Complete Nucleotide Sequence of a Conjugative Plasmid Carrying *bla*_{PER-1}

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The nucleotide sequence of a self-transmissible plasmid pVPH1 harboring *bla*_{PER-1} from *Vibrio parahaemolyticus* was determined. pVPH1 was 183,730 bp in size and shared a backbone similar to pAQU1 and pAQU2, differing mainly in an ~40-kb multidrug resistance (MDR) region. A complex class 1 integron was identified together with *ISCR1* and *bla*_{PER-1} (*ISCR1-bla*_{PER-1}-*gst-abct-qacEΔ1-sul1*), which was shown to form a circular intermediate playing an important role in the dissemination of *bla*_{PER-1}.

The emergence of extended-spectrum β-lactamase (ESBL)-harboring pathogens is of particular concern due to the limited options for eradicating such organisms in clinical settings. As a unique class A ESBL, the *bla*_{PER-1} gene product confers resistance to penicillins, oxyimino-cephalosporins, and aztreonam but not to oxacillins, cephamycins, and carbapenems and is susceptible to inhibition by clavulanic acid and sulbactam (1). The *bla*_{PER-1} gene was first recognized in the *Pseudomonas aeruginosa* strain RNL-1, which was recovered in France in 1991 (2). After that, other closely related variants, such as PER-2, -3, -4, -5, -6, and -7, have been identified (3, 4). PER-1-type β-lactamases were confined to relatively few locations and bacterial hosts (*Aeromonas* spp., *Acinetobacter baumannii*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae*) (5, 6). The *bla*_{PER-1} gene has been found in plasmids or chromosomes in diverse genetic environments and is often embedded within Tn1213 (6, 7). Recently, the *ISCR1* element was found to play a role in the dissemination of *bla*_{PER-1} (6). In a previous study, we reported the detection of *bla*_{PER-1} in *Vibrio parahaemolyticus* strains from seafood in Hong Kong (8). In order to further delineate the genetic background and dissemination mechanism of the *bla*_{PER-1} gene, we obtained the complete nucle-

otide sequence of the plasmid pVPH1 harboring *bla*_{PER-1} and determined its mechanisms of transmission.

V. parahaemolyticus strain V36 was isolated from a shrimp sample in Hong Kong in 2010. This strain was resistant to extended-spectrum β-lactams, including ceftazidime and cefepime. *bla*_{PER-1} was detected and shown to be located on an ~175-kb plasmid by S1 pulsed-field gel electrophoresis (S1-PFGE) and Southern hybridization. The plasmid carrying *bla*_{PER-1} was conjugated into *Escherichia coli* J53 and selected on LB plates (sodium

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FIG 1 Plasmid sequence alignment of pVPH1, pAQU1, and pAQU2 by Mauve software. Upper panel, sequence of pVPH1 obtained in this study. The representation of each sequence from Mauve contains, from top to bottom, the local colinear blocks (LCBs), forward catalytic domains (CDs), reverse CDs, and annotated features. Lines are used to link similar LCBs, and a colored similarity plot is drawn for each sequence, the height of which reflects the level of sequence identity. The unique regions for pVPH1 are labeled in the upper part of the figure.

FIG 2 Comparative analysis of complex class 1 integron harboring *bla*_{PER-1} from different bacterial species. Symbols: arrows, annotated coding sequences; shaded quadrangles, consensus sequences among different organisms; arrows without gene names, hypothetical open reading frames.

azide 100 µg/ml; ceftazidime 4 µg/ml). The nucleotide sequence of the plasmid was determined by the whole-genome shotgun approach, utilizing the Illumina HiSeq 2000 sequencing platform according to the manufacturer's recommendation. The sequencing reads were assembled into contigs with Velvet (<http://www.ebi.ac.uk/~zerbino/velvet/>). Gaps were closed using a PCR-based strategy and Sanger sequencing. Gene predictions and annotations were conducted with the RAST (Rapid Annotation using Subsystem Technology) tool and modified manually by BLAST online. Sequence comparisons and alignment were performed by Mauve software.

The complete plasmid sequence of pVPH1 was 183,730 bp in size and had an average G+C content of 45.2%, which was similar to that of the chromosome of *V. parahaemolyticus* (45.1% to 45.6%). Annotation results revealed that pVPH1 harbored 114 predicted coding sequences; among them, several functional regions were identified. After conducting the BLASTN alignment search against the NCBI database, the sequence organization of pVPH1 was found to be similar to that of pAQU1 (72%) and pAQU2 (76%). Both pVPH1- and pAQU-type plasmids belong to a group of self-transmissible plasmids known as MOB_(H12), which is prevalent in *Enterobacteriaceae* and *Vibrio-*

naceae (9–11). Although they harbored similar backbone sequences, the multidrug resistance (MDR) regions that constituted the remaining parts of the plasmid were structurally diverse (Fig. 1). This may indicate that the different MDR regions can be disseminated in plasmids with similar backbone structure.

The MDR regions of pVPH1 are centralized in a region ~40 kb in size that is flanked by two different Tn3 family transposase genes. There were many different genetic mobile elements in this mosaic region, such as IS26, class 1 integron, ISCR1, IS4321, and IS6100. A mercuric resistance operon (*mer* operon) similar to that of plasmid pR148 from *Aeromonas hydrophila* was also found between the Tn3 and TniA transposase elements (12). Downstream of the mercuric resistance operon was a ParDE type II toxin-antitoxin system (13).

A class 1 integron with an *arr3-dfrA23* cassette flanked by IS26 and ISCR1 was also found, with *bla*_{PER-1} located downstream of ISCR1 in an area that included *gst* (encoding a glutathione S-transferase), *abct* (encoding an ABC-type transporter), and three hypothetical genes, followed by the *qacEΔ1* and *sul1* genes. A similar *bla*_{PER-1} genetic arrangement was described in a previous study (6) with slight differences, as shown in Fig. 2. ISCR1 was always found to be linked with a class 1 integron, which played an impor-

FIG 3 Circular intermediate generated by ISCR1 detected by reverse PCR. The 3'-CS area (*qacEΔ1-sul1*) of class 1 integron probably was the homologous region for the excision of the circular intermediate. *oriIS* and *terIS*, origin and termination sites of ISCR element, respectively.

tant role in the mobilization of various antibiotic resistance genes (14). Resembling IS91-like elements that can generate free circular intermediates, ISCR1-mediated resistant genes are presumably translocated via rolling-cycle transposition with the help of *oriIS* and *terIS* (6, 14, 15). The genetic arrangement around *bla*_{PER-1} can be divided into two major groups: the conventional *bla*_{PER-1}-*gst* structure surrounded by ISPa12 and ISPa13 of Tn1213 (7) and an ISCR1-*bla*_{PER-1}-*gst*-*abct* structure in a class 1 integron. The first group may be derived from the chromosome of *P. aeruginosa* RNL-1 (GenBank accession no. AY779042), where *bla*_{PER-1} was first detected (7, 16). It has been detected in different pathogens and was found to disseminate mainly in European countries. However, the second group was always found to be fused with class 1 integron and distributed only in China to date (6, 8, 17, 18). This phenomenon indicates that the dissemination of *bla*_{PER-1} among bacterial populations undergoes different pathways in different geographic sites. In order to verify if this ISCR1 element can generate a circular DNA segment (19), a pair of primers (C-F, TTCGGCTTGACTCGGCTGA; C-R, TGCAGTAGAGGGTGC TGTTG) were designed to amplify the hypothetical circular intermediate with the reverse PCR method (Fig. 3). The result showed that it can be circularized, as seen in Fig. 3. It was speculated that ISCR1 may act like IS91 to generate circular intermediates and mobilize genes immediately upstream of ISCR1 (20). However, the sequencing results showed that the ISCR1 element in this plasmid can form the circular intermediate for the downstream region of ISCR1 (ISCR1-*per1*-*gst*-*abct*-*qac*Δ1-*sul1*). Examination of the nucleotide sequence revealed that the recombination site that triggered the excision of the circular intermediate was probably the 3'-conserved sequence (CS) area (*qac*Δ1-*sul1*) of the class 1 integron (Fig. 3). The results also suggested that this type of the circular intermediate may actually mediate the formation of the complex class 1 integron.

In conclusion, we determined the first complete nucleotide sequence of a *bla*_{PER-1}-carrying plasmid from *V. parahaemolyticus* and revealed its transmission mechanisms mediated by mobile elements.

Nucleotide sequence accession number. The annotated complete sequence of pVPH1 has been submitted to the GenBank nucleotide sequence database under the accession number KP688397.

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We have no conflicts of interests to declare.

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