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Identification and Characterization of IS*1411*, a New Insertion Sequence Which Causes Transcriptional Activation of the Phenol Degradation Genes in *Pseudomonas putida*

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A new insertion sequence (IS element), IS*1411*, was identified downstream of the phenol degradation genes *pheBA* that originated from plasmid DNA of *Pseudomonas* sp. strain EST1001. According to sequence analysis, IS*1411* belongs to a new family of IS elements that has recently been named the ISL*3* family (J. Mahillon and M. Chandler, Microbiol. Mol. Biol. Rev. 62:725–774, 1998). IS*1411* generates 8-bp duplication of the target DNA and carries 24-bp inverted repeats (IRs), highly homologous to the IRs of other IS elements belonging to this family. IS*1411* was discovered as a result of insertional activation of promoterless *pheBA* genes in *Pseudomonas putida* due to the presence of outward-directed promoters at the left end of IS*1411*. Both promoters located on the IS element have sequences that are similar to the consensus sequence of *Escherichia coli* σ^{70}. IS*1411* can produce IS circles, and the circle formation is enhanced when two copies of the element are present in the same plasmid.

Insertion sequences (IS elements) are the simplest transposable DNA elements and generally encode one protein required for transposition. They range in length from 800 to 2,500 bp and can be found in the genomes of a wide range of bacteria (reviewed in references 11 and 25). IS elements were originally identified as the causative agents of highly polar mutations because they reduced expression of genes downstream of the insertion points (26, 34). A number of IS elements, however, have been implicated in the transcriptional activation of silent genes (2, 6, 7, 10, 12, 23, 28, 32, 40, 47 [see also the references in reference 25]). Transposable DNA elements can move through bacterial populations horizontally, via transmission of genetic material from one bacterium to another, and play an important role in the dissemination and acquisition of accessory genes involved in antibiotic resistance, virulence, pathogenicity, and catabolic pathways (reviewed in references 11 and 25). Soil bacteria, e.g., *Pseudomonas* spp., are known to metabolize a broad range of aromatic compounds and are therefore ideal agents for environmental detoxification (15). Molecular characterization of plasmids carrying the genes for catabolism of aromatics has revealed the modular structure of these plasmids: the catabolic genes are usually parts of composite transposons or they are found to be flanked by genes having similarity to transposase genes of IS elements (41). This indicates that IS elements could play an important role in the evolution of catabolic pathways in soil bacteria and in the regulation of gene expression. However, little is known about the mechanism of transposition of these DNA elements in soil bacteria.

We have previously shown that introduction of a plasmid carrying the *pheBA* genes encoding catechol 1,2-dioxygenase and phenol monooxygenase, respectively, into *Pseudomonas putida* PaW85 enables the bacterium to use the hybrid plasmid-chromosome-encoded pathway for phenol degradation (18). Here we characterize a novel IS element—IS*1411* of *Pseudomonas* sp.—which is located downstream of the *pheBA* operon (Fig. 1) and has the potential to activate these genes due to outward-directed promoters on its left end. Sequence analysis of IS*1411* has revealed that this DNA element belongs to the ISL*3* family of IS elements (25). IS*1411* produces IS circles, and as a result of transposition of IS*1411* upstream to the promoterless *pheBA* operon, two copies of the element are present in the same plasmid. The possible mechanisms of transposition of this element will be discussed.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The bacterial strains and plasmids used in this study are described in Table 1. The DNA fragments containing the left end of IS*1411* were initially subcloned into the pBluescript SK(+) vector to obtain appropriate cloning sites for construction of pL1411END and pKTtnpA. To construct pL1411END, the 454-bp *Hin*dIII-*Rsa*I fragment (containing the left end of IS*1411* from the *Rsa*I site and the sequence that flanked the left end in pINS113 up to the *Hin*dIII site in the *pheB* gene) was cloned into pBluescript SK. Oligonucleotide 113 (5′-AAGGGTGTAGAAAAAAT-3′), complementary to nucleotides (nt) 14 to 31 relative to the left end of IS*1411* (Fig. 2), and a reverse primer complementary to a pBluescript SK sequence were used to amplify the 246-bp fragment containing the outward-oriented promoters of IS*1411*. The PCR-generated fragment was cut with *Bam*HI and cloned into pKTlacZ cleaved with *Bam*HI and *Sma*I. To construct pKTtnpA, the 665-bp *Hin*dIII fragment (Fig. 1B), cloned initially from pEST1414 into pBluescript, was inserted with *Bam*HI and *Xho*I ends into pKTlacZ. Bacteria were grown on Luria-Bertani medium (27). Antibiotics were added at the following final concentrations: for *Escherichia coli*, ampicillin at 100 μg/ml; for *P. putida*, carbenicillin at 1,500 μg/ml. *E. coli* was incubated at 37°C (for enzyme assays, at 30°C), and *P. putida* was incubated at 30°C. Early-stationary-phase cultures were used for enzyme assays. *E. coli* was transformed with plasmid DNA as described by Hanahan (14). *P. putida* was electrotransformed by using the protocol of Sharma and Schimke (35).

DNA sequencing and mRNA mapping. DNA sequencing was performed with a Sequenase version 2.0 DNA-sequencing kit (Amersham). A reverse transcriptase reaction was carried out to identify the 5′ ends of mRNA initiated from the outward-directed promoters and from the promoter of *tnpA* of IS*1411*. Total RNA (10 μg), purified from *P. putida* PaW85 and *E. coli* HB101 as described by Blomberg et al. (3), was used as the template in primer extension reactions. Primer 113 (described above) was used to map the outward-directed promoters. To map the transcription initiation from the *tnpA* promoter, the primer ORF2 (5′-CGAGGTTATTCAGTT-3′), complementary to nt 47 to 61 relative to the start codon GTG of the *tnpA* gene, was used in the primer extension analysis. Dideoxy sequencing standards of the double-stranded template (4 μg) were prepared by using the same primers.

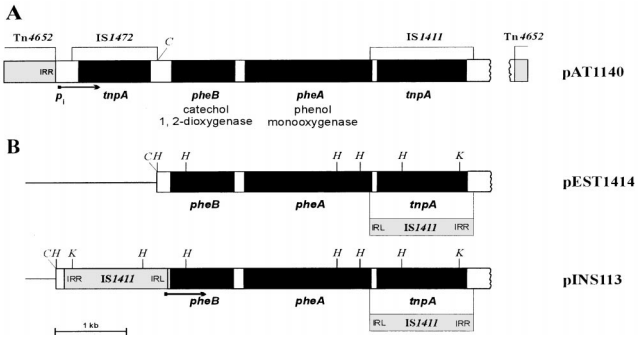

FIG. 1. Organization of the *pheBA* operon in plasmid pAT1140 (18). The *pheB* and *pheA* genes are flanked by two IS elements, IS*1472* and IS*1411* (GenBank accession no. M57500). The black boxes show the locations of the *pheBA* genes and the transposase genes (*tnpA*) of IS*1472* and IS*1411*. The open boxes represent the intergenic regions. The promoter of the *pheBA* operon (designated p_1) is located upstream of IS*1472*. The arrow indicates the direction of transcription of the genes. The right-end sequences of the transposon Tn*4652* (42, 43) are shown by shaded boxes. IRR indicates the 46-bp terminal IR of the right end of Tn*4652*. (B) Organization of the *pheBA* operon and IS*1411* in plasmids pEST1414 (19) and pINS113 (present study). The promoterless *pheBA* operon in pEST1414 is present, starting from the *Cla*I site. Only restriction sites relevant to the experiments presented in this paper are shown. *C*, *Cla*I; *H*, *Hin*dIII; *K*, *Kpn*I. The left and right IRs of IS*1411* are designated IRL and IRR, respectively. The arrow below the map of pINS113 indicates the direction of transcription of the *pheBA* genes from outward-directed promoters at the left end of the inserted IS*1411*.

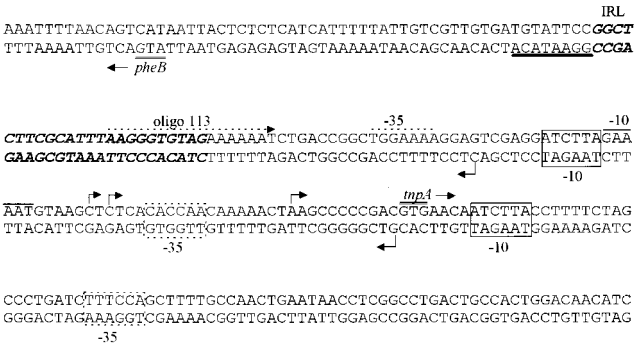

FIG. 2. Nucleotide sequence of the left end of IS*1411*. The 8-bp target sequence that was duplicated during transposition of IS*1411* upstream of the *pheBA* genes is underlined with a bold line. The 24-bp IR of the element is in boldface italics. The translation start sites of *pheB* and *tnpA* of IS*1411* are marked by double lines. The outward-directed promoters of IS*1411* are outlined with solid lines (−10 hexamers) and dashed lines (−35 hexamers). The transcription start sites for these promoters are indicated by arrows at the coding strand of *pheB*. The putative −10 and −35 hexamers of the *tnpA* promoter are shown by solid and dashed lines, respectively. Three 5′ ends of the *tnpA* mRNA, mapped by reverse transcriptase, are indicated by bent arrows at the coding strand of the *tnpA* gene. The location of oligonucleotide (oligo) 113, used for construction of plasmid pL1411END, is indicated by the dotted arrow.

Enzyme assays. The β-galactosidase (β-Gal) assay was performed as described by Miller (27). Protein concentration in cell lysates was measured by the Bradford method (5).

Detection of IS*1411* circles. Plasmid DNA was isolated by the alkaline lysis protocol (31). Southern blot analysis of DNA preparations of pINS113 and pEST1414 was carried out as described previously (31). One microgram of DNA was loaded onto an agarose gel. The radioactive DNA probe used in filter hybridization was an [α-^{32}P]dCTP-labeled 630-bp *Hin*dIII-*Eco*47III restriction fragment derived from the IS*1411* circle DNA containing both ends of IS*1411*. The hybridization signals at IS circles were quantitated by PhosphorImager (ImageQuant 4.2a software; Molecular Dynamics).

RESULTS

Insertional activation of the *pheBA* genes. We have previously shown that when plasmid pEST1414 carrying the promoterless phenol degradation genes *pheBA* was introduced into *P. putida* PaW85 and bacteria were selected for growth on phenol minimal plates, promoters for the transcription of these genes were created as a result of base substitutions, deletions, and the transposition of transposon Tn*4652* (19). Additionally, in one case (plasmid pINS113) activation of the *pheBA* genes was observed as a consequence of the insertion of a 1.4-kb-long DNA segment upstream of these genes. The nucleotide sequence of the inserted DNA revealed that a 1,419-bp element, bounded by 24-bp inverted repeats (IRs), had been inserted 35 nt upstream from the ATG start codon of the *pheB* gene. During this insertion, the 8-nt-long target sequence GGAAT ACA had been duplicated. The element was designated IS*1411*. The nucleotide sequence of the left end of IS*1411* is shown in Fig. 2.

IS*1411* originates downstream from the *pheBA* operon. The nucleotide sequence of the inserted element IS*1411* in plasmid pINS113 was identical to the sequence that was located downstream from the *pheBA* operon in the plasmid pEST1414 (Fig. 1). Interestingly, in its original location, the left IR of IS*1411* overlapped the *pheA* gene by 21 nt and the element lacked direct repeats of the target DNA. Both copies of IS*1411* were present in the plasmid pINS113. Because we observed no copies of IS*1411* in the chromosome of *P. putida* PaW85 (data not shown), we suppose that the IS element originated from the same plasmid, pEST1414 (Fig. 1).

Besides pINS113, we have described another plasmid carrying two copies of IS*1411* (data not shown). In that case (plasmid pM13) the insertion of IS*1411* (which also generated 8-bp direct repeats of the target DNA) led to the inactivation of the

TABLE 1. Bacterial strains and plasmids used

Strain or plasmid	Genotype or construction	Source or reference
E. coli HB101	*subE44 subF58 hsdS3* (r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	4
P. putida PaW85	Tn*4652*	1
Plasmids		
pBluescript SK(+)	Cloning vector (Ap^r)	Stratagene
pEST1414	Plasmid pAYC32 carrying promoterless *pheBA* operon	19
pINS113	pEST1414 carrying insertion of IS*1411* upstream of the *pheBA* genes	This work
pKTlacZ	Promoter probe vector containing *lacZ* gene in pKT240	16
pL1411END	PCR-generated 246-bp IS*1411* left-end fragment cloned into pKTlacZ by left terminus towards *lacZ*	This work
PKTtnpA	665-bp *Hin*dIII fragment from pEST1414 cloned into pKTlacZ by left terminus of IS*1411* in opposite direction to *lacZ*	This work

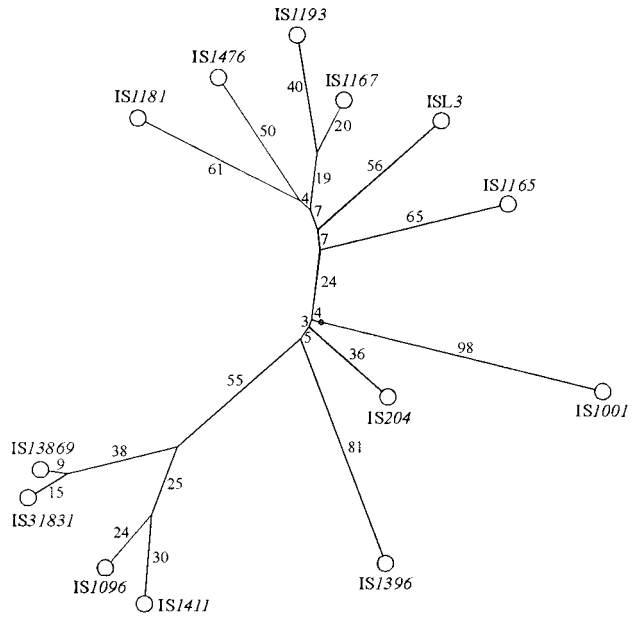

FIG. 3. Unrooted phylogenetic tree of the transposases of IS*1411* and its related elements. Multiple alignment of transposase sequences and construction of the phylogenetic tree were carried out via the CBRG server as described in the text. PAM distances are indicated at the branches of the tree. The tree-fitting index is 0.96. DNA sequence accession numbers and hosts (in parentheses) are as follows: IS*1411* (*Pseudomonas* sp.), M57500; IS*1096* (*M. smegmatis*), M76495; IS*31831* (*C. glutamicum*), D17429; IS*13869* (*B. lactofermentum*), Z66534; IS*1396* (*Serratia marcescens*), U13612; IS*1181* (*Staphylococcus aureus*), L14544; IS*1193* (*Streptococcus thermophilus*), Y13713; IS*1167* (*Streptococcus pneumoniae*), M36180; IS*1476* (*Enterococcus faecium*), U63997; IS*1165* (*Leuconostoc mesenteroides*), X62617; ISL*3* (*Lactobacillus delbrueckii*), X79114; IS*204* (*Nocardia asteroides*), U10634; IS*1001* (*Bordetella parapertussis*), X66858.

pheBA genes due to transposition into the constitutively expressed operon.

Comparison of the sequence of IS*1411* with the sequences of other IS elements. IS*1411* contains a 433-amino-acid-encoding open reading frame, designated ORFA, from nt 97 to 1405. The sequence of ORFA was compared with other sequences present in the GenBank and EMBL databases by using the TFASTA and FASTA programs. The predicted amino acid sequence of ORFA exhibited 60% identity with that of the transposase of IS*1096* from *Mycobacterium smegmatis* and approximately 40% identity with those of the transposases of IS*31831* from *Corynebacterium glutamicum* and IS*13869* from *Brevibacterium lactofermentum*. This analysis clearly indicated that ORFA of IS*1411* encodes transposase, and the gene was designated *tnpA*. TnpA of IS*1411* also displayed motifs similar to the transposase sequences of several other IS elements, i.e., IS*204*, IS*1476*, IS*1181*, IS*1165*, IS*1167*, IS*1396*, IS*1001*, IS*1193*, ISL*3*, IS*AE1*, and IS*T1*. The identity of the amino acid sequence of IS*1411* TnpA with the sequences of the TnpAs of these IS elements ranged from 20 to 24%. The phylogenetic tree of all of the TnpA sequences was constructed via the CBRG (Computational Biochemistry Research Group) server (http://cbrg.inf.ethz.ch/) by using the Darwin program. The tree-fitting index (1.16) when all of the sequences were compared was too high (above 1.0). Therefore, two TnpA sequences, those of IS*AE1* and IS*T1*, which exhibited the lowest level of similarity to the other TnpAs compared, were eliminated in the course of tree construction. The unrooted phylogenetic tree constructed without these sequences, shown in Fig. 3, had a tree-fitting index of 0.96. The tree demonstrated that the TnpAs of IS*1411*, IS*1096*, IS*31831*, and IS*13869* were more closely related to each other than to the rest of the TnpAs.

Multiple sequence alignment of the TnpAs of IS*1411*, IS*1096*, IS*31831*, and IS*13869* revealed strongly conserved amino acids over the entire protein (Fig. 4). The central region of the protein (the sequence from amino acids 209 to 273) contained seven residues (Fig. 4) that were conserved in all 13 TnpA sequences used for construction of the phylogenetic tree shown in Fig. 3. The IRs of the four IS elements compared in Fig. 4 also had a high degree of identity (Fig. 5). Interestingly, although the amino acid sequence of the TnpA of IS*204* exhibited similarity only in the central region of the protein, the IRs of IS*204* had a remarkable degree of homology to the sequences of the IRs of IS*1411* (Fig. 5). Moreover, the terminal 14 nt of the IRs of IS*204* were identical to the terminal sequences of the 24-bp IRs of IS*1096*.

IS*1411* activates transcription of the *pheBA* operon by outward-directed promoters at the left terminus of the element. Many transposons and IS elements carry promoters that can activate transcription of flanking genes (reviewed in references 11 and 25). Therefore, we mapped the transcriptional start site of the *pheBA* operon in the plasmid pINS113, constitutively expressing the *pheBA* genes and carrying an insertion of IS*1411* upstream of these genes, by using primer extension analysis. The 5′ ends of the mRNA were localized at C and G nucleotides, 51 and 105 nt inside the left end of IS*1411*, respectively (Fig. 2 and 6). The upper band was located just 8 nt downstream from the putative promoter sequence that resembled the σ^{70}-type promoter consensus TTGACAN$_{16-18}$TATAAT. The sequences TGGAAA, similar to the −35 hexamer, and TAAGAT, similar to the −10 hexamer, of this promoter were separated by the 18-nt-long spacer sequence. The proximal putative transcription start point was located 7 nt downstream from the sequence TAAGAT and was separated by 17 bp from the sequence TTGGTG, which resembles the −35 hexamer (Fig. 6).

The presence of the outward-directed promoters at the left end of IS*1411* was verified by subcloning the DNA fragment containing the left-end sequence of IS*1411* to the promoter-probe-plasmid pKTlacZ upstream from the promoterless *lacZ* gene (construct pL1411END). A high level of β-Gal expression, about 400 times higher than that of pKTlacZ, was observed in the cells of *P. putida* PaW85 carrying pL1411END (not shown).

Expression of the promoter of the IS*1411* transposase gene. We failed to map the transcription start site of IS*1411 tnpA* both in the plasmid pEST1414 and in plasmids where the genes *pheB* and *pheA* upstream from the element were actively transcribed (not shown). Because the potential promoter region of the *tnpA* gene can be located only within a narrow 100-bp DNA region in the left end of the element (the putative translation initiator codon GTG of the *tnpA* gene is located 105 nt inside the left end of the IS [Fig. 2]), it is possible that the IS element-encoded transposase could repress transcription from its own promoter due to the overlap of the transposase binding site and the *tnpA* promoter. To avoid that possibility, we cloned the DNA fragment containing the potential promoter sequence upstream of the *lacZ* gene into the plasmid pKTlacZ. Expression of the resulting plasmid, pKTtnpA, was investigated in the cells of both *E. coli* and *P. putida* (Fig. 7), and a modest level of expression of β-Gal was observed in the cells carrying pKTtnpA. In comparison with results obtained in the *E. coli* background the level of expression of β-Gal detected in the cells of *P. putida* was approximately three times lower.

To map the transcriptional start site of the *lacZ* transcription

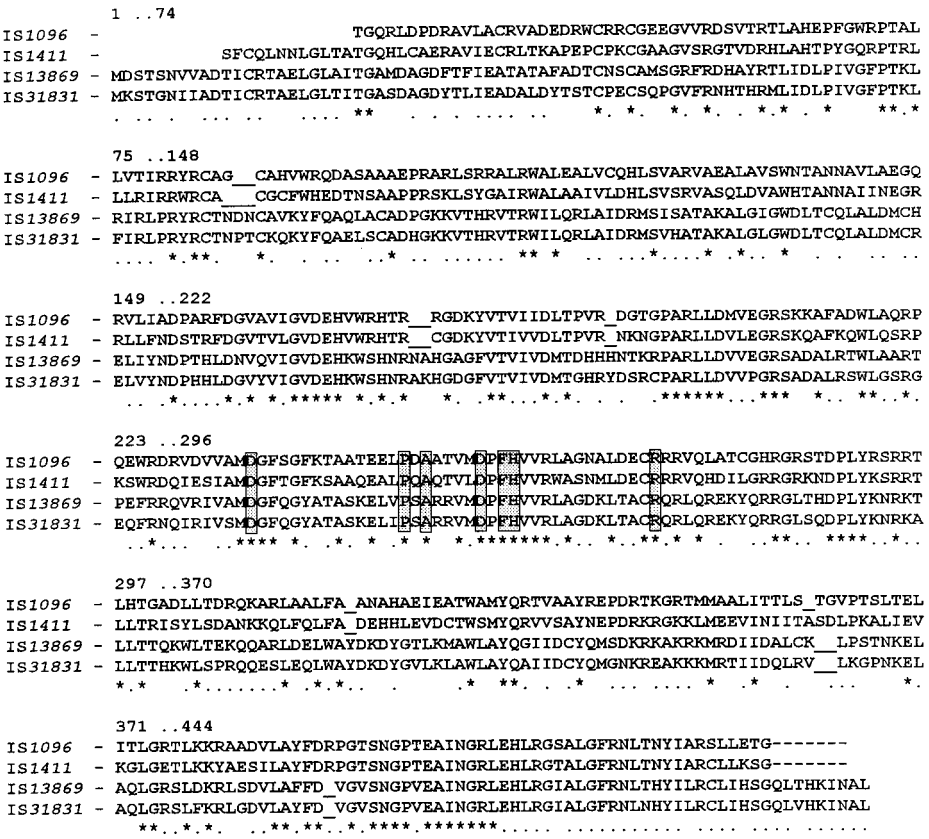

FIG. 4. Alignment of the deduced amino acid sequence of the transposase of IS*1411* with transposases of IS*1096*, IS*13869*, and IS*31831*. Gaps introduced to optimize the alignment are shown by lines. Identical amino acids are marked by asterisks, and similar amino acids are indicated by dots. The alignments were generated via the CBRG server by using the Darwin program. Identical amino acids that were conserved in all 13 transposases analyzed in Fig. 3 are shown by shaded boxes.

fusion in the cells carrying pKTtnpA, primer extension analysis of RNA was performed by using avian myeloblastosis virus reverse transcriptase. Three specific bands at positions −31, −29, and −11 from the *tnpA* translation initiation codon were revealed (these sites are indicated in Fig. 2). Due to the weak activity of the putative *tnpA* promoter, we did not expect to find promoter sequences exhibiting similarity to the well-known promoter consensus. The only candidate exhibiting similarity to the −10 consensus sequence for σ^{70} was the sequence GAAAAT located upstream from the primer extension products identified at nt −31 and −29 from the *tnpA* gene (Fig. 2). The sequence TGGAAA was found 17 bp upstream of this hexamer.

IS*1411* can form circular DNA molecules. If DNA was isolated from *E. coli* cells by an alkaline lysis procedure and electrophoresed in an agarose gel, a small weak band accompanied the band of plasmid pINS113. Analysis of *Hin*dIII digestion of the pINS113 preparation revealed that this band moved in the gel as a 1.4-kb DNA fragment and that it was weaker than the other DNA fragments derived from the *Hin*dIII digestion of pINS113. Formation of IS minicircles has been shown for several IS elements (24, 29, 33, 37, 44, 46). To study whether the 1.4-kb *Hin*dIII DNA fragment represents the linearized IS*1411* circle, we cloned this fragment into pBluescript SK and sequenced the insert. The DNA-sequencing data clearly demonstrated that the 1.4-kb *Hin*dIII fragment was derived from the IS*1411* circle. The IS*1411* circle was composed of the complete IS*1411*, with 5 bp separating the two IS ends. The 5-bp sequence AAACC was derived from the 3′ end of the *pheA* gene just flanking the left IR of IS*1411* at the original location of this element in the plasmid pINS113.

No visible band corresponding to the IS circle was observed in the agarose gel either by gel electrophoresis of the plasmid pEST1414 isolated from *E. coli* carrying the single copy of IS*1411* or by gel electrophoresis of pINS113 or pEST1414 isolated from *P. putida* (not shown). In order to reveal whether circularization of IS*1411* requires more than one copy of the element in the plasmid and whether IS*1411* could also circularize in the cells of *P. putida*, we performed Southern blot analysis of pINS113 and pEST1414 preparations from *P. putida* PaW85 and *E. coli* HB101. Both the uncut DNA and DNA digested with *Kpn*I were gel electrophoresed and transferred onto a nitrocellulose filter. The filter was hybridized with the radioactive probe derived from the IS*1411* circle (see Materials and Methods). The results of the hybridization are shown in Fig. 8. In the case of undigested DNA prepared from *E. coli*, the strong hybridization signal at the location of the IS*1411*

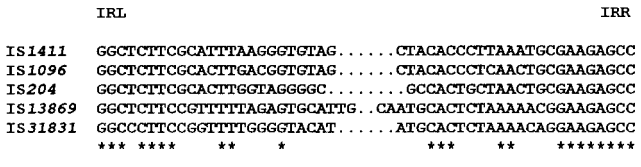

FIG. 5. Sequence alignment of IRs of IS*1411*, IS*1096*, IS*204*, IS*13869*, and IS*31831*. The asterisks indicate the nucleotides conserved in all of the IS elements compared. IRR and IRL, right and left IRs, respectively.

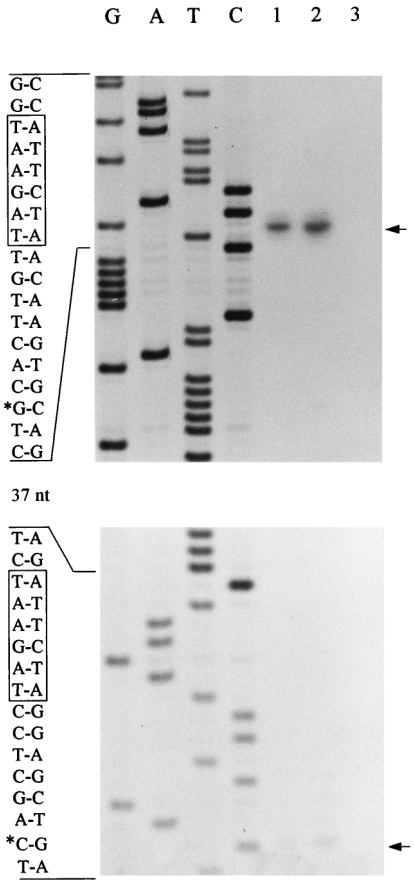

FIG. 6. Mapping of transcription initiation from outward-directed promoters of IS*1411* by reverse transcriptase. Lanes G, A, T, and C show DNA-sequencing reactions of the left end of IS*1411*. Lanes 1 to 3 represent primer extension reactions carried out with total RNA isolated from the following bacteria: lane 1, *P. putida* PaW85 carrying pINS113; lane 2, *E. coli* HB101 carrying pINS113; lane 3, *P. putida* PaW85 carrying pEST1414 (negative control). The primer extension products are indicated on the right by arrows. The interrupted sequence of the left end of IS*1411*, including the −10 sequences of the promoters (boxed) and the transcription start points (indicated by asterisks), are shown on the left.

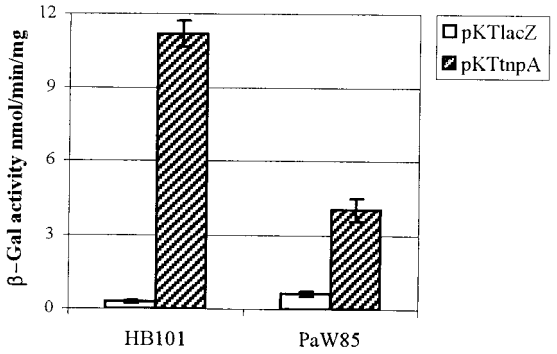

FIG. 7. β-Gal activity measured in *E. coli* HB101 and *P. putida* PaW85 carrying either the promoter-probe-vector pKTlacZ or pKTtnpA containing the *tnpA* promoter region. The data (means ± standard deviations) from at least four independent experiments are presented.

circle was detected if pINS113 was isolated (Fig. 8, lane 3). This signal was weaker in the case of pEST1414 (Fig. 8, lane 4). The strength of the hybridization signal in the pEST1414 preparation was 4% of that of the pINS113 preparation as quantitated by using the PhosphorImager. When DNA was isolated from *P. putida*, the IS*1411* circle became detectable in the pINS113 preparation only after overexposure of the hybridized filter (Fig. 8, lane 2).

Plasmid pEST1414 contains one *Kpn*I site within the *tnpA* gene of IS*1411* (Fig. 1B). Thus, the *Kpn*I digestion of the pEST1414 preparation opens the plasmid molecule and 1.4-kb IS circle. pINS113, carrying two IS elements, is cut into two fragments: an 11.5-kb fragment containing mostly the vector DNA and a 5.4-kb fragment covering sequences of the *pheBA* operon flanked at both sides by IS*1411* sequences up to the *Kpn*I sites (Fig. 1B). According to hybridization data, the 1.4-kb restriction fragment derived from the IS*1411* circle hybridized with the radioactive probe was visible in the case of pINS113 and pEST1414 prepared from *E. coli* (Fig. 8, lanes 7 and 8) and when pINS113 was prepared from *P. putida* (Fig. 8, lane 6). Again, the hybridization signal was strongest in analysis of pINS113 isolated from *E. coli* (Fig. 8, lane 7). If DNA was isolated from *P. putida*, the radioactive band corresponding to the IS circle after gel electrophoresis of the pINS113 preparation became visible after prolonged exposure of the filter (Fig. 8, lane 6), and in addition, an almost undetectable band also appeared in lane 5, containing the pEST1414 preparation.

DISCUSSION

Here we report on a novel IS element, IS*1411*, that was discovered by activation of the transcription of the phenol degradation genes *pheBA* in *P. putida*. We have used the plasmid pEST1414 carrying the promoterless *pheBA* operon as a reporter plasmid to study mutation processes in starving cells of *P. putida* (19). In contrast to the other promoter-creating genetic events (i.e., base substitutions, deletions, and generation of fusion promoters by Tn*4652*) we detected, the insertional activation of the *pheBA* genes by IS*1411* was rare. It has only been discovered once. The IS*1411* that activated the *pheBA* genes originated from the same plasmid, pEST1414, downstream from the activated genes (Fig. 1). The infrequency of the IS*1411* transposition event could be explained by a phenomenon called transposition immunity, i.e., the mobile DNA elements transpose much less frequently into a plasmid replicon that already contains a copy of the element than into a replicon lacking the element (reviewed in references 22 and 25). In addition, the level of expression of the *tnpA* gene of IS*1411* in *P. putida* was very low (Fig. 7).

Vertes et al. (45) suggested that IS*31831* and IS*1096* belong to a new family of IS elements. The homology search for

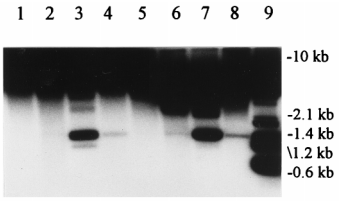

FIG. 8. IS*1411* forms circular DNA molecules. An autoradiograph of the Southern blot of the preparations of pINS113 and pEST1414 is shown. The radioactive DNA probe was prepared from IS*1411*. Marker sizes (lane 9) are indicated on the right of the autoradiograph. Lanes: 1 and 5, pEST1414 prepared from *P. putida*; 2 and 6, pINS113 prepared from *P. putida*; 3 and 7, pINS113 prepared from *E. coli*; 4 and 8, pEST1414 prepared from *E. coli*. Lanes 1 to 4 contain uncut DNA, and lanes 5 to 8 contain DNA cut with *Kpn*I. The additional weak bands on lane 3 are of unknown origin and are not discussed in this report.

IS*1411* revealed, in addition to IS*31831* and IS*1096*, several IS elements that encode transposases exhibiting similarities to the deduced amino acid sequence of the TnpA of IS*1411* (Fig. 3 and 4). The remarkable degree of similarity of the transposase sequences of IS elements analyzed in Fig. 3 indicates that these IS elements belong to the same family. According to the grouping of 443 IS elements in 17 families (25), this is the ISL*3* family. The DNA elements having higher degrees of similarity to IS*1411 tnpA* are distributed among soil bacteria (Fig. 4). These DNA elements also contain similar IRs (Fig. 5) and might constitute a distinct subgroup within the ISL*3* family.

The DNA segment containing the *pheBA* operon and IS*1411* has been cloned from plasmid DNA of *Pseudomonas* sp. strain EST1001, which is a derivative of *Pseudomonas* sp. strain S13 (20, 21). Strain S13 was isolated in 1976 by P. A. Williams and was not, therefore, of local origin. The *tnpA* gene identical to the *tnpA* of IS*1411* has been identified downstream of the chlorobiphenyl degradation genes *bph* in hybrid strain JHR22 of *Burkholderia capacia* (37a). Traces of IS*1411* have also been found in the 4-nitrotoluene-degrading *Pseudomonas* sp. strain TW3: the sequence of *xylB* homologue *ntnB** was interrupted by a piece of DNA identical to the left end of IS*1411* (17). However, we disagree with the authors' interpretation (17) that the disrupting sequence was derived from the Tn*4652*-encoded transposase.

Bacterial transposable elements utilize two major modes of transposition. In nonreplicative transposition (the cut-and-paste mechanism), transposase cuts both DNA strands at the element's two ends, and the element is transferred to the target (e.g., Tn*10* and Tn*7* [reviewed in reference 13]). The replicative transposition involves cointegrate formation, in which the donor and target sequences are fused, the element is duplicated, and then the cointegrate is resolved by recombination between the two copies of the element. This restores the initial donor molecule with one copy of the element and restores the target molecule with the second copy (e.g., Tn*3* family transposons and Mu phage [reviewed in reference 35]). In some cases the resolution step is carried out by element-encoded resolvase. There is no published data about mechanisms of transposition of IS elements similar to IS*1411*. For IS*1096*, it has been shown that this element encodes a putative resolvase (8). However, this is the only indication that IS*1096*-like elements could transpose replicatively. The other related IS elements are smaller, and they have not been shown to contain sequences encoding putative resolvases. The plasmids pINS113 and pM13 with insertions of IS*1411* that we isolated also retained the original copy of the element. Thus, one should discuss the fact that during insertion IS*1411* had duplicated in these plasmids, which indicates that the mechanism of transposition of IS*1411* might be replicative. However, the fact that the plasmids pINS113 and pM13 carry two copies of IS*1411* could just as well be the result of a conservative event in which the IS copy moved from one plasmid to a sibling plasmid.

One of the interesting features of IS*1411* is its formation of IS circles (Fig. 8). Circularization of the transposable element occurs during the transfer of conjugative transposons (30). The circular forms have also been observed among members of the IS*3* family (e.g., IS*3* itself [33], IS*911* [29], IS*2* [24], and IS*150* [46]) and also for other DNA elements (e.g., IS*1* [44], IS*117* [37], and Tn*4451* [9]). It has been supposed that circle formation could be an intermediate step of transposition (9, 37, 44). An unconventional pathway of transposition, i.e., transposition through a circular intermediate, has been experimentally confirmed for IS*2* and IS*911* (24, 38, 39). The formation of a figure eight molecule (in which only one of the IS strands has undergone cleavage and transfer to the opposite end, resulting in circularization of a single strand) as a precursor to the circle has been observed for these elements (24, 38). There is also data indicating that a circular transposition intermediate could arise replicatively (37, 44). Therefore, it is tempting to speculate that IS*1411*, which belongs to the distinct ISL*3* family, can follow (at least in some cases) a transposition pathway that utilizes an IS circle as an intermediate.

The frequency of circularization of IS*1411* was higher in *E. coli* than in *P. putida* (Fig. 8). The fact that formation of the IS*1411* circle was enhanced in the *E. coli* background indicates that the expression of the IS*1411* transposase may be downregulated in *P. putida*. Our attempts to map the IS*1411 tnpA* promoter revealed that the level of expression of the reporter gene *lacZ* under the *tnpA* promoter was also higher in *E. coli* than in *P. putida* (Fig. 7). The basis for these differences is at present unclear. It is possible that transcription of the *tnpA* of IS*1411* is more tightly controlled in *P. putida* than in heterologous hosts. In comparison with plasmid pEST1414 carrying a single copy of IS*1411*, the frequency of IS*1411* circle formation was higher in the case of plasmid pINS113 carrying two copies of the element (Fig. 8). The IS circles can also be easily detected in analysis of the pM13 preparation (not shown). Thus, it is possible that a difference in circularization frequency can be a simple gene dosage effect. The fact that elevated levels of transposase stimulated circle formation has been demonstrated for IS*2* (24) and for IS*911* (38).

Transposable elements isolated from different soil bacteria can be of importance in regulating gene expression due to silencing or activating certain genes. They take part in genomic rearrangements and can be involved in the evolution of new catabolic operons. So far, little is known about the mechanisms and regulation of the transposition of DNA elements in soil bacteria. As discussed above, the IS element IS*1411* is distributed in different aromatic-compound-degrading bacteria. This DNA element is also capable of driving expression of promoterless genes due to the presence of the outward-directed promoters in the left end. Therefore, the study of the transposition processes of IS*1411* would extend our awareness of genetic processes in soil bacteria.

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REFERENCES

1. **Bayley, S. A., C. J. Duggleby, M. J. Worsey, P. A. Williams, K. G. Hardy, and P. Broda.** 1977. Two modes of loss of the TOL function from *Pseudomonas putida* mt-2. Mol. Gen. Genet. **154:**203–204.
2. **Blazey, D., and R. O. Burns.** 1982. Transcriptional activity of the transposable element Tn*10* in the *Salmonella typhimurium ilvGEDA* operon. Proc. Natl. Acad. Sci. USA **79:**5011–5015.
3. **Blomberg, P., E. G. Wagner, and K. Nordström.** 1990. Control of replication of plasmid R1: the duplex between the antisense RNA, CopA, and its target, CopT, is processed specifically *in vivo* and *in vitro* by RNase III. EMBO J. **9:**2331–2340.
4. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:**459–472.
5. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
6. **Charlier, D., J. Piette, and N. Glansdorff.** 1982. IS*3* can function as a mobile promoter in *E. coli*. Nucleic Acids Res. **10:**5935–5948.
7. **Ciampi, M. S., M. B. Schmid, and J. R. Roth.** 1982. Transposon Tn*10* provides a promoter for transcription of adjacent sequences. Proc. Natl. Acad. Sci. USA **79:**5016–5020.

8. **Cirillo, J. D., R. G. Barletta, B. R. Bloom, and W. R. Jacobs, Jr.** 1991. A novel transposon trap for mycobacteria: isolation and characterization of IS*1096*. J. Bacteriol. **173:**7772–7780.
9. **Crellin, P. K., and J. I. Rood.** 1997. The resolvase/invertase domain of the site-specific recombinase TnpX is functional and recognizes a target sequence that resembles the junction of the circular form of the *Clostridium perfringens* transposon Tn*4451*. J. Bacteriol. **179:**5148–5156.
10. **Dodd, H. M., N. Horn, and M. J. Gasson.** 1994. Characterization of IS*905*, a new multicopy insertion sequence identified in lactococci. J. Bacteriol. **176:** 3393–3396.
11. **Galas, D. J., and M. Chandler.** 1989. Bacterial insertion sequences, p. 109–162. *In* D. E. Berg and M. H. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
12. **Glansdorff, N., D. Charlier, and M. Zafarullah.** 1981. Activation of gene expression by IS*2* and IS*3*. Cold Spring Harbor Symp. Quant. Biol. **45:**153–156.
13. **Hallet, B., and D. J. Sherratt.** 1997. Transposition and site-specific recombination: adapting DNA cut-and-paste mechanisms to a variety of genetic rearrangements. FEMS Microbiol. Rev. **21:**157–178.
14. **Hanahan, D.** 1983. Studies on the transformation of *E. coli* with plasmids. J. Mol. Biol. **166:**577–580.
15. **Harayama, S., and K. N. Timmis.** 1989. Catabolism of aromatic hydrocarbons by *Pseudomonas*, p. 151–174. *In* D. A. Hopwood and K. E. Chater (ed.), Genetics of bacterial diversity. Academic Press Ltd., London, England.
16. **Hõrak, R., and M. Kivisaar.** 1998. Expression of the transposase gene *tnpA* of Tn*4652* is positively affected by integration host factor. J. Bacteriol. **180:** 2822–2829.
17. **James, K. D., and P. A. Williams.** 1998. *ntn* genes determining the early steps in the divergent catabolism of 4-nitrotoluene and toluene in *Pseudomonas* sp. strain TW3. J. Bacteriol. **180:**2043–2049.
18. **Kasak, L., R. Hõrak, A. Nurk, K. Talvik, and M. Kivisaar.** 1993. Regulation of the catechol 1,2-dioxygenase- and phenol monooxygenase-encoding *pheBA* operon in *Pseudomonas putida* PaW85. J. Bacteriol. **175:**8038–8042.
19. **Kasak, L., R. Hõrak, and M. Kivisaar.** 1997. Promoter-creating mutations in *Pseudomonas putida*: a model system for the study of mutation in starving bacteria. Proc. Natl. Acad. Sci. USA **94:**3134–3139.
20. **Kivisaar, M., J. Habicht, and A. Heinaru.** 1989. Degradation of phenol and *m*-toluate in *Pseudomonas* sp. strain EST1001 and its transconjugants is determined by a multiplasmid system. J. Bacteriol. **171:**5111–5116.
21. **Kivisaar, M., R. Hõrak, L. Kasak, A. Heinaru, and J. Habicht.** 1990. Selection of independent plasmids determining phenol degradation in *Pseudomonas putida* and the cloning and expression of genes encoding phenol monooxygenase and catechol 1,2-dioxygenase. Plasmid **24:**25–36.
22. **Kleckner, N.** 1990. Regulation of transposition in bacteria. Annu. Rev. Cell Biol. **6:**297–327.
23. **Lessie, T. G., M. S. Wood, A. Byrne, and A. Ferrante.** 1990. Transposable gene-activating elements in *Pseudomonas cepacia*, p. 279–291. *In* S. Silver, A. M. Chakrabarty, B. Iglewski, and S. Kaplan (ed.), *Pseudomonas*: biotransformations, pathogenesis, and evolving biotechnology. American Society for Microbiology, Washington, D.C.
24. **Lewis, L. A., and N. D. F. Grindley.** 1997. Two abundant intramolecular transposition products, resulting from reactions initiated at a single end, suggest that IS*2* transposes by an unconventional pathway. Mol. Microbiol. **25:**517–529.
25. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
26. **Malamy, M. H.** 1970. Some properties of insertion mutations in the *lac* operon, p. 359–373. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Podglajen, I., J. Breuil, and F. Collatz.** 1994. Insertion of a novel DNA sequence, 1S1186, upstream of the silent carbapenemase gene *cfiA*, promotes expression of carbapenem resistance in clinical isolates of *Bacteroides fragilis*. Mol. Microbiol. **12:**105–114.
29. **Polard, P., M. F. Prere, O. Fayet, and M. Chandler.** 1992. Transposase-induced excision and circularization of the bacterial insertion sequence IS*911*. EMBO J. **11:**5079–5090.
30. **Salyers, A. A., N. B. Shoemaker, A. M. Stevens, and L.-Y. Li.** 1995. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. Microbiol. Rev. **59:**579–590.
31. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Schwartz, E., C. Herberger, and B. Rak.** 1988. Second-element turn-on of gene expression in an IS1 insertion mutant. Mol. Gen. Genet. **211:**282–289.
33. **Sekine, Y., N. Eisaki, and E. Ohtsubo.** 1994. Translational control in production of transposase and in transposition of insertion sequence IS*3*. J. Mol. Biol. **235:**1406–1420.
34. **Shapiro, J. A.** 1969. Mutations caused by the insertion of genetic material into the galactose operon of *Escherichia coli*. J. Mol. Biol. **40:**93–105.
35. **Sharma, R. C., and R. T. Schimke.** 1996. Preparation of electro-competent *E. coli* using salt-free growth medium. BioTechniques **20:**42–44.
36. **Sherratt, D.** 1989. Tn*3* and related transposable elements: site-specific recombination and transposition, p. 163–184. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
37. **Smokvina, T., D. J. Henderson, R. E. Melton, D. F. Brolle, T. Kieser, and D. A. Hopwood.** 1994. Transposition of IS*117*, the 2.5 kb *Streptomyces coelicolor* A3(2) "minicircle": roles of open reading frames and origin of tandem insertions. Mol. Microbiol. **12:**459–468.

37a. **Springael, D.** Personal communication.

38. **Ton-Hoang, B., M. Betermier, P. Polard, and M. Chandler.** 1997. Assembly of a strong promoter following IS*911* circularization and the role of circles in transposition. EMBO J. **16:**3357–3371.
39. **Ton-Hoang, B., P. Polard, and M. Chandler.** 1998. Efficient transposition of IS*911* circles *in vitro*. EMBO J. **17:**1169–1181.
40. **Trinh, S., A. Haggoud, G. Reysset, and M. Sebald.** 1995. Plasmids pIP419 and pIP421 from Bacteroides: 5-nitroimidazole resistance genes and their upstream insertion sequence elements. Microbiology **141:**927–935.
41. **Tsuda, M.** 1996. Catabolic transposons in pseudomonads, p. 219–228. *In* T. Nakazawa, K. Furukawa, D. Haas, and S. Silver (ed.), Molecular biology of pseudomonads. ASM Press, Washington, D.C.
42. **Tsuda, M., and T. Iino.** 1987. Genetic analysis of a transposon carrying toluene degrading genes on a TOL plasmid pWWO. Mol. Gen. Genet. **210:**270–276.
43. **Tsuda, M., K.-I. Minegishi, and T. Iino.** 1989. Toluene transposons Tn*4651* and Tn*4653* are class II transposons. J. Bacteriol. **171:**1386–1393.
44. **Turlan, C., and M. Chandler.** 1995. IS*1*-mediated intramolecular rearrangements: formation of excised transposon circles and replicative deletions. EMBO J. **14:**5410–5421.
45. **Vertes, A. A., M. Inui, M. Kobayashi, Y. Kurusu, and H. Yukawa.** 1994. Isolation and characterization of IS*31831*, a transposable element from *Corynebacterium glutamicum*. Mol. Microbiol. **11:**739–746.
46. **Welz, C.** 1993. Functionelle analyse des Bacteriellen Insertionelements IS*150*. Ph.D. thesis. Fakultät für Biologie der Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.
47. **Wood, M. S., A. Byrne, and T. G. Lessie.** 1991. IS*406* and IS*407*, two gene-activating insertion sequences from *Pseudomonas cepacia*. J. Bacteriol. **172:**1719–1724.