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Functional Analysis of Unique Class II Insertion Sequence IS*1071*

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Various xenobiotic-degrading genes on many catabolic plasmids are often flanked by two copies of an insertion sequence, IS*1071*. This 3.2-kb IS element has long (110-bp) terminal inverted repeats (IRs) and a transposase gene that are phylogenetically related to those of the class II transposons. However, the transposition mechanism of IS*1071* has remained unclear. Our study revealed that IS*1071* was only able to transpose at high frequencies in two environmental β-proteobacterial strains, *Comamonas testosteroni* and *Delftia acidovorans*, and not in any of the bacteria examined which belong to the α- and γ-proteobacteria. IS*1071* was found to have the functional features of the class II transposons in that (i) the final product of the IS*1071* transposition was a cointegrate of its donor and target DNA molecules connected by two directly repeated copies of IS*1071*, one at each junction; (ii) a 5-bp duplication of the target sequence was observed at the insertion site; and (iii) a *tnpA* mutation of IS*1071* was efficiently complemented by supplying the wild-type *tnpA* gene in *trans*. Deletion analysis of the IS*1071* IR sequences indicated that nearly the entire region of the IRs was required for its transposition, suggesting that the interaction between the transposase and IRs of IS*1071* might be different from that of the other well-characterized class II transposons.

Bacterial class II (Tn*3*-like) transposons generally carry the genes for their transposition (*tnpA*, *tnpR*, and *res*) and one or more phenotypic traits between their terminal inverted repeats (IRs), which have sizes of less than 50 bp (Fig. 1A and B) (23). These transposons move by a two-step and replicative mechanism (6, 23). In the first step, the *tnpA* product (transposase) acts at the IRs to generate a cointegrate of the donor and target molecules connected by two directly repeated copies of the transposon, one at each junction. In the second step, the cointegrate resolves at the resolution (*res*) sites by means of the *tnpR* product (resolvase). A 5-bp duplication of the target sequence is generated upon transposition. The transposases of the class II transposons are able to catalyze their transposition even when the *tnpA* gene and cognate IRs are located on separate molecules (6, 23). Several class II transposons have been reported to play an important role in the wide dissemination of various catabolic gene clusters, such as toluene-xylenes, naphthalene, and carbazole (17, 29–31). To date, three major groups (Tn*3*, Tn*21*, and Tn*4651*) of class II transposons have been characterized in detail with respect to their structural and functional aspects (Fig. 1A).

IS*1071* is a 3.2-kb insertion sequence (IS) that was originally identified in a chlorobenzoate-catabolic transposon, Tn*5271*, from *Comamonas testosteroni* BR60 (20). On the basis of structural features of its 110-bp IRs and 2,913-bp *tnpA* gene, IS*1071* has been considered to belong to the class II transposons (7, 20). However, this IS element shows the uniqueness in its long (110-bp) IRs and its lack of the resolution function. The identification of many IS*1071* sequences in close proximity to various xenobiotic-degrading genes on self-transmissible plasmids from environmental bacteria, e.g., *Pseudomonas* (18), *Comamonas* (2, 13), and *Wautersia* (3), indicates that IS*1071* must have been involved in the recruitment of catabolic genes to these plasmids and in the dissemination of these genes among various host strains. We have also identified a haloacetate-catabolic IS*1071*-composite transposon, Tn*Had1*, on an IncP-1β plasmid, pUO1, from *Delftia acidovorans* strain B (Fig. 1A) (24, 25). Tn*Had1* is located within a defective class II transposon, Tn*Had2*, which is a Tn*21*-related transposon that lacks the *tnpA* and *tnpR* genes (Fig. 1A) (24). We have previously reported that the two intact copies and one truncated copy of IS*1071* in Tn*Had2* might have been incorporated into an ancestor of Tn*Had2* (24). However, no clear transposition events of the Tn*Had1*-specified IS*1071* element were observed.

No functional analysis of IS*1071* has been carried out since its discovery more than a decade ago. Our functional analysis of IS*1071* in this study has indicated that (i) efficient transposition of IS*1071* occurred in two specific host strains, (ii) IS*1071* had the functional features of the class II transposons, and (iii) almost the entire region of the 110-bp IR was required for transposition.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The strains and plasmids used are listed in Table 1. Luria broth (LB) and LB agar (1) were used throughout this study. *Escherichia coli* cells were cultivated at 37°C and the others at 30°C. The agents added to the media were as follows: ampicillin, 100 μg/ml; chloramphenicol, 50 μg/ml; kanamycin, 50 μg/ml; nalidixic acid, 30 μg/ml; tetracycline, 10 μg/ml; sulfathiazole, 350 μg/ml.

DNA methodology. Standard methods were used for extracting plasmid DNA, DNA digestion with restriction endonucleases, ligation, gel electrophoresis, and transformation of bacterial cells (1). The PCR was carried out

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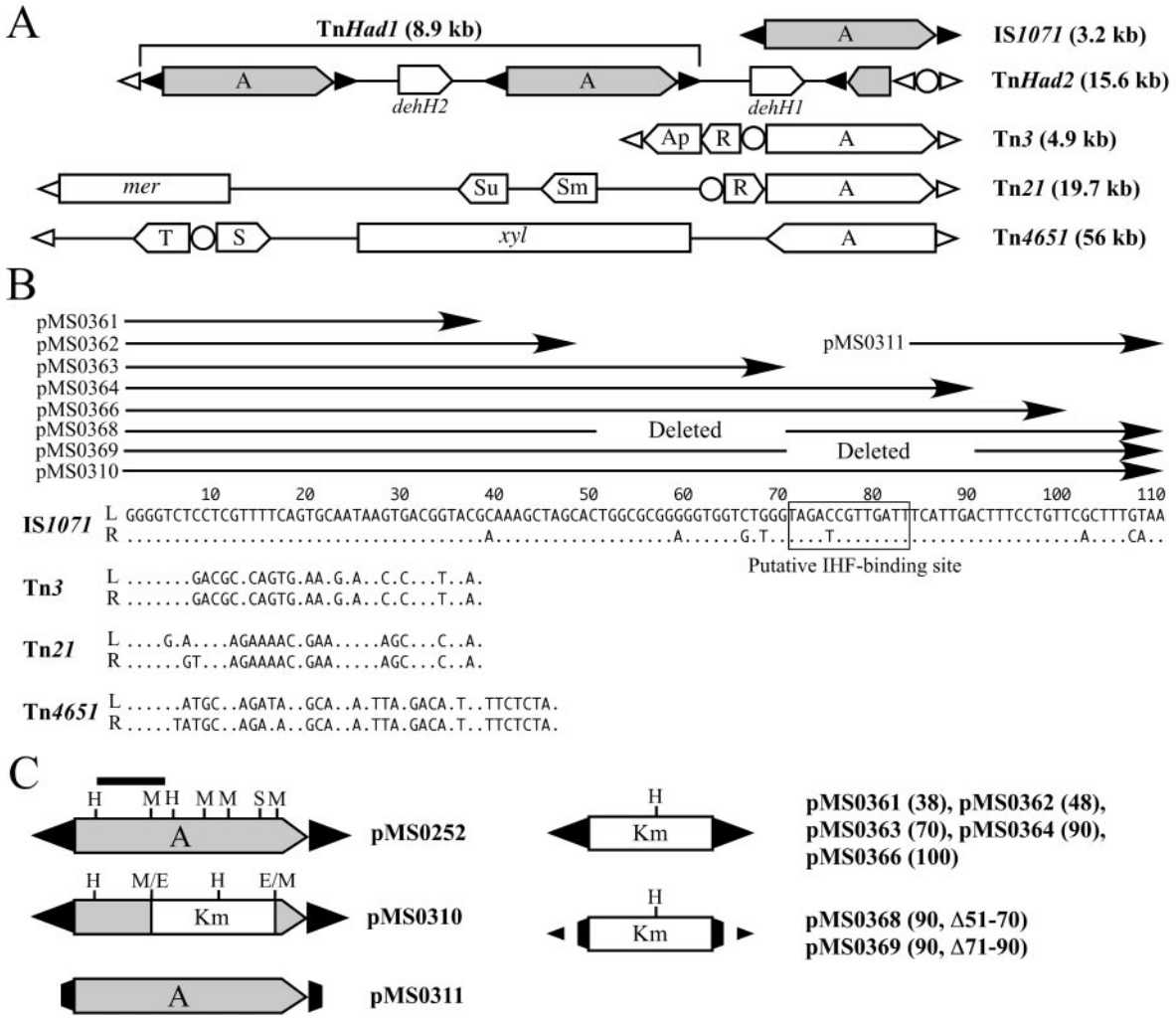

FIG. 1. Structures of class II transposons, their IR sequences, and IS*1071* derivatives. (A) Schematic structures of the class II transposons. The sizes are arbitrary. The black and white arrowheads indicate the terminal IR sequences of IS*1071* and those of the representative transposons, respectively, and the circle represents the *res* site. The pentagon shows the orientation of the gene. Abbreviations: A, *tnpA* gene; R, S, and T, genes for cointegrate resolution; Ap, Sm, Su, and *mer*, genes for resistance to ampicillin, streptomycin, sulfonamide, and mercury, respectively; *xyl*, genes for degradation of toluene and xylenes. (B) Comparison of IR sequences. The left (L) and right (R) ends of IS*1071* are defined as those located upstream and downstream, respectively, of the *tnpA* gene, and those of other transposons are defined as the distal and proximal ends, respectively, to the *tnpA* gene. A dot indicates a nucleotide identical to that in the left IR of IS*1071*. The arrows indicate the IR sequences that are carried in the IS*1071* derivatives on the plasmids depicted. The boxed sequence represents the putative IHF-binding site. (C) Structures of the IS*1071* derivatives on the pMS plasmids. The black bar indicates the DNA fragment used as a probe for the Southern analysis (Fig. 3). E, H, M, and S represent EcoRI, HindIII, MunI, and SalI sites, respectively. The values in parentheses are the lengths of the mutant IRs in the pMS plasmids.

with *ExTaq* DNA polymerase (TAKARA BIO). Purification of PCR-amplified DNA fragments was done with a GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) according to the manufacturer's protocol. Nucleotide sequencing was performed with an ABI PRISM model 310 sequencer (Applied Biosystems). Southern hybridization (1) was done with an ECL Random-Prime Labeling and Detection System (Amersham Biosciences) according to the manufacturer's protocol.

PCR amplification and construction of plasmids. Primer 1071Kpn (5′-ACG TGGTACC**GGGGTCTCCTCGTTTTCAGT**-3′) contained a KpnI site (underlined) and the outermost 20-base sequence of the 110-bp IR of IS*1071* (bold letters). This primer was used for PCR amplification of the entire IS*1071* sequence with pUO1 as the template. The product was cloned into the KpnI site of pBBR1MCS-3 (15) to generate pMS0252 (Fig. 1C). The MunI fragment in the IS*1071* element on pMS0252 was replaced by the 1.3-kb pUC4K-derived EcoRI fragment carrying a Km^r determinant (26), and the resulting Km^r-IS*1071* derivative was inserted into the KpnI site on broad-host-range plasmid pNIT6012 (Table 1) to construct pMS0310 (Fig. 1C). Seven pNIT6012-based plasmids, pMS0361, pMS0362, pMS0363, pMS0364, pMS0366, pMS0368, and pMS0369, carried the Km^r-IS*1071* derivatives with mutant IRs (38-, 48-, 70-, 90-, 100-, 90-, and 90-bp IRs, respectively), and the IRs in the last two plasmids lacked internal 20-bp sequences at different positions (Fig. 1B and C). These plasmids were constructed by PCR with appropriate primers. Primer 1071-84 (5′-GGCCGC TAGC**TCATTGACTTTCCTGTTC**-3′) had an NheI site (underlined) and the 18-bp sequence (bold letters) that annealed to the internal nucleotides (positions 84 to 101, Fig. 1B) of the 110-bp IRs of IS*1071*. This primer was used to amplify an IS*1071* derivative lacking the outermost 83-bp sequences at both ends. Cloning of the amplified product into the XbaI site of pBBR1MCS (16) generated pMS0311 (Fig. 1C). Primers 1071pro1 (5′-TTTTGTCGACGGGGTCTCCTC GTTTTCAGT-3′) and 1071pro3 (5′-TTTTAGATCTCGTGAACCTCAAAA GTGGGA-3′) were used to amplify the 145-bp sequence upstream of the putative *tnpA* gene of IS*1071* (Fig. 2A). The product flanked by SalI (GTCGAC, underlined in 1071pro1) and BglII (AGATCT, underlined in 1071pro3) sites was inserted between the corresponding sites in promoter-probe vector pCB182 (22) to construct pMS0321 (Fig. 2B). The BamHI-PstI fragment of pMS0321 con-

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Source or reference
Strains		
Agrobacterium tumefaciens C58	Free of IS*1071*	35
Comamonas testosteroni JCM5832	Free of IS*1071*; type strain	JCM[a]
Delftia acidovorans B	Harboring pUO1	14
D. acidovorans B123	pUO1-cured derivative of strain B, more than one copy of IS*1071* in the chromosome	14
Escherichia coli DH5α	*recA lacZ*Δ*M15*; free of IS*1071*	1
E. coli HB101	*recA*; free of IS*1071*	1
E. coli JM109	Nal^r *recA hsdR17*; free of IS*1071*	1
Pseudomonas alcaligenes JCM5967	Free of IS*1071*; type strain	JCM[a]
P. putida PpN1	Free of IS*1071*	4
Plasmids		
pUO1	Hg^r, haloacetate degradation, Tn*Had1*, Tn*Had2* (Fig. 1A)	14
pBBR1MCS	Cm^r, broad-host-range cloning vector	16
pBBR1MCS-3	Tc^r, broad-host-range cloning vector	15
pCB182	Ap^r, promoter-probe vector, *lacZ* gene without its promoter sequence (Fig. 2B)	22
pME6012	Tc^r, broad-host-range vector, pVS1 and p15A replicons	8
pNIT6012	Tc^r, pME6012 derivative carrying the *oriT* sequence of RP4 at BamHI site	Laboratory collection
pMS0252	Tc^r, pBBR1MCS-3 derivative carrying IS*1071* (Fig. 1C)	This study
pMS0310	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with 110-bp IRs (Fig. 1B and C)	This study
pMS0311	Cm^r, pBBR1MCS derivative carrying an IS*1071* derivative that lacked the outermost 83-bp sequence in both IRs (Fig. 1B and C)	This study
pMS0321	Ap^r, pCB182 derivative carrying the 145-bp sequence upstream of the *tnpA* gene in IS*1071* between BglII and SalI sites (Fig. 2A and B)	This study
pMS0324	Cm^r, pBBR1MCS derivative carrying the BamHI-PstI fragment of pMS0321 (Fig. 2B)	This study
pMS0326	Cm^r, pBBR1MCS derivative carrying the BglII-PstI fragment of pCB182 (Fig. 2B)	This study
pMS0361	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the outermost 38-bp sequences of the IRs (Fig. 1B and C)	This study
pMS0362	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the outermost 48-bp sequences of the IRs (Fig. 1B and C)	This study
pMS0363	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the outermost 70-bp sequences of the IRs (Fig. 1B and C)	This study
pMS0364	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the outermost 90-bp sequences of the IRs (Fig. 1B and C)	This study
pMS0366	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the outermost 100-bp sequences of the IRs (Fig. 1B and C)	This study
pMS0368	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative whose IRs have a 20-bp deletion at positions 51 to 70 (Fig. 1B and C)	This study
pMS0369	Tc^r Km^r, pNIT6012 derivative carrying a Km^r-IS*1071* derivative whose IRs have a 20-bp deletion at positions 71 to 90 (Fig. 1B and C)	This study
pUC4K	Ap^r Km^r, cloning vector, Km^r gene is flanked by the EcoRI, BamHI, SalI, and PstI sites	26
R388	Tp^r Su^r, conjugal plasmid free of transposons, IncW replicon	32

[a] JCM, Japan Collection of Microorganisms.

taining the 145-bp fragment and a promoterless *lacZ* gene was cloned to the corresponding sites in pBBR1MCS to construct pMS0324 (Fig. 2B). The BglII-PstI fragment of pCB182 that carried the promoterless *lacZ* gene was cloned between the BamHI and PstI sites in pBBR1MCS to generate pMS0326 (Fig. 2B). Primers 1071-287 (5′-GGTCTGGCGCTCCATATTGGTTTCCTGCGC-3′) and 1071-1358 (5′-GCAGCTTGGCAAGGTACTCGATGGCAGGAT-3′) were used to amplify the 1.1-kb portion of IS*1071* (Fig. 1C). The product was used as a probe for Southern hybridization.

Transposition assays. Transposition of the IS*1071* derivatives was assayed by the "mating-out" experiments described previously (24). For this purpose, we introduced pMS0252 and R388 (32) into the bacterial strains listed in Table 2. The resulting strains were employed as donors to mate with *E. coli* JM109 on a membrane filter, and Tc^r Nal^r transconjugants were selected. The transposition frequency was expressed as the number of Tc^r Nal^r transconjugants per number of Su^r Nal^r transconjugants. The Tc^r Nal^r transconjugants were analyzed for their plasmid profiles. Complementation of the IS*1071 tnpA* mutation and transposition of the Km^r-IS*1071* derivatives with mutant ends were also examined by the mating-out experiment. The donor strain was *C. testosteroni* JCM5832 harboring the following three plasmids: (i) R388; (ii) pMS0311, a pBBR1MCS-based plasmid carrying the *tnpA* gene of IS*1071*; and (iii) pMS0310 or one of the other pNIT6012-based plasmids carrying a Km^r-IS*1071* derivative with mutant ends (Fig. 1B and C). Such a JCM5832 derivative was mated with JM109, and Km^r Nal^r transconjugants were selected and analyzed for their plasmid profiles.

β-Gal assays. The bacterial strains harboring pMS0324 or pMS0326 (Fig. 2B) were grown to the early stationary phase in LB containing chloramphenicol and used for β-galactosidase (β-Gal) assays according to the method of Miller (19).

Nucleotide sequence accession number. Our partial sequencing and the previously deposited sequences of IncW plasmid R388 revealed its complete sequence (33,913 bp), and the sequence has been deposited in the DDBJ/EMBL/GenBank databases under accession number BR000038.

RESULTS

Transposition of IS*1071*. Although we previously detected a very low frequency of transposition (1.3×10^{-7}) of the pUO1-

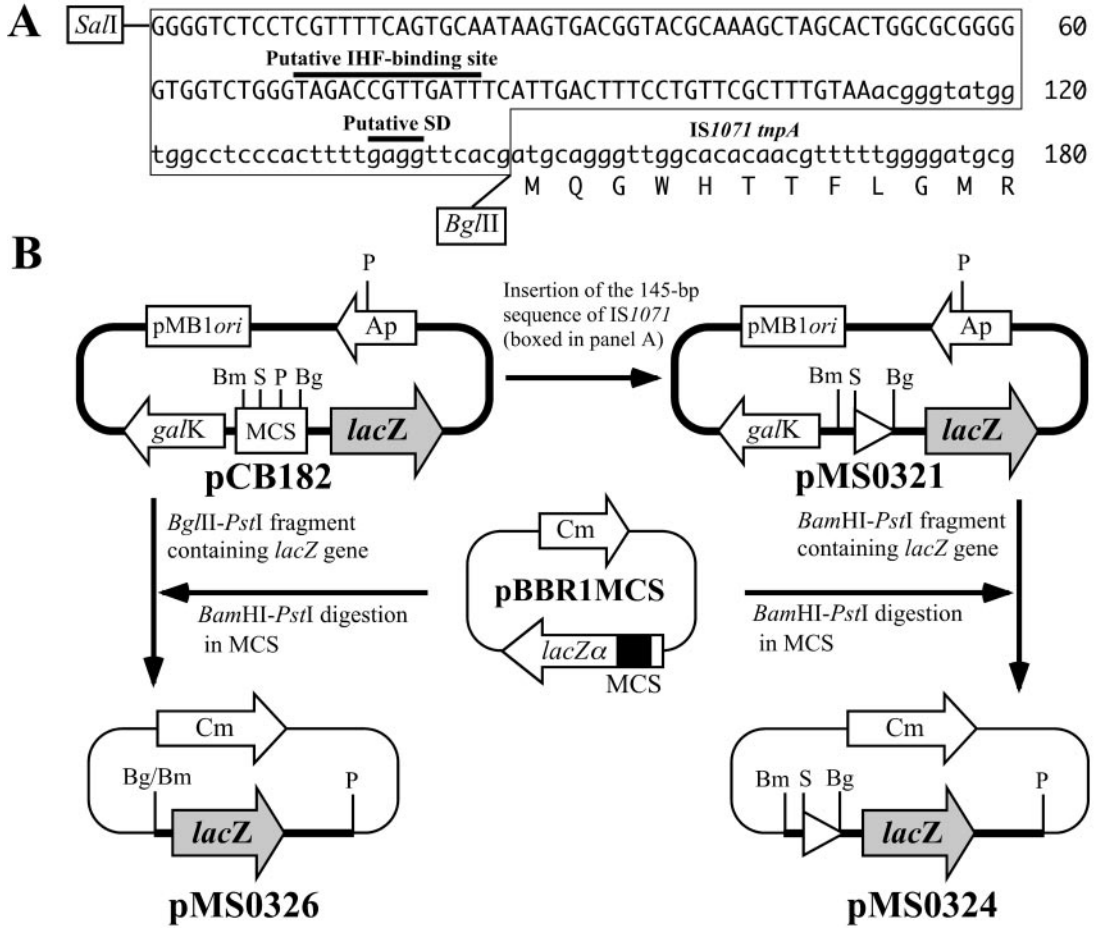

FIG. 2. Construction of plasmids used for *tnpA* promoter analysis. (A) Nucleotide sequence upstream of the *tnpA* gene of IS*1071*. Capital letters indicate the 110-bp IR sequence of IS*1071*. The boxed region (145 bp) was amplified by PCR with primers 1071pro1 and 1071pro3 and used to construct pMS0321. (B) Construction of plasmids. Abbreviations for restriction endonucleases are as follows: B, BamHI; Bg, BglII; P, PstI; S, SalI. Plasmid pCB182 is a promoter-probe vector and carries a multiple-cloning site (MCS) between the *galK* and *lacZ* genes, each of which lacks its promoter sequence (22). The BglII-PstI fragment of pCB182 that carried the promoterless *lacZ* gene was cloned between the BamHI and PstI sites in pBBR1MCS (16) to generate pMS0326. The 145-bp sequence of IS*1071* (white arrowhead) flanked by SalI and BglII sites (see panel A) was inserted between the corresponding sites in pCB182 to generate pMS0321. Subsequent cloning of the BamHI-PstI fragment of pMS0321 containing the *lacZ* gene between the corresponding sites in pBBR1MCS led to the construction of pMS0324.

derived IS*1071* element in *E. coli* (24), our subsequent and repeated attempts to detect such a transposition event in any *E. coli* strains were unsuccessful. Therefore, in this study we investigated IS*1071* transposition in several environmental bacterial strains listed in Table 2. These strains, harboring pMS0252 (=pBBR1MCS-3::IS*1071*) and R388, were employed as the donor host strains in the mating-out experiments. Tc^r Nal^r transconjugants were obtained at high frequencies when *C. testosteroni* JCM5832 and *D. acidovorans* B123 were employed as the donor host strains (Table 2) but not with any of the other donor strains. Restriction and Southern analyses of the plasmids from several Tc^r Nal^r transconjugants revealed that these plasmids were cointegrates of pMS0252 and R388 connected by two directly repeated copies of IS*1071*, one at each junction, since each plasmid had (i) two (4.2- and 3.5-kb) fragments identical to those from pMS0252 (Fig. 3A) and (ii) two copies of IS*1071* (Fig. 3B). It was theoretically possible that these cointegrates were generated by nonreplicative transposition of an IS*1071*-composite transposon from dimers of

TABLE 2. Transposition of IS*1071*

Host strain[a]	Proteobacterial division	Transposition frequency[b]	
		pBBR1MCS-3	pMS0252
A. tumefaciens C58	α	$<1.2 \times 10^{-7}$	$<8.7 \times 10^{-8}$
C. testosteroni JCM5832	β	$<3.3 \times 10^{-7}$	1.5×10^{-3}
D. acidovorans B123[c]	β	$<1.4 \times 10^{-5}$	4.0×10^{-4}
E. coli HB101	γ	$<9.8 \times 10^{-9}$	$<1.6 \times 10^{-8}$
P. alcaligenes JCM5967	γ	$<9.7 \times 10^{-8}$	$<2.1 \times 10^{-8}$
P. putida PpN1	γ	$<1.9 \times 10^{-8}$	$<1.4 \times 10^{-8}$

[a] *D. acidovorans* B123 carried more than one copy of IS*1071* in its chromosome, and the other strains were free of IS*1071* (data not shown).

[b] Transposition of IS*1071* was detected by the cointegration between pMS0252 and R388 (32). Plasmid pMS0252 is a pBBR1MCS-3 derivative carrying IS*1071* (Fig. 1C). The transposition frequency is expressed as the number of Tc^r Nal^r transconjugants per number of Su^r Nal^r transconjugants. A value with the symbol < means that no Tc^r Nal^r transconjugants were obtained in the experiment. All values are averages from at least three independent experiments.

[c] The transfer frequency of R388 was low (2×10^{-4} per donor cell) for an unknown reason(s) when *D. acidovorans* B123 was used as the donor host.

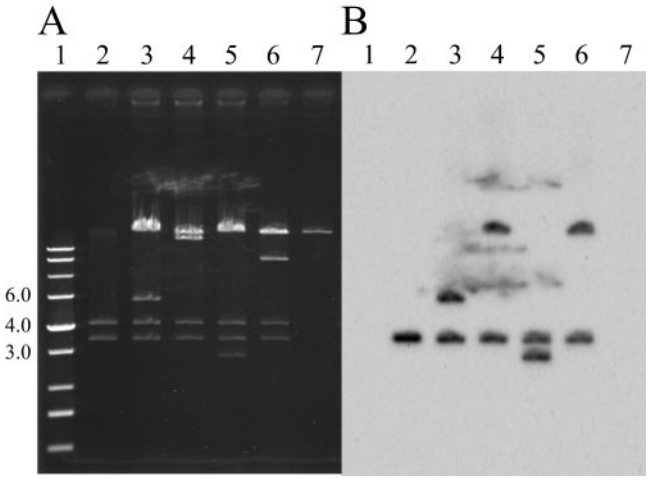

FIG. 3. Analysis of IS*1071*-mediated cointegrates by agarose gel electrophoresis (A) and Southern hybridization patterns with an internal 1.1-kb fragment of IS*1071* (Fig. 1C) as a probe (B). All samples were digested with SalI, which cleaved IS*1071* at one site (Fig. 1C). Lane 1, marker DNA; lane 2, pMS0252; lanes 3 to 6, cointegrates of pMS0252 and R388 obtained in the independent experiments; lane 7, R388. The sizes (in kilobases) of three fragments in the marker DNA are shown on the left.

pMS0252. This possibility was unlikely since agarose gel analysis did not reveal the preferential presence of the pMS0252 dimers in the two host strains (data not shown). Sequence analysis of the insertion sites indicated that IS*1071* transposed to various sites in R388 with concomitant generation of a 5-bp duplication of the target sequence. These results demonstrated that (i) IS*1071* is highly mobile, but only in specific bacterial strains; (ii) IS*1071* is duplicated upon transposition; and (iii) IS*1071* generates a 5-bp duplication of the target sequence. Subsequent transposition experiments in this study were carried out with *C. testosteroni* JCM5832 since this strain was free of IS*1071*.

Complementation of cointegration function. The *tnpA* mutations of the class II transposons are usually complemented efficiently by supplying their cognate wild-type *tnpA* genes in *trans* (23). To investigate whether this was the case with IS*1071*, we conducted a mating-out experiment with *E. coli* JM109 as a recipient strain and a *C. testosteroni* JCM5832 derivative harboring pMS0311 (the supplier of *tnpA*), pMS0310 (the Km^r-IS*1071* carrier) (Fig. 1B and C), and R388 as a donor strain. Km^r Nal^r transconjugants were obtained at a frequency of 3.7×10^{-3} (Table 3, experiment 1). All of the 100 transconjugants examined showed resistance to tetracycline, which was encoded by the vector portion (pNIT6012) of pMS0310. This suggested that all the transconjugants carried the fusion plasmids that have portions of pMS0310 and R388. Detailed analysis of the plasmids from several transconjugants with restriction endonucleases further revealed that they were cointegrates of the two plasmids connected by two directly repeated copies of the Km^r-IS*1071* derivative, one at each junction (data not shown). The absence of the R388::Km^r-IS*1071* plasmids in the 100 transconjugants indicated that the *C. testosteroni* JCM5832-encoded DNA recombination systems (e.g., RecA system) did not function efficiently to resolve the cointegrates rapidly. These results indicated that (i) the wild-type *tnpA* gene of IS*1071* is able to complement its mutation in *trans* and (ii) the final product of the IS*1071* transposition is a cointegrate of its donor and target DNA molecules. The Km^r Nal^r transconjugants were also obtained at very low frequencies when the *tnpA* gene was not supplied (Table 3, experiment 1). However, all the plasmids residing in these transconjugants had an identical structure in which pMS0310 and R388 were fused but not connected by the two copies of Km^r-IS*1071* (data not shown). Such a structure also supported the idea that the IS*1071* derivative was not involved in the cointegration. We did not further examine the mechanism by which such a fusion product was formed because IS*1071* transposition was not involved.

Transposition of IS*1071* derivatives with mutant ends. IS*1071* has 110-bp IRs, the outermost 38-bp sequences of which are similar to the IRs of Tn*3* and Tn*21* (Fig. 1B). Considering that Tn*3* and Tn*21* are able to transpose by using their 38-bp IRs (23), we constructed several Km^r-IS*1071* derivatives with shorter IRs and investigated their transposition. A *C. testosteroni* JCM5832 derivative harboring pMS0311, R388, and a pNIT6012-based plasmid carrying a Km^r-IS*1071* derivative with mutant ends (Fig. 1B and C) was mated with JM109. Use of the seven pNIT6012 derivatives generated the Km^r Nal^r transconjugants at frequencies of 10^{-6} to 10^{-7}, and these frequencies were very similar regardless of the presence or absence of the *tnpA* gene (Table 3, experiments 2 to 8). Furthermore, the transconjugants in each experiment had a fusion product of R388 and the pNIT6012-based plasmid that was not generated by Km^r-IS*1071* (data not shown). These results strongly suggest that IS*1071*-mediated cointegration required almost the entire region of its 110-bp IRs.

Transcriptional activity of the *tnpA* gene. To know why IS*1071* was transposable in the *Comamonas* and *Delftia* cells but not in the *Agrobacterium*, *E. coli*, and *Pseudomonas* cells (Table 2), we investigated the promoter activity of the IS*1071*-specified 145-bp sequence that was located just upstream of its *tnpA* gene (Fig. 2A). Plasmid pMS0324, which carried this sequence in front of a promoterless *lacZ* gene (Fig. 2B), was introduced into the strains listed in Table 2, and the β-Gal activities of the resulting strains were assayed. As shown in Table 4, *C. testosteroni* JCM5832, *Agrobacterium tumefaciens*

TABLE 3. Transposition of Km^r-IS*1071* derivatives with mutant IRs[a]

Expt	Donor of Km^r-IS*1071*[b] (IR length, bp)	Transposition frequency[c]	
		pBBR1MCS	pMS0311
1	pMS0310 (110)	8.5×10^{-7}	3.7×10^{-3}
2	PMS0366 (100)	1.5×10^{-6}	8.7×10^{-6}
3	pMS0364 (90)	2.4×10^{-6}	6.0×10^{-6}
4	pMS0363 (70)	8.0×10^{-7}	4.2×10^{-7}
5	pMS0362 (48)	8.7×10^{-7}	3.8×10^{-6}
6	pMS0361 (38)	4.7×10^{-7}	2.9×10^{-6}
7	pMS0368 (90, Δ51–70)	3.7×10^{-6}	8.7×10^{-6}
8	pMS0369 (90, Δ71–90)	3.1×10^{-6}	2.8×10^{-6}

[a] The donor strain was a *C. testosteroni* JCM5832 derivative harboring the three following plasmids: (i) R388, (ii) a pNIT6012 derivative carrying a Km^r-IS*1071* derivative with the mutant IRs, and (iii) either pBBR1MCS or pMS0311, a pBBR1MCS derivative that carried the *tnpA* gene of IS*1071* (Fig. 1B and C).

[b] The IR sequences and structure, respectively, of each Km^r-IS*1071* derivative are shown in Fig. 1B and C.

[c] The transposition frequency is expressed as the number of Km^r Nal^r transconjugants per number of Su^r Nal^r transconjugants. All values are averages from at least three independent experiments.

TABLE 4. Promoter analysis of the *tnpA* gene[a]

Host strain	IS*1071*-mediated cointegration	β-Gal activity (Miller units)	
		pMS0324	pMS0326
A. tumefaciens C58	No	142 ± 31	7.6 ± 2.1
C. testosteroni JCM5832	Yes	9.2 ± 0.6	ND[b]
D. acidovorans B123	Yes	ND	ND
E. coli DH5α	No	ND	ND
P. alcaligenes JCM5967	No	ND	ND
P. putida PpN1	No	33 ± 4.0	ND

[a] β-Gal activity was assayed by the method of Miller (19). Plasmid pMS0324 carries a *tnpA* promoter-*lacZ* transcriptional fusion, whereas pMS0326 lacks the *tnpA* promoter region and was used as a negative control. The nucleotide sequence upstream of the *tnpA* gene is shown in Fig. 2A, and the structures of the plasmids used in this analysis are shown in Fig. 2B. The values are averages from three independent experiments and are shown with the standard deviations.

[b] ND, not detected in a 60-min reaction.

C58, and *Pseudomonas putida* PpN1 cells harboring pMS0324 showed low β-Gal activities but the activities were not detected in *E. coli* DH5α, *D. acidovorans* B123, and *Pseudomonas alcaligenes* JCM5967. These results were inconsistent with the transposition experiments since transposition of IS*1071* in *A. tumefaciens* C58 and *P. putida* PpN1 was not detected despite the positive transcriptional activity of the *tnpA* promoter in these hosts. Thus, differences in promoter activity cannot be the cause of the observed differences in transposition activity among the strains examined.

DISCUSSION

In this study, we showed that (i) IS*1071* transposed by a replicative mode to generate a cointegrate of its donor and target molecules as the final product, (ii) a 5-bp duplication of the target sequence was generated upon transposition, and (iii) a *tnpA* mutation of IS*1071* was efficiently complemented by the supply of the wild-type *tnpA* gene in *trans*. The experimental data obtained in this study confirm that IS*1071* is classified as a class II transposon. A remarkable difference between IS*1071* and other typical class II transposons is the absence of the resolution function. Such a function is not absolutely required for completion of the transposition reaction of IS*1071* since the host-specified RecA system is also able to resolve the cointegrate by homologous recombination between two directly repeated copies of a transposon (6). It has been proposed that the class II transposons would have evolved from an IS*1071* or IS*1071*-like element by acquisition of the resolution systems (7, 20). This is consistent with the fact that the phylogenetic tree of the enzymes for the resolution systems does not agree with that of the transposases from various class II transposons (7); for example, the transposases of Tn*5403* (accession no. X75779) and Tn*5393* (M95402) have 62% amino acid identity but their resolvases share only 35% identity. It would be interesting to examine by what special molecular mechanisms the resolution function was acquired.

The length (110 bp) of the IRs is another remarkable characteristic of IS*1071* since the IRs of other prokaryotic class II transposons are less than 50 bp long (Fig. 1B). Our deletion analysis in this study revealed that the outermost 100-bp part of the IRs was not sufficient for the transposition of IS*1071* (Table 3). It is known that transposases of several class II transposons cooperatively bind to their IRs with the integration host factor (IHF) (12, 34). A well-studied example is Tn*4652*, a deletion derivative of Tn*4651* from *P. putida* KT2440 (12, 34). Tn*4652* requires the IHF for its efficient transposition (10, 12). It has been considered that an unidentified *Pseudomonas*-specific host factor(s) activates the transcription of the *tnpA* gene promoter and that binding of the *Pseudomonas* IHF to the ends of Tn*4652* is also required for its transposition (10, 12). Since the IHF generally binds to the flanking region of its target sequence (12) and IS*1071* carries a putative IHF-binding site in its both IRs (Fig. 1B), it is likely that lack of the IHF-binding sites in the mutant IRs of IS*1071* might be a reason why our IS*1071* mutants were unable to transpose. The apparent requirement of the long IR sequences for IS*1071* transposition also indicates that the specific recognition and binding of the IS*1071* transposase to its IRs might be different from those of other typical class II transposons such as Tn*3* and Tn*21*. In vivo binding experiments of the IS*1071* transposase and the IHF with its cognate IRs will provide some clues to clarify the unique interaction between the transposase and IRs.

In this study, high-frequency transposition of IS*1071* was detected only in two β-proteobacteria and not in any of the other bacteria tested (Table 2), which belong to the α- and γ-proteobacteria. Since *E. coli* JM109, used as the recipient cell in the transposition experiments, has an *hsdR* (restriction-defective) mutation, the host-specific detection of the IS*1071* transposition was not due to the restriction of the transferred cointegrates in JM109. It should be noted that our experiment with pMS0252 and R388 cannot detect the IS*1071* transposition if (i) IS*1071* transposes into R388 by a simple insertion mechanism or (ii) the cointegrate of pMS0252 and R388 resolves rapidly by the host-encoded DNA recombination systems (6). However, this idea is not valid because (i) we never obtained the R388::Km^r-IS*1071* plasmid and (ii) the cointegrate of pMS0252 and R388 was stably maintained in *A. tumefaciens*, *P. putida*, and *P. alcaligenes* (data not shown). The strain-specific transposition of IS*1071* is consistent with our database searches, which showed that IS*1071* and its remnants are, in addition to their preferential localization on the broad-host-range IncP-1β plasmids (18, 21, 27, 28), mainly distributed on the chromosomes of several β-proteobacterial strains such as *D. acidovorans* P4a (9), *Wautersia metallidurans* CH34 (accession no. X90708), and *Burkholderia xenovorans* LB400 (5). Moreover, the nucleotide sequence of IS*1071* is highly conserved (>99%) in many bacterial strains, indicating that this element might have been maintained only in a limited number of closely related bacterial strains. One possible explanation for the host-specific transposition of IS*1071* is that its transposition requires or is inhibited by some host-specific factor(s). The other possibility is the defect in posttranscriptional steps of the *tnpA* transcript (translation, holding, and maturation, etc.). It could also be envisioned that the host specificity is involved in the codon usages of the host cells, because (i) taxonomically closely related organisms have similar codon usages (11) and (ii) both *C. testosteroni* and *D. acidovorans* are members of the family *Comamonadaceae* (33). These ideas are consistent with our preliminary experiment in that IS*1071* transposed only at a very low frequency (9.6×10^{-7}) in *E. coli* cells, even when its *tnpA* gene was expressed under the control of a *tac* promoter. We do not know which one of the three ideas is

the most plausible. It is of great interest to uncover the specific host factor(s) of *Comamonas* and *Delftia* cells that plays a crucial role in IS*1071* transposition.

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