Antimicrobial Agents and Chemotherapy

Molecular Analysis of the Sequences Surrounding *bla*$_{OXA-45}$ Reveals Acquisition of This Gene by *Pseudomonas aeruginosa* via a Novel IS*CR* Element, IS*CR5*

Hongyang Li, Timothy R. Walsh and Mark A. Toleman
Antimicrob. Agents Chemother. **2009, 53(3):1248. DOI: 10.1128/AAC.00480-08.**
Published Ahead of Print 8 December 2008.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 2009, p. 1248–1251 Vol. 53, No. 3
0066-4804/09/$08.00+0 doi:10.1128/AAC.00480-08

Molecular Analysis of the Sequences Surrounding bla_{OXA-45} Reveals Acquisition of This Gene by *Pseudomonas aeruginosa* via a Novel IS*CR* Element, IS*CR5*▽

Hongyang Li,[1] Timothy R. Walsh,[2] and Mark A. Toleman[2]*

Department of Cellular and Molecular Medicine, University of Bristol, Bristol BS8 1TD, United Kingdom,[1] and Department of Medical Microbiology, School of Medicine, University of Cardiff, Cardiff CF14 4XN, United Kingdom[2]

Received 11 April 2008/Returned for modification 6 July 2008/Accepted 28 November 2008

The bla_{OXA-45} gene of *Pseudomonas aeruginosa* 07-406 is driven by a promoter found within a truncated IS*Pme*1 insertion sequence. The gene is located between nonidentical repeats of a new IS*CR* element, IS*CR5*, which is likely responsible for its acquisition. Sequence analysis indicates that IS*CR5* is a chimera of IS*CR3* and IS*CR16*.

bla_{OXA-45}, a gene encoding a class 2d′ β-lactamase, was initially characterized from a *Pseudomonas aeruginosa* isolate 07-406 (12, 13).

A number of genetic mechanisms that enable resistance genes to be acquired by bacteria have been reported. These genetic mechanisms include plasmids, transposons, integrons, classical insertion sequences (IS), and IS*CR* elements (2, 4, 11).

IS*CR* elements are an unusual group of insertion sequences that have similarities to the IS*91* family of insertion sequences (10, 11). They differ considerably from classical insertion sequences in both their structure and transposition mechanism (5, 6), and studies on the IS*91* family have demonstrated that these elements can cotranspose DNA adjacent to their terminal ter*IS* sequence, mediated by a single copy of the element (so-called one-ended transposition) (8, 9). At present, the IS*CR* family comprises 19 members that vary in identity between 18 and 96% (http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html). They are found adjacent to a wide variety of antibiotic resistance genes of foreign origin and are thus implicated in their acquisition (11).

P. aeruginosa strains 07-406 and 07-408 were isolated in 2001 from two patients via the CANCER Antimicrobial Surveillance Program (12, 13). *Escherichia coli* strain DH5α *recA1* (3) was used for library construction as described previously (13), and clones that included bla_{OXA-45} were selected by growth on plates containing ceftazidime (50 μg/ml) plus 50 mM EDTA. EDTA was included to exclude clones containing the metallo-β-lactamase gene bla_{VIM-7}, a gene also found in this strain (12). The bla_{OXA-45} locus was constructed from multiple clones sequenced on both strands using custom-designed primers, and the 7,018-bp bla_{OXA-45} locus has been deposited in the EMBL database under the accession number AM849110. The sequence included 2,557 bp of upstream DNA and 3,671 bp of downstream DNA flanking the bla_{OXA-45} structural gene. Sequence analysis revealed that immediately upstream of the bla_{OXA-45} gene is a truncated copy of a classical insertion sequence that displays 99% identity to the insertion sequence IS*Pme1* from *Xanthobacter autotrophicus* (15). This element was located 19 bp upstream from the start codon of bla_{OXA-45} and consists of 369 bp of the right-hand end encoding the last 48

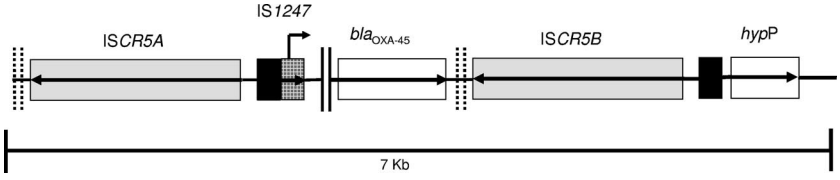

FIG. 1. Schematic of the genetic locus of bla_{OXA-45}. ORFs are depicted as boxes with arrows indicating the direction of transcription of the ORF. The truncated IS*Pme*1 element is depicted as a checkered box indicating the truncated ORF together with vertical parallel lines representing the right-hand end inverted repeat sequence. The promoter driving the transcription of bla_{OXA-45} is drawn as a bent horizontal arrow. Solid black boxes indicate the repeated 376-bp section of DNA found upstream of both IS*CR5* elements. The *ori*IS of IS*CR5A* and IS*CR5B* are depicted as dotted vertical lines found downstream of the IS*CR5* ORFs.

* Corresponding author. Mailing address: Department of Medical Microbiology, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, United Kingdom. Phone: 44 (0) 29 2074 3129. Fax: 44 (0) 29 2074 2161. E-mail: TolemanMA@Cardiff.ac.uk.

▽ Published ahead of print on 8 December 2008.

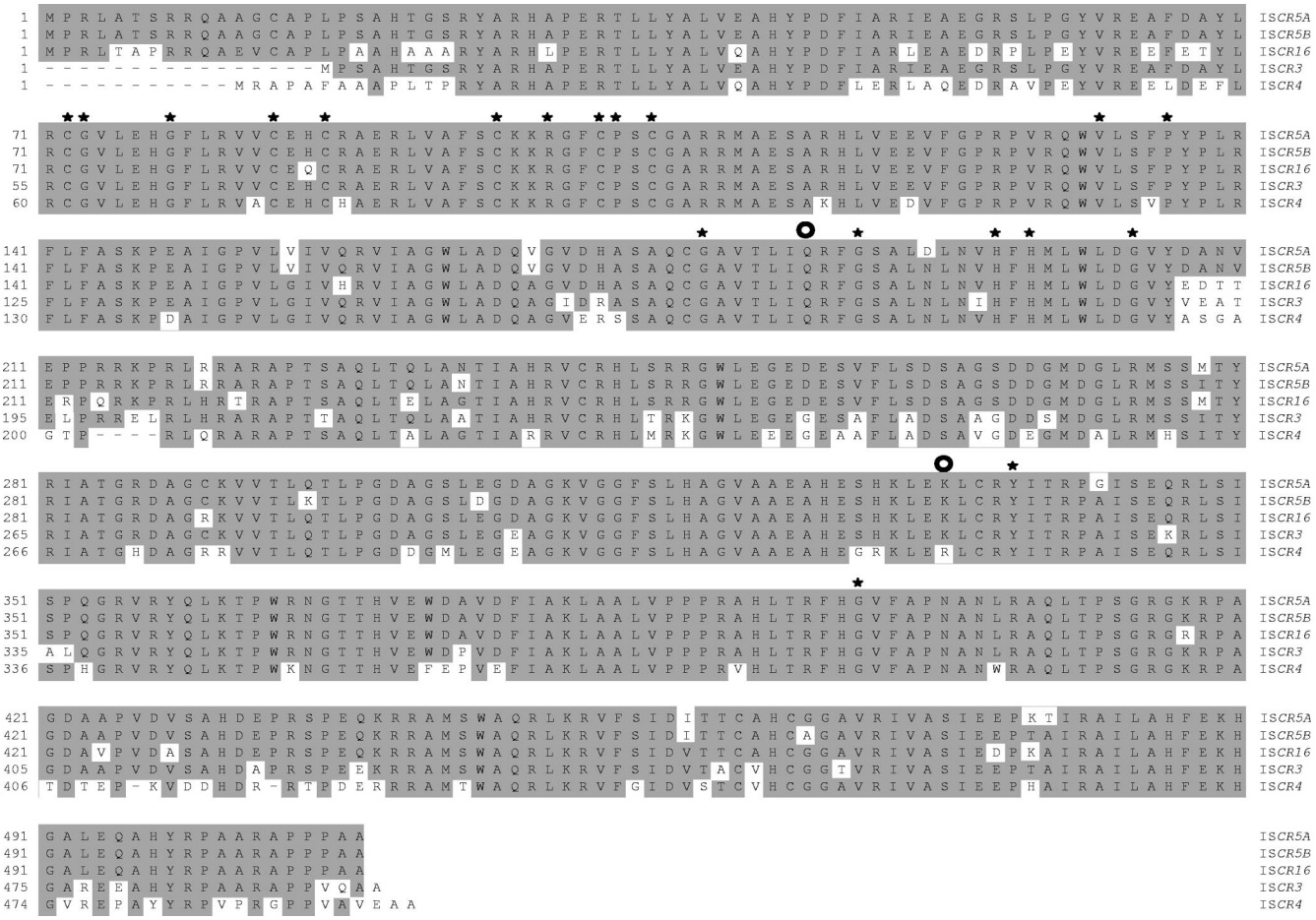

FIG. 2. Alignment of transposases of IS*CR* elements displaying the highest identity to IS*CR5A* and IS*CR5B*. Residues found in the majority of sequences are shown on a gray background. The residues that are found conserved in all IS*91* family transposases are indicated by black stars above the sequences. Two key residues that differ from those in the transposases of the IS*91* family are indicated by open circles above the sequences. The first residue, Q85 in the IS*CR5* sequence, is a histidine residue in all IS*91* family elements, and the lysine residue K333 (IS*CR5*) is a tyrosine in all IS*91* family elements and an arginine in IS*CR1*, IS*CR2*, and IS*CR4*. (see reference 11 for a discussion of these differences). Gaps introduced to maximize alignment of the sequences are indicated by the dashes.

amino acids of the transposase gene together with the inverted repeat sequence. As 19 bp is too short to contain promoter sequences, the promoter driving $bla_{\text{oxa-45}}$ expression must be located within this IS.

This IS/$bla_{\text{OXA-45}}$ section is sandwiched between tandem repeats of approximately 2,100 bp (Fig. 1). These include two nearly identical open reading frames (ORFs), encoding putative proteins of 509 amino acids. Comparison of these two ORFs revealed 25 single base pair differences. Most of these nucleotide polymorphisms are silent, but seven account for amino acid differences between the putative encoded proteins (Fig. 2). Searches via the protein databases at EMBL-EBI (FASTA protein similarity search at http://www.ebi.ac.uk/fasta33) revealed high identity with the IS*CR* transposase ORFs IS*CR16* (14), IS*CR3*, IS*CR4*, and IS*CR1* with identities of 92%, 91%, 78%, and 51%, respectively. Alignment of these various protein sequences (Fig. 2) reveals the large sections of identity and the key motifs conserved among IS*CR* transposases. The DNA sequences found on either side of these putative IS*CR* transposase ORFs also display high identity to the corresponding *ter*IS and *ori*IS of IS*CR16*, IS*CR3*, and IS*CR4* (Fig. 3). Therefore, the $bla_{\text{OXA-45}}$ structural gene is flanked by nearly identical copies of a new IS*CR* element, IS*CR5*, and these copies will hereafter be referred to as IS*CR5A* and IS*CR5B*.

A 376-bp section of unidentified DNA is found 5′ adjacent to both copies of IS*CR5* (Fig. 1). This is different from IS*CR3*, IS*CR4*, and IS*CR16*, which all have *groEL* sequences in the same position (14). Curiously, the *ori*IS end of the IS*CR5* elements displays higher identity (98%) to the respective end of IS*CR16* than to the same section of IS*CR3* (80%) (Fig. 3). This same difference is seen in the IS*CR5* transposase, in that the sequence coding for the N-terminal section of the IS*CR5* transposase displays higher identity to IS*CR3* than to IS*CR16* (Fig. 2), and the sequence coding for the carboxy terminus of the protein displays the opposite, i.e., higher identity to IS*CR16* than to IS*CR3*. This observation is consistent with a homologous recombination event between IS*CR3* and IS*CR16*-like elements resulting in the chimeric element IS*CR5*. Further upstream of IS*CR5B* is an additional 1,400 bp of sequence that displays identity to

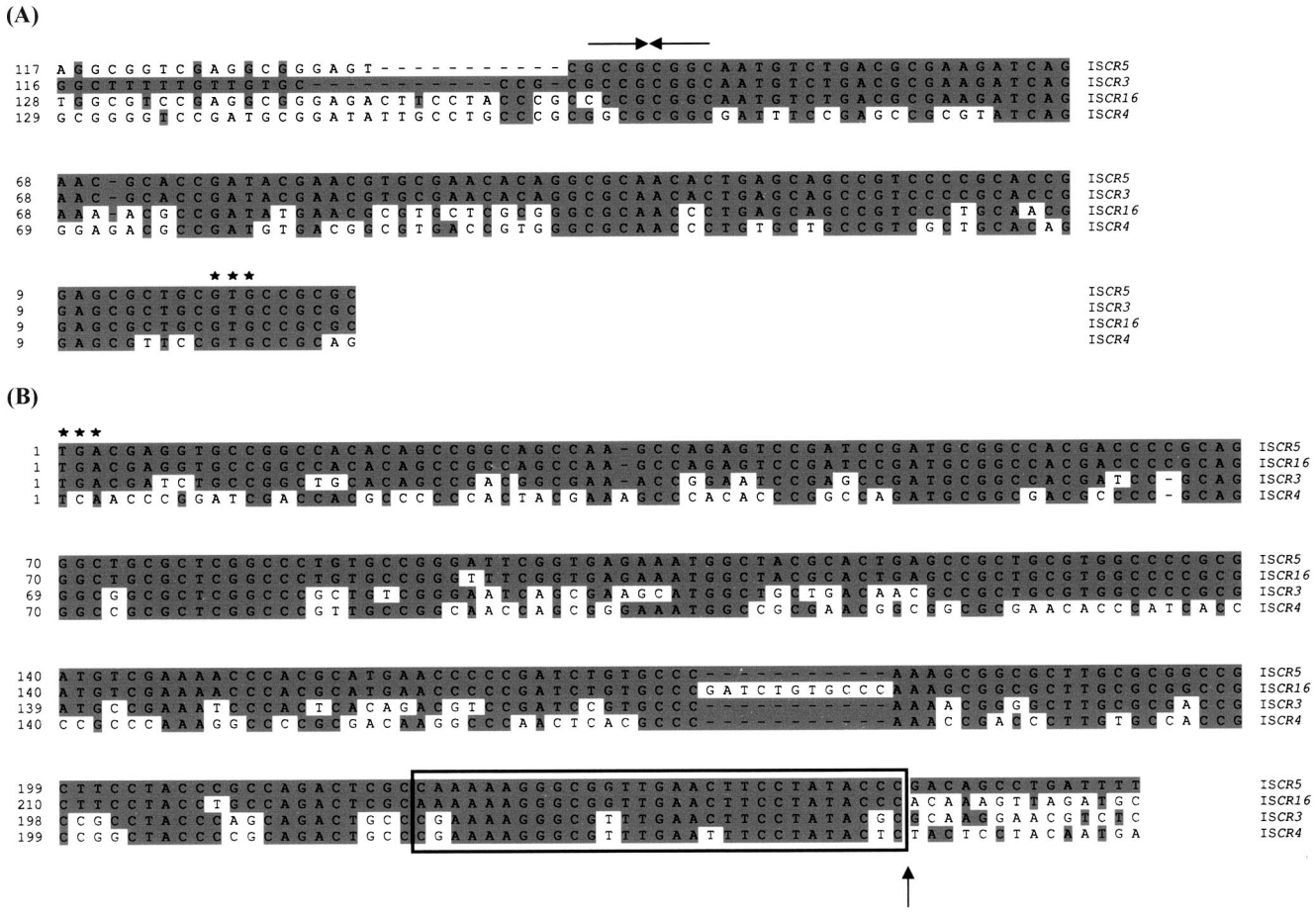

FIG. 3. (A) Alignment of the 5′ DNA sequences of IS*CR* elements displaying the highest identity to IS*CR5*. Nucleotides that are identical to those in the sequence of IS*CR3* are shown on a gray background. The GTG start codons of the various IS*CR* element transposase genes are indicated by asterisks, and nucleotides are numbered upstream starting at this codon. Sequences were collected from the following data banks and accession numbers: GenBank AF261825 (IS*CR3*), CP000604 (IS*CR16*), GenBank AY341249 (IS*CR4*), and AM849110 (IS*CR5B* [this study]). The IS*CR5* 5′-terminal sequence displays 100% identity to the respective sequence from IS*CR3* until 98 bp upstream of the start codon where identity to IS*CR3* and other elements is abruptly lost. The same sequence displays 86% identity to the equivalent sequence from IS*CR16*. A short 4-bp inverted repeat sequence at this position is indicated by inverted arrows above the nucleotide sequence which is similar to that found in the *ter*IS of IS*91*. (B) Alignment of the 3′-terminal DNA sequences of IS*CR* elements displaying the highest identity to IS*CR5*. Nucleotides that are identical to those in the sequence of IS*CR5* are shown on a gray background. The stop codons of IS*CR5*, IS*CR16*, and IS*CR3* transposase genes are indicated by black stars, and the *ori*IS sequence found at the extreme terminus of the various IS*CR* elements is boxed. The vertical arrow indicates the junction between the IS*CR* elements and carrier DNA. The IS*CR5* 3′-terminal sequence displays the highest identity (98%) to IS*CR16* but only 80% identity to the respective sequence from IS*CR3*.

hypothetical protein-encoding regions found in several genome-sequencing projects (for example, 68% and 77% identity over ~500 bp to two hypothetical proteins found in *Stenotrophomonas maltophilia* genome sequence CP00111 and 67% identity over ~500 bp to a hypothetical protein from *Cellvibrio japonicus* Ueda 107 genome sequence CP000934).

The copy number and genomic location of $bla_{\text{OXA-45}}$ were determined by preparation and digestion of genomic DNA in agar plugs and separated using pulsed-field gel electrophoresis as described previously (7). The plugs were digested with enzymes SpeI, I-Ceu1, and S1. S1 is known to nick single-stranded DNA and is used to unwind closed circular DNA so that plasmids run true to size in agarose gels (1). Hybridization of the $bla_{\text{OXA-45}}$ probe to DNA of high molecular weight (>1 Mb) from S1- and I-Ceu1-digested DNA indicates that $bla_{\text{OXA-45}}$ is chromosomally encoded (Fig. 4). SpeI digests show that the two isolates (isolates 07-406 and 07-408) are identical. However, probing of these digests indicated that the copy number of $bla_{\text{OXA-45}}$ is greater in isolate 07-408 than in 07-406 and also that their chromosomal positions are different (Fig. 4). This is interesting because isolates 07-406 and 07-408 were collected in 2001 from separate patients in the same ward of the M. D. Anderson Cancer Center in Texas (12). This observation is consistent with replicative transposition events of IS*CR5A* comobilizing $bla_{\text{OXA-45}}$. It is also likely that these events are of recent origin, presumably occurring on transfer from one patient to another.

In summary, two IS*CR5* elements are found flanking the $bla_{\text{OXA-45}}$ gene in *P. aeruginosa* isolate 07-406. IS*CR5A* found upstream of the $bla_{\text{OXA-45}}$ gene is implicated in the acquisition of this gene by *P. aeruginosa* by virtue both of its position and

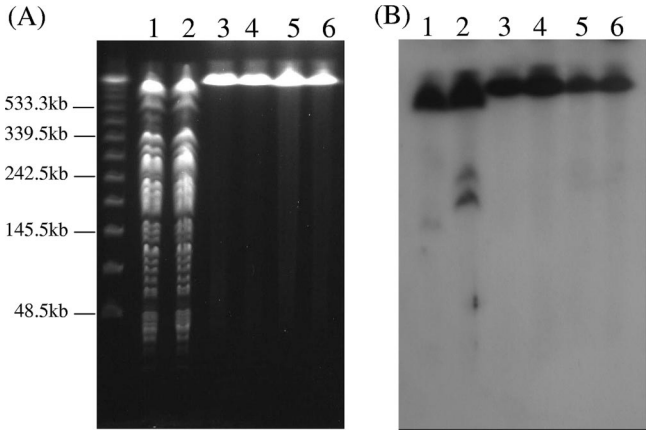

FIG. 4. (A) Pulsed-field gel of genomic DNA from *P. aeruginosa* strains 07-406 and 07-408. Lane 1, lambda ladder pulsed-field gel markers; lanes 2, 4, and 6, strain 07-406 DNA digested with SpeI, I-Ceu1, and S1, respectively; lanes 3, 5, and 7, strain 07-408 DNA digested with SpeI, I-Ceu1, and S1, respectively. (B) Autoradiograph of the gel shown in panel A probed with bla_{OXA-45}.

structure. There are now 19 IS*CR* elements with distinct sequences, and the evidence that these elements are of central importance in the acquisition of resistance mechanisms by pathogenic bacteria is steadily increasing.

This work was funded in part by EU grant LSHM-CT-2005-018705 and The Wellcome Trust project grant WT084627MF. H. Li was the recipient of an overseas studentship from the University of Bristol.

REFERENCES

1. **Barton, B. M., G. P. Harding, and A. J. Zuccarelli.** 1995. A general method for detecting and sizing large plasmids. Anal. Biochem. **226:**235–240.
2. **Datta, N., and R. W. Hedges.** 1972. Trimethoprim resistance conferred by W plasmids in Enterobacteriaceae. J. Gen. Microbiol. **72:**349–355.
3. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557–580.
4. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
5. **Mendiola, M. V., and F. de la Cruz.** 1992. IS*91* transposase is related to the rolling-circle-type replication proteins of the pUB110 family of plasmids. Nucleic Acids Res. **20:**3521.
6. **Mendiola, M. V., Y. Jubete, and F. de la Cruz.** 1992. DNA sequence of IS*91* and identification of the transposase gene. J. Bacteriol. **174:**1345–1351.
7. **Patzer, J. A., and D. Dzierzanowska.** 2007. Increase of imipenem resistance among *Pseudomonas aeruginosa* isolates from a Polish paediatric hospital (1993–2002). Int. J. Antimicrob. Agents **29:**153–158.
8. **Schlör, S., S. Riedl, J. Blass, and J. Reidl.** 2000. Genetic rearrangements of the regions adjacent to genes encoding heat-labile enterotoxins (*eltAB*) of enterotoxigenic *Escherichia coli* strains. Appl. Environ. Microbiol. **66:**352–358.
9. **Tavakoli, N., A. Comanducci, H. M. Dodd, M. C. Lett, B. Albiger, and P. Bennett.** 2000. IS*1294*, a DNA element that transposes by RC transposition. Plasmid **44:**66–84.
10. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. Common regions e.g. orf*513* and antibiotic resistance: IS*91*-like elements evolving complex class 1 integrons. J. Antimicrob. Chemother. **58:**1–6.
11. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:** 296–316.
12. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2004. bla_{VIM-7}, an evolutionarily distinct metallo-β-lactamase gene in a *Pseudomonas aeruginosa* isolate from the United States. Antimicrob. Agents Chemother. **48:** 329–332.
13. **Toleman, M. A., K. Rolston, R. N. Jones, and T. R. Walsh.** 2003. Molecular and biochemical characterization of OXA-45, an extended-spectrum class 2d′ β-lactamase in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **47:**2859–2863.
14. **Toleman, M. A., and T. R. Walsh.** 2008. Evolution of the IS*CR3* group of IS*CR* elements. Antimicrob. Agents Chemother. **52:**3789–3791.
15. **van der Ploeg, J., M. Willemsen, G. van Hall, and D. B. Janssen.** 1995. Adaptation of *Xanthobacter autotrophicus* GJ10 to bromoacetate due to activation and mobilization of the haloacetate dehalogenase gene by insertion element IS*1247*. J. Bacteriol. **177:**1348–1356.