Short Communication

Genetic Environments of the Transferable Plasmid-Mediated *bla*_{CTX-M-3} Gene in *Serratia marcescens* Isolates

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SUMMARY: In this study, genetic environments of the transferable plasmid-mediated *bla*_{CTX-M-3} gene were characterized among 14 isolates of cefotaxime-resistant *Serratia marcescens* using PCR and BLAST DNA sequence analysis. A total of 3 types of genetic architectures in the regions surrounding this *bla*_{CTX-M-3} gene were identified. Type I architecture was characterized by the presence of a complete insertion sequence of *tnpA*-*ISEcp1*, identified as interrupting a reverse IS26 sequence in the upstream region of the *bla*_{CTX-M-3} gene. A reverse-directional *orf477* fragment was located downstream of the *bla*_{CTX-M-3} gene, which was in the same direction of the *mucA* gene. A common region containing the *orf513* element was located upstream of the *mucA* gene. Moreover, a copy of the 3'-CS2 element was located immediately upstream of the *orf513* element. A novel complex class 1 integron was characterized by the presence of the *dfrA19* gene, which was flanked by two copies of class 1 integrons. This is the first report to describe the *dfrA19* gene within a novel complex class 1 integron in *S. marcescens* isolates from Taiwan. This novel complex class 1 integron structure was located distantly upstream of the *bla*_{CTX-M-3} gene.

Human nosocomial infections caused by extended-spectrum β -lactamase (ESBL)-positive *Serratia marcescens* have become increasingly prominent in recent years (1). The CTX-M-3 enzyme is the most common ESBL type among *S. marcescens* isolates in Taiwan (2,3). Conjugative plasmids encoding CTX-M-3 ESBL from *Enterobacteriaceae*, but not *S. marcescens* isolates, have been previously described in Taiwan (4). In our previous study, the gene cassettes of class 1 integrons among CTX-M-3-producing *S. marcescens* isolates from southern Taiwan were described (5). However, we were unable to determine the localization of the *bla*_{CTX-M-3} gene in the surrounding region of the class 1 integron schemes. In this study, the genetic environments of the transferable plasmid-mediated *bla*_{CTX-M-3} gene in *S. marcescens* isolates were further characterized.

The genetic ISCR elements found within the 3'-CS region of class 1 integrons, which contribute to the formation of a novel complex of class 1 integrons, have been previously investigated (6). The *orf513* (*ISCR1*) element has been regarded as a novel gene-capturing system that mobilizes resistance genes (6). Complex class 1 integrons are involved in the development of multidrug resistances mediated by transferable plasmid DNA in clinical bacterial isolates (7). Many different alleles of *bla*_{CTX-M} genes associated with class 1 integrons containing *ISCR1* elements, which encode CTX-M-type β -lactamases and promote higher frequency of

*bla*_{CTX-M} gene mobilization among the *Enterobacteriaceae* isolates (8). The aim of the present study was to characterize the region surrounding the *bla*_{CTX-M-3} gene among the cefotaxime-resistant *S. marcescens* isolates from southern Taiwan. Novel complex class 1 integrons bearing *ISCR1* elements in *S. marcescens* isolates carrying the transferable plasmid-mediated *bla*_{CTX-M-3} gene were also investigated.

All cefotaxime-resistant *S. marcescens* specimens were isolated at Kaohsiung Medical University Hospital (Kaohsiung, Taiwan) from 2005 to July 2006. These isolates were identified using the ATB system (ID32GN strip; bioMérieux, Marcy l'Etoile, France). The transferable plasmid-mediated *bla*_{CTX-M-3} gene in the cefotaxime-resistant *S. marcescens* isolates was identified by the filter mating method with recipient rifampicin-resistant *Escherichia coli* MV 10 cells (*Lys*⁻, *His*⁻, *Trp*⁻, *Lac*⁻, *Rec A*⁻, *Rif*^R) (9). Detection of the CTX-M-3 β -lactamase gene was performed by PCR with genomic DNA (8,10). The primer sets, sizes of the expected PCR products, and annealing temperatures used for the PCR assay are listed in Table 1. Positive amplifications for the *bla*_{CTX-M-3} gene were obtained using primers MA and MB (Table 1). The genetic organization of the *bla*_{CTX-M-3} gene was also investigated using a series of primer pairs for PCR techniques, PCR cloning, and walking-DNA sequencing of the regions surrounding these genes. All PCR products were subjected to direct sequencing and analyzed using the BLAST and Clustal W programs (11).

The genetic environments of the transferable plasmid-mediated *bla*_{CTX-M-3} genes from the cefotaxime-resistant *S. marcescens* isolates are shown in Fig. 1. Three different architectures (Type I [2 isolates], Type II [7 isolates], and Type III [5 isolates]) were identified for the genetic environments of the plasmid-mediated *bla*_{CTX-M-3} genes.

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Table 1. Primers used for PCR amplification in this study

PCR target	Primer	Primer sequence	Accession no.
CTX-M group I	MA	5'-CGC TTT GCG ATG TGC AG-3'	X92506
CTX-M group I	MB	5'-ACC GCG ATA TCG TTG GT-3'	X92506
CTX-M-1	M1-F	5'-AAG GCG TTT TGA CAG ACT ATT C-3'	X92506
CTX-M-1	M1-R	5'-CAC CCG TTT CCG CTA TTA CA-3'	X92506
CTX-M-3	CTXM3 F	5'-GAA CCC ATG GTT AAA AAA TCA CTG C-3'	AF550415
CTX-M-3	CTXM3 R	5'-CCG CTA TTA CAA ACC GTC GGT GAC-3'	AF550415
ISEcp1	TnpA1 ISEcp1	5'-AAT ACT ACC TTG CTT TCT GA-3'	EF382672.1
ISEcp1	ISEcp1R	5'-CAA CCA CCT TTC AAT CAT TTT T-3'	AF550415
IS26	IS26-5'	5'-CAA AGT TAG CGA TGA GGC AG-3'	AF550415
IS26	tnpA IS26	5'-AGC GGT AAA TCG TGG AGT GA-3'	AM003906
<i>sul</i> I-	S1F	5'-TTCCGCTATTGGTCTCGGTGTC-3'	EU091084
<i>orf</i> 513	So513 R	5'-AAA CCA GCA TGG TTG GCT AC-3'	EU117158
<i>Orf477</i>	Orf477R	5'-ACT TCA AAA ATT ATG CCA CC-3'	EF382672.1
<i>Orf477</i>	orf477R	5'-ACT TCA AAA ATT ATG CCA CC-3'	AM003906
<i>mucA</i>	<i>mucA</i> lower	5'-GGC ATC AGG CAG GCG TAA GG-3'	AF550415
CTX-M3	CTX-M3 F	5'-GAA CCC ATG GTT AAA AAA TCA CTG C-3'	AF550415
<i>orf</i> 477	<i>orf</i> 477 R	5'-ACT TCA AAA ATT ATG CCA CC-3'	AF550415
CTX-M3	CTX-M3 F	5'-GAA CCC ATG GTT AAA AAA TCA CTG C-3'	AF550415
<i>muc</i> A	<i>muc</i> A R	5'-GGC ATC AGG CAG GCG TAA GG-3'	AF550415
<i>orf</i> 477	orf477R	5'-ACT TCA AAA ATT ATG CCA CC-3'	AM003906
<i>mucA</i>	<i>mucA</i> lower	5'-GGC ATC AGG CAG GCG TAA GG-3'	AF550415
<i>orf</i> 513	so 513 F	5'-GTA TTG CGC CGC TCT TAG AC-3'	EU117158
	so 513 R	5'-AAA CCA GCA TGG TTG GCT AC-3'	EU117158
<i>orf</i> 513	orf 513 F	GCATCTGAAACAGAAAACAGCCAAGGCGAATG	EU117158
	orf 513 R	TGGCGTTTCGTAACCGTTTGTGTTGAGTGG	EU117158
CTX-M3	CTX-M3 F	5'-GAA CCC ATG GTT AAA AAA TCA CTG C-3'	AF550415
<i>orf</i> 477	<i>orf</i> 477 R	5'-ACT TCA AAA ATT ATG CCA CC-3'	AF550415
<i>dfrA19</i>	<i>dfrA19</i> FA	5'-ATGAGTCACCCACAACCTGAGCTAA-3'	AJ310778
<i>dfrA19</i>	<i>dfrA19</i> RA	5'-TTACTGATTGTTACGCACGTAAGTA-3'	AJ310778

The Type II architecture was the most common and found in 7 (50%) of 14 *S. marcescens* isolates. The Type I architecture was characterized by the presence of a complete insertion sequence of *tnpA*-*ISEcp1*, identified as interrupting a reverse IS26 sequence in the upstream region of the *bla*_{CTX-M-3} gene. A reverse-directional *orf477* fragment was located downstream of the *bla*_{CTX-M-3} gene, which was in the same direction of the *mucA* gene. An *orf513*-containing common region was located upstream of the *mucA* gene. Moreover, a second copy of the 3'-CS (3'-CS2) was located immediately upstream of the *orf513* element. The genetic environments of Type II architecture had similarities to the Type I architecture with the exception of the deleted 3'-CS2 structure. Lastly, the Type III architecture of the *bla*_{CTX-M-3} gene genetic environment, with the exception of the *orf513* region, was identical to the Type II architecture.

Simultaneously, a novel complex class 1 integron structure was detected in all 14 cefotaxime-resistant *S. marcescens* isolates positive for the presence of the *bla*_{CTX-M-3} gene. However, the complex class 1 integron was found distantly upstream of the *bla*_{CTX-M-3} gene. A novel complex class 1 integron was characterized by the presence of two copies of class 1 integrons that were linked by the *dfrA19* gene. In addition, the *orf513* element was identified immediately downstream of each class 1 integron. Consequently, the novel complex class 1 integron was characterized by the presence of the

dfrA19 gene flanked by two copies of class 1 integrons (Fig. 1).

Although the plasmid-mediated *bla*_{CTX-M-3} gene was commonly found among the *S. marcescens* isolates, in our isolates, the *bla*_{CTX-M-3} gene was not inserted into the variable region of the class 1 integron (5). From the genetic environments, the complete *tnpA*-*ISEcp1* insertion sequence was identified upstream of the *bla*_{CTX-M-3} gene. The location of the *ISEcp1* insertion sequence was in contrast with that reported in previous results, which identified the location as being downstream of the *bla*_{CTX-M-3} gene in isolates from France (12). However, the presence of the *orf513*, *orf477*, and *mucA* genes were similar to the results of previous reports (13,14). Further, the IS26 insertion sequence detected upstream of the *ISEcp1* element was associated with the promotion of the dissemination of the *bla*_{CTX-M-3} gene. As described previously, other insertion sequences (i.e., IS10 and IS1) were constantly substituted for IS26 linked to the *bla*_{CTX-M-3} gene (15,16). Although IS26 was commonly found adjacent to the *ISEcp1* element, the location of IS26 could be in different directions (reverse and/or forward) upstream of the *ISEcp1* element (17,18). In our *S. marcescens* isolates, Type II architecture was the most common genetic environment of the *bla*_{CTX-M-3} gene. Therefore, the presence of *ISEcp1*, IS26, *orf477*, and the *mucA* gene were highly associated with mobilization of the *bla*_{CTX-M-3} gene.

To our knowledge, the *bla*_{CTX-M} genes close to the

Type I

Type II

Type III

Fig. 1. Surrounding regions of transferable plasmid-mediated *bla*_{CTX-M-3} gene (right) and their complex class 1 integrons (left) in *Serratia marcescens* isolates. (Right) Type I architecture was characterized by the presence of a complete insertion sequence of *tnpA*-*ISEcp1*, identified in the upstream region of the *bla*_{CTX-M-3} gene with interrupting a reverse *IS26* sequence. And the reverse-directional *orf477* fragment was located downstream of the *bla*_{CTX-M-3} gene, which was connected with the same direction of the *mucA* gene. An *orf513*-containing common region was located upstream next to the *mucA* gene. Moreover, a copy of 3'-CS2 element was located immediately upstream of the *orf513* element. Type II architecture had genetic environments similarities to Type I architecture except for deletion of the 3'-CS2 structure. And Type III architecture for genetic environment of the *bla*_{CTX-M-3} gene, with the exception of *orf513* region, is identical to Type II architecture. (Left) Novel complex class 1 integron was characterized by the presence of the *dfrA19* gene, which was flanked by two copies of class 1 integrons linking with *orf513* element.

ISCR1 element located between the 3'-CS and the second 3'-CS (3'-CS2) structures are inclined to generate novel complex class 1 integrons (6). The 3'-CS2 structure may play an important role in the generation of an unusual class 1 integron (7,14). It is located upstream of the *bla*_{CTX-M-3} gene in the schematic of the Type I architecture from the tested *S. marcescens* isolates. The occurrence of the 3'-CS2 structure together with the *ISCR1* element in complex class 1 integrons is probably a potential specific-recombination site for the incorporation of foreign resistance genes into the integron structure (14).

To describe the presence of a novel complex class 1 integron bearing two *orf513* elements, two class 1 integrons, and the *dfrA19* gene is interesting. These two class 1 integrons are linked in series and separated by the *dfrA19* gene. Therefore, the *dfrA19* gene is flanked by one copy of a class 1 integron in direct orientation. This novel complex class 1 integron is located within the conjugative plasmid, which can play an important role in genetic dissemination through conjugation to other bacterial pathogens. This is the first study to report the *dfrA19* gene within a novel complex class 1 integron in *S. marcescens* isolates from Taiwan. Although a novel complex class 1 integron bearing an *ISCR1* element was characterized in *S. marcescens* carrying the *bla*_{CTX-M-3} gene, PCR analysis and the genome walking method

demonstrated the presence of this complex class 1 integron distantly upstream of the *bla*_{CTX-M-3} gene.

Conflict of interest None to declare.

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