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A Novel Integron-Like Element Carrying the Metallo-β-Lactamase Gene *bla*$_{IMP}$

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A plasmid-mediated metallo-β-lactamase gene was cloned from a carbapenem-resistant *Serratia marcescens* strain, AK9373. The metallo-β-lactamase gene was identical to the *bla*$_{IMP}$, and it was located in the space between an integrase-like gene and an *aac*(*6′*)-*Ib*-like gene. The deduced amino acid sequence for the putative integrase gene showed considerable identity (60.9%) to that of the *Escherichia coli* integrase reported. Sequences similar to the GTTRRRY and an atypical 59-base element containing a 67-bp inverted repeat sequence, which were peculiar to the integrase-dependent recombination, were also conserved in the flanking regions of the *bla*$_{IMP}$ gene. These findings imply that the metallo-β-lactamase gene in *S. marcescens* AK9373 is carried by a novel integron-like element that is mediated by a transferable large plasmid.

Carbapenems, such as imipenem, are potent agents for chemotherapy in infectious diseases caused by gram-negative bacteria including the family *Enterobacteriaceae*, since they are quite stable to hydrolysis by β-lactamases produced by these organisms (3, 4, 9, 10, 12). However, several clinical isolates of *Bacteroides fragilis*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa* were reported to be resistant to these agents because of production of metallo-β-lactamases (7, 13, 15, 19) belonging to Ambler's class B (1). Recently, we also isolated an imipenem-resistant *Serratia marcescens* strain, TN9106, and characterized a novel enterobacterial metallo-β-lactamase, IMP-1, produced by this strain (14). Transfer of carbapenem resistance was observed in some strains of *B. fragilis* (5) and *P. aeruginosa* (19), though genetic characterization has not been done yet. In the present study, we investigated the structural features of an element carrying the metallo-β-lactamase gene mediated by a transferable large plasmid harbored by an imipenem-resistant *S. marcescens* strain, AK9373 (11).

(This study was presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., 4 to 7 October 1994 [2].)

Cloning of the imipenem resistance gene. *S. marcescens* AK9373 showing high resistance to various broad-spectrum β-lactams including imipenem was isolated from a patient with a urinary tract infection at a general hospital in Anjyo, Japan, in 1993 (11). The total DNA of this strain was prepared and digested with *Bam*HI; then, the resultant fragments were ligated in plasmid vector pMK16 (14). *Escherichia coli* HB101 was transformed with these recombinant plasmids, and several colonies grown on Luria-Bertani agar plates supplemented with 8 μg of ceftazidime per ml were isolated (14). A 9-kb insert was generally found among the recombinant plasmids harbored by these ceftazidime-resistant transformants. The restriction sites of several endonucleases in the recombinant plasmid pSMB731 were determined as shown in Fig. 1, and the imipenem resistance gene was localized near the *Sma*I site by deletion analysis.

Sequence analyses and identification of ORFs. The *Bam*HI-*Sac*I fragment was subcloned into M13 phage, and the nucleotide sequence was determined on both strands by using M13 phage (21). An open reading frame (ORF) identical to the *bla*$_{IMP}$ gene of *S. marcescens* TN9106 (14) was found. A sequence similar to the *aac*(6′)-*Ib* (16, 18) gene was also found in the downstream region of the *bla*$_{IMP}$; however, introduction of

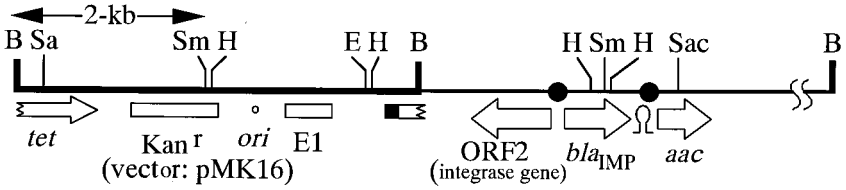

FIG. 1. Restriction map of pSMB731. The positions and transcriptional directions of each ORF are indicated (open arrows). The ORF2 product demonstrated a considerable similarity at the amino acid level with the integrase found in *E. coli* (17). The positions of the sequences similar to the GTTRRRY sequence and the inverted repeat are also indicated (● and ⟟, respectively). The thick line represents the vector plasmid pMK16, and the positions of the tetracycline resistance gene (*tet*), kanamicin resistance gene (Kan^r), and colicin E1 (E1) are indicated, together with the promoter of the *tet* gene (■) and the replication origin (*ori*) of pMK16. The 9-kb insert carrying *bla*$_{IMP}$ was ligated in the *Bam*HI site in *tet.* Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Sa, *Sal*I, Sac, *Sac*I; Sm, *Sma*I.

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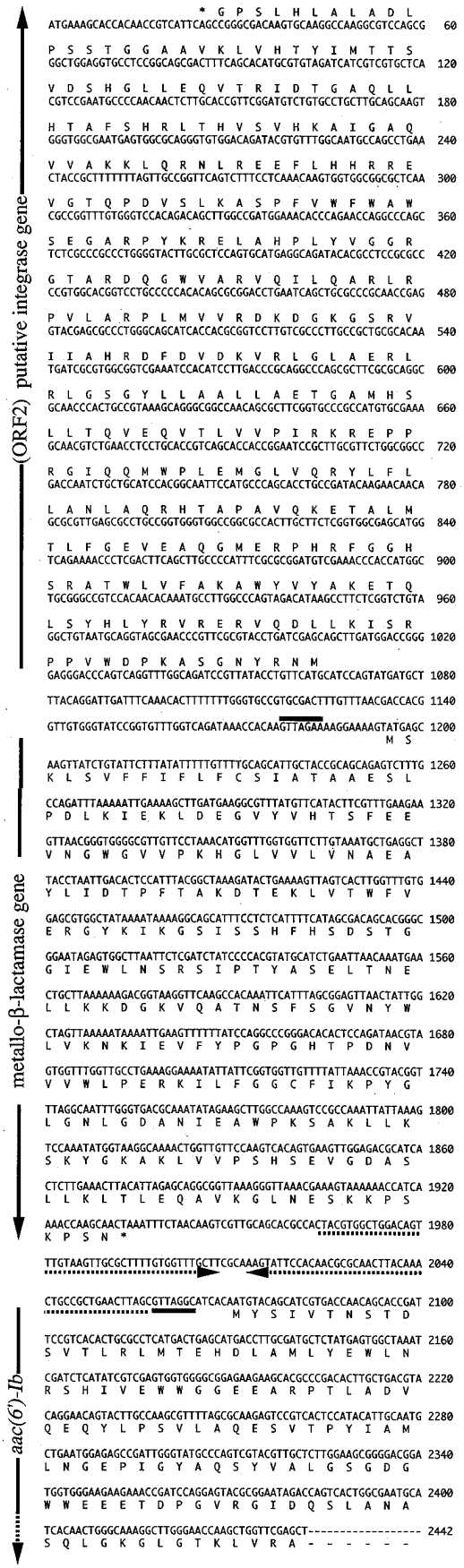


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1'  MNRYNGSAKPDWVPPRSIKLLDQVRERVRYLHYSLQTEKAYVYWAKAFVLWTARSHGGFR
              * **.*.***.***.*******.**.***.*..**.    * * * *
1"      MKTATAPLPPLRSVKVLDQLRERIRYLHYSLRTEQAYVHWVRAFI----RFH-GVR

61'  HPREMGQAEVEGFLTMLATEKQVAPATHRQALNALLFLYRQVLGMELPWMQQIGRPPERK
     ** ..* .***.**. **.*..*. .******.****.* .**  .***.*.****. ..
52"  HPATLGSSEVEAFLSWLANERKVSVSTHRQALAALLFFYGKVLCTDLPWLQEIGRPRPSR

121' RIPVVLTVQEVQTLLSHMAGTEALLAALLYGSGLRLREALGLRVKDVDFDRHAIIVRSGK
     *.***** .**  .*. ..*.. *.*.****.*.*..*.* *****.***. .****.**
112" RLPVVLTPDEVVRILGFLEGEHRLFAQLLYGTGMRISEGLQLRVKDLDFDHGTIIVREGK

181' GDKDRVVMLPRALVPRLRAQLIQVRAVWGQDRATGRGGVYLPHALERKYPRAGESWAWFW
     *.***..*** .*.*.**.** ..** * .*.*..**.** **.**********.**.***
172" GSKDRALMLPESLAPSLREQLSRARAWWLKDQAEGRSGVALPDALERKYPRAGHSWPWFW

241' VFPSAKLSVDPQTGVERRHHLFEERLNRQLKKAVVQAGIAKHVSVHTLRHSFATHLLQAG
     **.  . *.**..** ****.... ..*..*.** ****.*... ********* **..*
232" VFAQHTHSTDPRSGVVRRHHMYDQTFQRAFKRAVEQAGITKPATPHTLRHSFATALLRSG

301' TDIRTVQELLGHSDVSTTMIYTHVLKVAAGGTSSPLDALALHLSPG
      ******.******************...*..******.                [60.9% / 327 aa ]
292" YDIRTVQDLLGHSDVSTTMIYTHVLKVGGAGVRSPLDALPPLTSER
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FIG. 3. Comparison of the amino acid sequence deduced from ORF2 with that of the integrase of *E. coli*. The deduced amino acid sequence of a newly identified putative integrase (upper strand) is compared with that of the *E. coli* integrase (lower strand) (17). A 60.9% identity was observed between the two amino acid sequences. Identical (*) and similar (·) amino acid residues are indicated. Dashes, gaps.

pSMB731 in *E. coli* HB101 did not give resistance to aminoglycosides such as amikacin, gentamicin, and tobramicin. An opposite-orientation ORF2, was identified in the upstream region of the bla_{IMP} gene (Fig. 1 and 2). The amino acid sequence deduced from ORF2 demonstrated an identity of 60.9% to that of an integrase carried by an R plasmid found in a clinical isolate of *E. coli* (17) (Fig. 3).

Consensus sequences involved in the integrase-dependent recombination. The specific sequences similar to the GTTR RRY consensus sequence peculiar to the integrase-dependent recombination site (6) were conserved in both 5′-side and 3′-side flanking regions of bla_{IMP} (Fig. 2), while the sequence similar to the 59-base element involved in the integrase-dependent recombination (8) was not found in the downstream region of bla_{IMP}. However, an atypical 59-base element containing a 67-bp inverted repeat sequence was found in the downstream region of bla_{IMP} (Fig. 4).

Comparison of the nucleotide sequences in the upstream regions of two bla_{IMP} genes. The 5′-side flanking sequences of two bla_{IMP} genes, cloned from strains TN9106 (14) and AK9373 (11), respectively, were compared. The nucleotide sequence ^{1178}GTTAGAA in the downstream region of AK9373 was identical to the corresponding sequence of strain TN9106, while no considerable sequence similarity was found in the upstream regions (Fig. 5). This observation suggested that an integrase-dependent recombination of the gene cassette carrying bla_{IMP} might occur at this position (^{1178}GTTA GAA---), as described by Hall et al. (8).

The amino acid sequence of the putative integrase found in this study demonstrated a relatively low identity (60.9%) with

FIG. 2. DNA sequence of the region containing ORF2 (putative integrase gene), bla_{IMP}, and a part of the *aac(6′)-Ib*-like gene. The 2,442-bp sequence determined is shown, together with the amino acid sequences deduced from each ORF. The sequences similar to the GTTRRRY sequences at which the integrase-dependent recombination might occur are indicated (horizontal lines). The region containing a large inverted repeat sequence is also indicated (horizontal arrows), and the sequence similar to the 59-base element was found in this region as shown in Fig. 4. The position and transcriptional direction of each gene is indicated on the left.

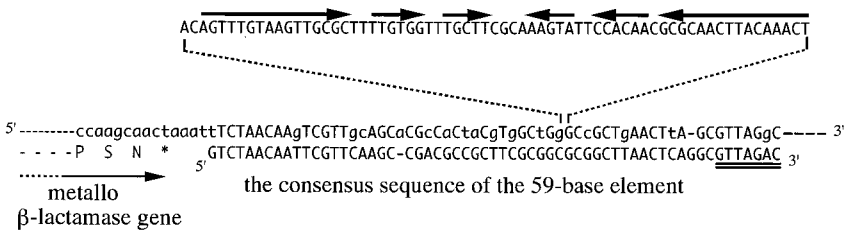

FIG. 4. Analysis of the 3′-side flanking regions of bla_{IMP} gene. The nucleotide sequence of the region containing a sequence similar to the 59-base element is the upper of the two lower sequences, and the consensus sequence of the 59-base element reported (8) is the lower sequence. Identical bases are uppercased, and the ^{2058}GTTAGGC sequence, similar to the GTTRRRY sequence, is doubly underlined. The inverted repeat sequence is shown at the top, with the positions of dyad symmetries indicated (arrows).

that of the integrase in *E. coli*, although high sequence identities, above 90%, have been observed among other integrases reported. Sequences similar to the GTTRRRY sequence, which are located generally in both terminals of the gene cassette, were also found in the both 5′-side and 3′-side flanking regions of the bla_{IMP} gene of strain AK9373. However, no sequence similar to the typical 59-base element was found in the downstream region of bla_{IMP}, while a 59-base element-like sequence appeared when a 67-bp inverted repeat sequence was removed from the downstream region of the bla_{IMP} gene. These findings imply that the metallo-β-lactamase gene bla_{IMP} mediated by a large plasmid of strain AK9373 is carried by a novel integron-like element.

In this study, an inverted repeat sequence was found within the atypical 59-base element that followed the bla_{IMP} gene of strain AK9373, though the 59-base element sequences were well conserved among various gene cassettes reported (8). We are not sure whether the inverted sequence was inserted in the 59-base element-like sequence after the integration of the gene cassette carrying the bla_{IMP} gene. High-level sequence similarities were observed among integrases reported (6, 8, 17, 18, 20), while the putative integrase found in strain AK9373 showed relatively low similarity to these integrases. Hence, the putative integrase may be a novel integrase that recognizes the long sequence consisting of the 59-base element-like sequence and the inverted repeat sequence. Further analysis of the enzyme activity upon DNA recombination should be continued.

In our previous work, we failed to transfer imipenem resistance from strain TN9106 to *E. coli*, and no plasmid was found in this strain. Moreover, the bla_{IMP}-specific DNA probes hybridized to the chromosomal position of strain TN9106 on the blot (14). These findings strongly suggested that the bla_{IMP} gene of strain TN9106 was encoded on the chromosome. However, in the present study, the metallo-β-lactamase gene of strain AK9373 was found to be carried by a large plasmid. This observation suggests a potential translocation of the metallo-β-lactamase gene between the chromosome and a resident large plasmid. By the comparative analysis of nucleotide sequences in the 5′-side flanking region of two bla_{IMP} genes cloned individually from different strains, it was strongly suggested that the GTTRRRY sequences might work as the recombination junction in the newly identified integron-like element. These findings imply that the 5′ and 3′ borders of the gene cassette carrying the bla_{IMP} gene are ^{1178}GTTAGAA-- and ---^{2058}GTTAGGC, respectively.

In this study, a sequence similar to *aac(6′)-Ib* was found in the downstream region of bla_{IMP}. The aminoglycoside acetyltransferase genes, such as *aacA* and *aacC1*, have also been found in In5 carried by pSCH884 and In4 found in Tn*1696*, respectively (8). This observation may reveal an evolutionary relation between these integrons and the newly identified integron-like element. The sequence analysis in the downstream region should be continued to characterize the phylogenic relation among them.

Nucleotide sequence accession number. The nucleotide sequence data for Fig. 2 will appear in the GSDB, DDBJ, EMBL, and NCBI databases under accession number D50438.

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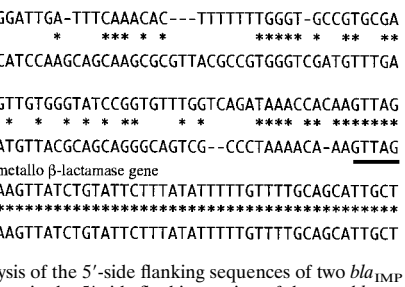

FIG. 5. Comparative analysis of the 5′-side flanking sequences of two bla_{IMP} genes. The nucleotide sequences in the 5′-side flanking region of the two bla_{IMP} genes cloned from strains TN9106 (14) and (lower sequence) and AK9373 (this study) (upper sequence) are aligned. Identical bases are indicated (*), and the ^{1178}GTTAGAA sequence that was speculated to be the site of integrase-dependent recombination is underlined.

REFERENCES

1. **Ambler, R. P.** 1980. The structure of β-lactamases. Philos. Trans. R. Soc. Lond. Ser. B **289:**321–331.
2. **Arakawa, Y., H. Ito, S. Ohsuka, N. Kato, and M. Ohta.** 1994. Genetic analyses of an enterobacterial metallo β-lactamase gene carried by a large plasmid of Serratia marcescens, abstr. C64. *In* Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
3. **Arakawa, Y., M. Ohta, N. Kido, Y. Fujii, T. Komatsu, and N. Kato.** 1986. Close evolutionary relationship between the chromosomally encoded β-lactamase gene of *Klebsiella pneumoniae* and the TEM β-lactamase gene mediated by R plasmids. FEBS Lett. **207:**69–74.
4. **Arakawa, Y., M. Ohta, N. Kido, M. Mori, H. Ito, T. Komatsu, Y. Fujii, and N. Kato.** 1989. Chromosomal β-lactamase of *Klebsiella oxytoca*, a new class A enzyme that hydrolyzes broad-spectrum β-lactam antibiotics. Antimicrob. Agents Chemother. **33:**63–70.
5. **Bandoh, K., K. Watanabe, Y. Muto, Y. Tanaka, N. Kato, and K. Ueno.** 1992. Conjugal transfer of imipenem resistance in *Bacteroides fragilis*. J. Antibiot. (Tokyo) **45:**542–547.
6. **Collis, C. M., and R. M. Hall.** 1992. Site-specific deletion and rearrangement of integron insert genes catalyzed by the integron DNA integrase. J. Bacteriol. **174:**1574–1585.
7. **Cuchural, G. J., Jr., M. H. Malamy, and F. P. Tally.** 1986. β-Lactamase-mediated imipenem resistance in *Bacteroides fragilis*. Antimicrob. Agents Chemother. **30:**645–648.
8. **Hall, R. M., D. E. Brookes, and H. W. Stokes.** 1991. Site-specific insertion of genes into integrons: role of the 59-base element and determination of the recombination crossover point. Mol. Microbiol. **5:**1941–1959.
9. **Horii, T., Y. Arakawa, M. Ohta, S. Ichiyama, R. Wacharotayankun, and N. Kato.** 1993. Plasmid-mediated AmpC-type β-lactamase isolated from *Klebsiella pneumoniae* confers resistance to broad-spectrum β-lactams, including

moxalactam. Antimicrob. Agents Chemother. **37:**984–990.
10. **Horii, T., Y. Arakawa, M. Ohta, T. Sugiyama, R. Wacharotayankun, H. Ito, and N. Kato.** 1994. Characterization of a plasmid-borne and constitutively expressed bla_{MOX-1} gene encoding AmpC-type β-lactamase. Gene **139:**93–98.
11. **Ito, H., Y. Arakawa, S. Ohsuka, R. Wacharotayankun, N. Kato, and M. Ohta.** 1995. Plasmid-mediated dissemination of the metallo-β-lactamase gene bla_{IMP} among clinically isolated strains of *Serratia marcescens*. Antimicrob. Agents Chemother. **39:**824–829.
12. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
13. **Massidda, O., G. M. Rossolini, and G. Satta.** 1991. The *Aeromonas hydrophila cphA* gene: molecular heterogeneity among class B metallo-β-lactamases. J. Bacteriol. **173:**4611–4617.
14. **Osano, E., Y. Arakawa, R. Wacharotayankun, M. Ohta, T. Horii, H. Ito, F. Yoshimura, and N. Kato.** 1994. Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob. Agents Chemother. **38:**71–78.
15. **Rasmussen, B. A., Y. Gluzman, and F. P. Tally.** 1990. Cloning and sequencing of the class B β-lactamase gene (*ccrA*) from *Bacteroides fragilis* TAL3636. Antimicrob. Agents Chemother. **34:**1590–1592.
16. **Shaw, K. J., C. A. Cramer, M. Rizzo, R. Mierzwa, K. Gewain, G. H. Miller, and R. S. Hare.** 1989. Isolation, characterization, and DNA sequence analysis of an AAC(6′)-II gene from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **33:**2052–2062.
17. **Stokes, H. W., and R. M. Hall.** 1989. A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. Mol. Microbiol. **3:**1669–1683.
18. **Van Nhieu, G. T., and E. Collatz.** 1987. Primary structure of an aminoglycoside 6′-*N*-acetyltransferase, AAC(6′)-4, fused in vivo with the signal peptide of the Tn*3*-encoded β-lactamase. J. Bacteriol. **169:**5708–5714.
19. **Watanabe, S., S. Iyobe, M. Inoue, and S. Mitsuhashi.** 1991. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **35:**147–151.
20. **Wohlleben, W., W. Arnord, L. Bissonnette, A. Pelletier, A. Tanguay, P. H. Roy, G. C. Gamboa, G. F. Barry, E. Aubert, J. Davies, and S. A. Kagan.** 1989. On the evolution of the Tn*21*-like multiresistant transposon: sequence analysis of the gene (*aac1*) for gentamicin acetyltransferase-3-I(AAC(3)-I), another member of the Tn*21*-based expression cassette. Mol. Gen. Genet. **217:**202–208.
21. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.