactamases in *Proteus mirabilis*. J Clin Microbiol 40:1549–1552. https://doi.org/10.1128/JCM.40.4.1549-1552.2002.
12. Lahlaoui H, Ben Moussa M. 2014. Detection of PEr-1 extended-spectrum β-lactamase among nosocomial *Providencia stuartii* isolates in Tunisia. Tunis Med 92:258–261.
13. Bahar G, Erac B, Mert A, Gulay Z. 2004. PER-1 production in a urinary isolate of *Providencia rettgeri*. J Chemother 16:343–346. https://doi.org/10.1179/joc.2004.16.4.343.
14. Power P, Di Conza J, Rodriguez MM, Ghiglione B, Ayala JA, Casellas JM, Radice M, Gutkind G. 2007. Biochemical characterization of PER-2 and genetic environment of bla_{PER-2}. Antimicrob Agents Chemother 51:2359–2365. https://doi.org/10.1128/AAC.01395-06.
15. Picão RC, Poirel L, Demarta A, Petrini O, Corvaglia AR, Nordmann P. 2008. Expanded-spectrum ß-lactamase PER-1 in an environmental *Aeromonas media* isolate from Switzerland. Antimicrob Agents Chemother 52:3461–3462. https://doi.org/10.1128/AAC.00770-08.
16. Wu CJ, Chuang YC, Lee MF, Lee CC, Lee HC, Lee NY, Chang CM, Chen PL, Lin YT, Yan JJ, Ko WC. 2011. Bacteremia due to extended-spectrum-β-lactamase-producing *Aeromonas* spp. at a medical center in Southern Taiwan. Antimicrob Agents Chemother 55:5813–5818. https://doi.org/10.1128/AAC.00634-11.
17. Almuzara M, Montaña S, Lazzaro T, Uong S, Parmeciano Di Noto G, Traglia G, Bakai R, Centrón D, Iriarte A, Quiroga C, Ramirez MS. 2017. Genetic analysis of a PER-2 producing *Shewanella* spp. strain harboring a variety of mobile genetic elements and antibiotic resistant determinants. J Glob Antimicrob Resist 11:81–86. https://doi.org/10.1016/j.jgar.2017.06.005.
18. Wu J, Xie L, Zhang F, Ni Y, Sun J. 2015. Molecular characterization of IS*CR1*-mediated bla_{PER-1} in a non-O1, non-O139 *Vibrio cholerae* strain from China. Antimicrob Agents Chemother 59:4293–4295. https://doi.org/10.1128/AAC.00166-15.
19. Poirel L, Bonnin RA, Nordmann P. 2012. Genetic support and diversity of acquired extended-spectrum beta-lactamases in Gram-negative rods. Infect Genet Evol 12:883–893. https://doi.org/10.1016/j.meegid.2012.02.008.
20. Al-Hassan L, El Mahallawy H, Amyes SG. 2013. First report of bla_{PER-3} in *Acinetobacter baumannii*. Int J Antimicrob Agents 41:93–94. https://doi.org/10.1016/j.ijantimicag.2012.09.010.
21. Poirel L, Cabanne L, Vahaboglu H, Nordmann P. 2005. Genetic environment and expression of the extended-spectrum ß-lactamase bla_{PER-1} gene in gram-negative bacteria. Antimicrob Agents Chemother 49:1708–1713. https://doi.org/10.1128/AAC.49.5.1708-1713.2005.
22. Xie L, Wu J, Zhang F, Han L, Guo X, Ni Y, Sun J. 2016. Molecular epidemiology and genetic characteristics of various bla_{PER} genes in Shanghai, China. Antimicrob Agents Chemother 60:3849–3853. https://doi.org/10.1128/AAC.00258-16.
23. De Palmenaer D, Siguier P, Mahillon J. 2008. IS*4* family goes genomic. BMC Evol Biol 8:18. https://doi.org/10.1186/1471-2148-8-18.
24. Poirel L, Kieffer N, Nordmann P. 27 June 2017. In vitro study of ISA*pl1*-mediated mobilization of the colistin resistance gene *mcr-1*. Antimicrob Agents Chemother https://doi.org/10.1128/AAC.00127-17.
25. Derbyshire KM, Hwang L, Grindley ND. 1987. Genetic analysis of the interaction of the insertion sequence IS*903* transposase with its terminal inverted repeats. Proc Natl Acad Sci U S A 84:8049–8053. https://doi.org/10.1073/pnas.84.22.8049.