Applied and Environmental Microbiology, Apr. 2005, p. 1822–1828 Vol. 71, No. 4
0099-2240/05/$08.00+0 doi:10.1128/AEM.71.4.1822–1828.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

High-Temperature-Induced Transposition of Insertion Elements in *Burkholderia multivorans* ATCC 17616

Yoshiyuki Ohtsubo,* Hiroyuki Genka, Harunobu Komatsu, Yuji Nagata, and Masataka Tsuda

Department of Environmental Life Sciences, Graduate School of Life Sciences, Tohoku University, Sendai, Japan

Received 29 July 2004/Accepted 5 November 2004

An efficient and quantitative method to analyze the transposition of various insertion sequence (IS) elements in *Burkholderia multivorans* ATCC 17616 was devised. pGEN500, a plasmid carrying a *Bacillus subtilis*-derived *sacB* gene, was introduced into ATCC 17616 cells, and 25% of their sucrose-resistant derivatives were found to carry various IS elements on pGEN500. A PCR-based experimental protocol, in which a mixture of several specific primer pairs was used, revealed that pGEN500 captured, in addition to five previously reported IS elements (IS*401*, IS*402*, IS*406*, IS*407*, and IS*408*), three novel IS elements, IS*Bmu1*, IS*Bmu2*, and IS*Bmu3*. The global transposition frequency of these IS elements was enhanced more than sevenfold under a high-temperature condition (42°C) but not under oxidative stress or starvation conditions. To our knowledge, this is the first report demonstrating the elevated transposition activities of several IS elements at a high temperature. The efficient experimental protocol developed in this study will be useful in quantitatively and simultaneously investigating various IS elements, as well as in capturing novel functional mobile elements from a wide variety of bacteria.

The transposition of insertion sequence (IS) elements within a genome plays important roles in the evolution of host cells (23, 26). IS transposition usually completely inactivates a gene at a target site but sometimes leads to the constitutive expression of an adjacently located cryptic or regulated gene by delivering IS-loaded promoter sequences (16, 22, 23). Moreover, two or more copies of an identical IS element dispersed over a genome promote various genetic rearrangements, including inversion, deletion, and duplication of the intervening region, and fusion of two replicons residing in the same cell (1, 10, 23, 29). Therefore, IS transposition is one of the most important driving forces that enhance the variability and consequently the adaptive and evolutionary capacities of their hosts.

The *Burkholderia cepacia* complex (Bcc) consists of several species of closely related bacteria belonging to the β-subgroup of proteobacteria (5, 28). The Bcc species are widely disseminated throughout various environments, including animals, plants, and soil (3, 17, 20, 34). Such wide dissemination of the Bcc has been proposed to be due to their extraordinary metabolic versatility per se and their capability to adapt to new environments as a result of a high degree of IS-mediated genome rearrangements (21). A soil-derived *Burkholderia multivorans* strain, ATCC 17616 (20), which belongs to the Bcc, has three circular chromosomes and a 170-kb plasmid, pTGL1 (4, 19). This strain has been reported to carry 10 active IS elements (IS*401*, IS*402*, IS*403*, IS*404*, IS*405*, IS*406*, IS*407*, IS*408*, IS*411*, and IS*415*) (22). IS*402*, IS*403*, IS*404*, and IS*405* have been identified based on their ability to allow the expression of a promoterless gene by providing outwardly directed promoter sequences present on the IS elements (8, 32). IS*411* has been found to be an active IS element on pTGL1 (1). The remaining IS elements have been identified in a screening protocol, in which their insertion into the λ*cI* repressor gene resulted in the derepression of the *cI*-repressed genes (1, 30, 35, 36). However, the available sequences of the IS elements in the public databases are limited to the complete sequences of IS*401*, IS*402*, IS*406*, and IS*407* as well as the incomplete sequence of IS*408*.

The transposition of IS elements under certain growth conditions is likely to be beneficial for the survival of host cells and for the expression of new genetic traits. The aim of this study was to test whether any of several growth conditions would affect the transposition activities of the IS elements in *B. multivorans* ATCC 17616 cells. For this purpose, we employed a *sacB*-based strategy (11) for the entrapment of active IS elements from the ATCC 17616 genome and a PCR-based strategy, in which several specific primer pairs were simultaneously used to classify the entrapped IS elements. Four growth conditions were examined; we entrapped various IS elements that included three novel IS elements (IS*Bmu1*, IS*Bmu2*, and IS*Bmu3*), and a high-temperature condition was found to enhance the transposition activities of several IS elements. The experimental scheme employed in this study will be generally applied for the quantitative and simultaneous detection of the transposition activities of various IS elements in a wide variety of bacteria.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The strains used in this study were *B. multivorans* ATCC 17616 (34) and three *Escherichia coli* strains, JM109, S17-1, and HB101 (24). JM109 was used for the construction and preparation of plasmids. S17-1 had a chromosomal copy of a trimethoprim resistance gene (19). *E. coli* and *B. multivorans* cells, unless otherwise stated, were cultivated at 37 and 30°C, respectively. The liquid media used were M9 minimal medium containing 10% sucrose as a carbon source and 1/3 Luria-Bertani (LB) medium (6.66 g of Bacto Tryptone per liter, 3.33 g of yeast extract per liter, and 5 g of NaCl per liter). The solid media were prepared by the addition of 1.5% agar. Selective

* Corresponding author. Mailing address: Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Sendai 980-8577, Japan. Phone: 81-22-217-5743. Fax: 81-22-217-5699. E-mail: yohtsubo@ige.tohoku.ac.jp.

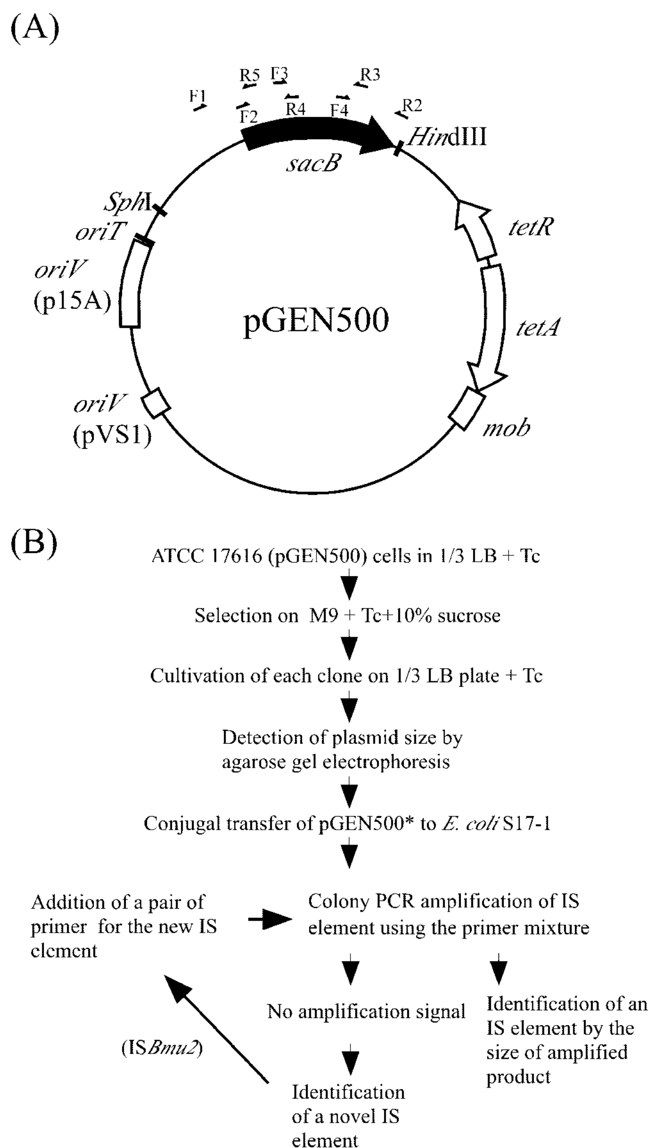

FIG. 1. Strategy to entrap IS elements from ATCC 17616. (A) Structure of pGEN500. The arrows indicate the primer-annealing regions. F1 to F4 and R2 to R5 denote primers listed in Table 2. (B) The experimental scheme developed and used in this study. See the text for the details of this scheme. The pGEN500::IS derivative is indicated as pGEN500*. Tc, tetracycline.

agents added to the media were as follows: kanamycin at 30 μg/ml, tetracycline (TET) at 20 μg/ml, and trimethoprim at 100 μg/ml.

Basic DNA manipulation. Established protocols (19) were used for the following procedures: preparation of genomic and plasmid DNA, DNA digestion with restriction endonucleases, ligation, agarose gel electrophoresis, Southern hybridization, and transformation of *E. coli* and *B. multivorans* cells. PCR was performed with *ExTaq* DNA polymerase (TaKaRa, Kyoto, Japan) using the reaction solution provided by the manufacturer.

Entrapment of IS elements from ATCC 17616. A TET-resistant plasmid, pNIT6012 (15), carries the replication origins of a *Pseudomonas* plasmid, pVS1, and an *E. coli* plasmid, p15A. The *oriT* region from p15A and the *mob* region from RP4 were also present on pNIT6012, therefore allowing its conjugal mobilization by supplying in *trans* the RK2-specified transfer genes from, for example, pRK2013 (9). A 2.7-kb SphI-HindIII fragment containing the *Bacillus subtilis*-derived *sacB* gene was excised from pMOB3 (31) and inserted into the corresponding sites of pNIT6012 to generate pGEN500 (Fig. 1A). The frozen stock cells of *B. multivorans* ATCC 17616(pGEN500) were streaked onto a 1/3 LB agar plate containing TET, and a single colony was cultivated in 1/3 LB broth for 12 h with vigorous shaking. A 150-μl portion of the culture was transferred to 5 ml of fresh 1/3 LB broth and incubated under various growth conditions. Viable cells in the resulting culture were counted by plating appropriate dilutions onto 1/3 LB agar plates containing TET. A portion of the same culture was plated onto M9 agar plates containing TET and 10% sucrose, and the colonies thus formed were transferred to and grown on 1/3 LB agar plates containing TET. Each colony was directly transferred by a sterilized toothpick in a microcentrifuge tube containing 20 μl of electrophoresis loading buffer with RNase A (10 μg/ml). After the subsequent addition of 20 μl of phenol-chloroform reagent, the tube was vortexed for 3 min and centrifuged for 5 min at 15,000 rpm (20,400 × *g*), and the supernatant was used for agarose gel electrophoresis. The colonies that contained plasmids larger than pGEN500 were retained for further analysis.

Conjugal transfer of plasmid from ATCC 17616 into *E. coli* S17-1. Individual ATCC 17616 clones containing the pGEN500 derivatives were grown overnight in 50 μl of 1/3 LB broth in a 96-well microtiter plate. Each 50-μl portion of the recipient (S17-1) and the helper [HB101(pRK2013)] cells grown overnight was then added to each well. The 20-μl portions of the mixture were transferred to a new 96-well microtiter plate filled with LB agar. The plate was air dried to facilitate the conjugation and incubated for 24 h at 30°C. The cells in the wells were thereafter suspended in 20 μl of LB and transferred to an LB agar plate containing TET and trimethoprim in order to select the transconjugants that received the pGEN500 derivatives.

Analysis of IS elements entrapped on pGEN500. Classification of the IS elements entrapped on pGEN500 was carried out by colony PCR. The S17-1 cells carrying the pGEN500 derivative were transferred by a toothpick into a tube containing the PCR solution. The primers listed in Table 1 were used, each at a final concentration of 0.2 pmol/μl of reaction mixture. PCR was carried out under the following conditions: 1 cycle of 94°C for 5 min; 30 cycles of 94°C for 15 s, 60°C for 15 s, and 72°C for 30 s; and 1 cycle of 72°C for 5 min. The PCR products were analyzed by electrophoresis through 2% agarose gel, and the IS elements entrapped on pGEN500 were classified by the size of the amplified products (Table 1). When no PCR amplification of any of the expected sizes was detected, the corresponding plasmid was prepared by a standard protocol from a liquid culture. For the determination of the insertion sites of the novel IS elements on such plasmids, four PCRs that were designed to amplify different parts of the *sacB* region on pGEN500 were carried out (Table 2 and Fig. 1A). The primers used in these PCRs were also used for DNA sequencing.

Nucleotide sequencing and accession numbers of the IS elements. The nucleotide sequencing was carried out using an ABI310 sequencer (Applied Biosystems Japan, Tokyo, Japan). The DNA sequences of novel IS elements were determined by primer walking. The complete nucleotide sequences of IS*Bmu1*, IS*Bmu2*, IS*Bmu3*, and IS*408* have been deposited in the DDBJ/EMBL/GenBank databases under the accession numbers AB159773, AB159774, AB159775, and AB182267, respectively.

RESULTS

Experimental scheme for the entrapment and identification of IS elements. We have previously observed that the expression of the *sacB* gene in the presence of 10% sucrose was severely toxic to the growth of *B. multivorans* ATCC 17616 cells (19). This observation led us to expect that the *sacB* gene might be suitable to entrap active IS elements from this strain. Since pNIT6012 (15) was found to be stably maintained in ATCC 17616, the *sacB* gene was inserted into pNIT6012 to obtain pGEN500 (Fig. 1A).

The quantitative analysis of the transposition of various IS elements usually requires the multistep and laborious characterization of a large number of candidate plasmids that might have entrapped the IS elements (13, 14, 18, 25, 30). To overcome these inconveniences, we developed an efficient experimental scheme, depicted in Fig. 1B. The sucrose-resistant derivatives of ATCC 17616(pGEN500) were selected on M9 agar plates containing TET and 10% sucrose. The plasmids residing in these derivatives were directly extracted from the colonies and electrophoresed in agarose gel in order to examine their

TABLE 1. Primers for classification of entrapped IS elements

Target	Primer	Sequence (5′ to 3′)	Primer-annealing region[a]	Expected length of the product (bp)
IS*401*	IS401X	GAGGAGGCTCCAACTCTTGAGAGAAT	19 to 44	100
	IS401Y	CGGTGCTCTTGCACCATGCGCA	118 to 97	
IS*402*	IS402X	GCGGTGAGCTGTTAACCTCAGGCA	41 to 64	150
	IS402Y	AAACAGGCAGGCGGCCCGGGTTCT	190 to 167	
IS*406*	IS406X	AAGGAGTGTATCCCTCATGGCGAT	145 to 168	200
	IS406Y	CATTGGCGTAATCGCGCCGTGTTT	344 to 321	
IS*407*	IS407X	ACAGGGCCAGCCGGAGTCTAGTAA	22 to 45	250
	IS407Y	TCTTCAGCCGGGCATTCTCCACCT	271 to 248	
IS*408*	IS408X	GCTGTCGTGCGGCATCTGCTGA	1552 to 1573	300
	IS408Y	GACTGCATCAACATCGTGACCTCCTT	1851 to 1826	
IS*Bmu2*	ISBmu2X	GGAACGGACCCACGACGATGAAGA	70 to 93	350
	ISBmu2Y	GCGCCGGAACTTCAACAGCGTGGT	419 to 396	

[a] The numbers indicate the primer-annealing region on the IS element. The left end of each IS element was defined as position 1.

sizes. The plasmids larger than pGEN500 were thereafter conjugally transferred from *B. multivorans* ATCC 17616 to *E. coli* S17-1 by triparental mating using pRK2013 as a helper plasmid. The pGEN500 derivatives transferred were then analyzed by colony PCR. For this PCR analysis, a mixture of six primer pairs was designed based on the nucleotide sequences of five reported IS elements (IS*401*, IS*402*, IS*406*, IS*407*, and IS*408)* as well as on that of an IS element newly identified in this study (IS*Bmu2*) (Table 1). Each primer pair was specifically able to amplify a part of one of the six IS elements, with a size unique to each element, thereby allowing the rapid identification of an IS element entrapped on pGEN500. No amplification by the colony PCR suggested the presence of novel IS elements on pGEN500. Each of the plasmids was prepared by the standard protocol, and the approximate insertion site of the IS element in each plasmid was determined by the results of four PCRs, in which four partially overlapping regions in the *sacB* gene on pGEN500 were targeted for amplification. No PCR amplification in one of the four regions identified the region carrying the IS element (Fig. 1A and Table 2). The primers used for the determination of the insertion site were also used for the determination of the nucleotide sequence of the entrapped IS elements.

Entrapment of IS elements and their analysis. Since some growth conditions have been reported to affect the transposition of mobile genetic elements (23, 27), the entrapment of IS elements from ATCC 17616 was examined under the normal growth conditions (i.e., overnight growth at 30°C) and under high-temperature, oxidative stress, and starvation growth conditions (overnight growth at 42°C, overnight growth at 30°C in the presence of 0.1 mM paraquat [0.5 mM was lethal to the cells], and growth for 3 days at 30°C [which led to a 10-fold decrease in the number of viable cells], respectively). After the cultivation of ATCC 17616(pGEN500) cells under each of the four conditions, the cells were plated onto M9 medium containing TET and sucrose. For each of the four conditions, three independent experiments were performed, and 32 sucrose- and TET- resistant clones were randomly chosen in each experiment. Consequently, we collected 96 clones for each condition and 384 clones in total (Tables 3 and 4). Among the 384 clones, 97 clones had plasmids larger than pGEN500. The PCR analysis using mixed primers for IS*401*, IS*402*, IS*406*, IS*407*, and IS*408* indicated that 23 plasmids carried one of the five elements (Table 4). The IS elements on the remaining 74 plasmids differed from the four previously reported IS elements, IS*403*, IS*404*, IS*411*, and IS*415* (10, 32, 36), in terms of their size and/or restriction pattern (data not shown). Subsequent PCR and sequencing analyses revealed that 71 of the 74 plasmids had an insert of a novel 1.2-kb IS element, IS*Bmu2* (accession no. AB159774) and that the remaining three plasmids carried two novel IS elements, IS*Bmu1* (accession no. AB159773) and IS*Bmu3* (accession no. AB159775) (Table 4).

The 3′ portion of the IS*408* sequence has not yet been reported. The determination in this study of the complete sequence indicated the following characteristics regarding IS*408*: (i) it was 2,798 bp in size, (ii) it possessed 47-bp terminal inverted repeats (IRs) with 9-bp mismatches, (iii) it encoded the two putative transposition-related proteins, IstA and IstB, with sizes of 518 and 231 amino acids, respectively, and (iv) it generated the 7-bp duplication of its target sequences (Fig. 2). These properties supported the previously proposed classification of IS*408* to the IS*21* family (2). The less-conserved multiple terminal repeats commonly present at both ends of members of the IS*21* family (2) were also found in three and two copies at the left and right ends, respectively (Fig. 2). There existed between the 3′ end of *istB* and the right end of IS*408* three copies of the canonical −35 and −10 sequences of the *E.*

TABLE 2. Primers to investigate the IS insertion site on pGEN500

Primer	Sequence (5′ to 3′)	Primer-annealing region[a]
sacB-F1	GATTTTTTAGTTCTTTAGGCC	−311 to −291
sacB-R5	GTAAAGGTTAATACTGTTGC	47 to 28
sacB-F2	AACATCAAAAAGTTTGCAAAAC	4 to 25
sacB-R4	TCATTTGCATCGAATTTGTC	458 to 439
sacB-F3	GCCGCGTCTTTAAAGACAGC	419 to 438
sacB-R3	CGTTTAGCTCAATCATACCG	939 to 958
sacB-F4	TTAGCAAACGGCGCTCTCGG	922 to 941
sacB-R2	TTATTTGTTAACTGTTAATTGTCC	1422 to 1399

[a] The numbers indicate the primer-annealing region on pGEN500. The first A residue of the *sacB* start codon was defined as position +1.

TABLE 3. IS transposition frequency under several growth conditions

Condition and experiment no.	TET-resistant cells/ml of culture (A)	Sucrose-resistant clones/ml of culture (B)	Ratio of IS-mediated-sucrose-resistant clones among 32 sucrose-resistant clones (C)	IS transposition frequency[a] (B × C/A)	Average	Standard deviation
Normal						
1	2.4×10^9	2.9×10^4	9/32	3.4×10^{-6}	2.1×10^{-6}	1.3×10^{-6}
2	3.4×10^9	2.5×10^4	9/32	2.1×10^{-6}		
3	3.7×10^9	2.8×10^4	3/32	7.0×10^{-7}		
Oxidative						
1	2.8×10^9	2.1×10^4	6/32	1.5×10^{-6}	2.3×10^{-6}	1.3×10^{-6}
2	3.6×10^9	2.7×10^4	7/32	1.6×10^{-6}		
3	2.7×10^9	2.5×10^4	13/32	3.8×10^{-6}		
High temperature						
1	3.8×10^9	1.1×10^5	9/32	8.6×10^{-6}	1.6×10^{-5}	7.2×10^{-6}
2	3.8×10^9	1.9×10^5	10/32	1.5×10^{-5}		
3	2.1×10^9	1.3×10^4	12/32	2.3×10^{-5}		
Starvation						
1	2.0×10^9	5.2×10^3	10/32	8.1×10^{-7}	1.6×10^{-6}	1.9×10^{-6}
2	1.8×10^9	4.4×10^3	5/32	3.8×10^{-6}		
3	2.0×10^9	5.5×10^4	4/32	3.1×10^{-7}		

[a] The number of sucrose- and TET-resistant clones per milliliter of culture (B) was multiplied by the ratio of IS-mediated-sucrose-resistant clones among 32 sucrose-resistant clones (C) and then divided by the number of TET-resistant cells per milliliter of culture (A). For each condition, three independent experiments were performed (experiments 1 to 3), and the average and standard deviation were calculated.

coli promoter, and all of these putative promoters were rightward directed.

The two novel IS elements, IS*Bmu1* (2,581 bp) and IS*Bmu3* (2,697 bp), generated 5-bp target duplication, and the ends showed the 5′-TG-3′ dinucleotide and seven copies of the multiple terminal repeats that were approximately 20 bases in length (Fig. 2). IS*Bmu1* and IS*Bmu3* had long terminal IRs (i.e., 51-bp IRs with 12-bp mismatches and 49-bp IRs with 8-bp mismatches, respectively) and carried two genes (*istA* and *istB*) that were separated by 4 and 15 bases, respectively, bearing putative ribosome-binding sequences. The phylogenetic analysis of the IstA and IstB proteins of the two IS elements revealed that their IstA and IstB proteins were transposases and helper proteins, respectively, of the IS*21* family. A BLAST search of the databases and subsequent phylogenetic analysis (data not shown) revealed that the IstA and IstB proteins of IS*Bmu1* showed the highest identities with Rv3428c (34%) and RV3427c (37%), respectively, of *Mycobacterium tuberculosis* H37Rv (6), and the proteins of IS*Bmu3* showed the highest identities with Reut5638 (73%) and Reut5637 (77%), respectively, of *Ralstonia metallidurans*. While the DNA region between the *istB* gene and the right end of IS*Bmu3* carried one copy of the rightward directed promoter sequence, no such sequence was found in the corresponding region of IS*Bmu1*. IS*Bmu3* showed 75% nucleotide identity with IS*408*.

IS*Bmu2* was 1,210 bp in size and shared the structural properties commonly associated with the IS*5* subgroup in the IS*5* family, since this element (i) had the 16-bp terminal IRs with 4-bp mismatches and one *orf* (*ins*) encoding a 330-amino-acid transposase with a DDE motif and (ii) generated 4-bp duplication of the target sequences (Fig. 2). This Ins protein showed the highest (82%) identity with that of IS*Rso3*, another member of the IS*5* subgroup from *Ralstonia solanacearum* GMI1000. Since IS*Bmu2* accounted for more than 75% of the IS elements entrapped in this study, eight randomly chosen pGEN500::IS*Bmu2* plasmids were analyzed with respect to their insertion sites. Six out of the eight IS*Bmu2* inserts generated the target duplication of the CTAA sequence at five different positions in the *sacB* gene. This finding indicated that IS*Bmu2* had a preference for its target site selection.

To determine the copy numbers of IS*408*, IS*Bmu1*, IS*Bmu2*, and IS*Bmu3* in ATCC 17616, its genomic DNA was digested by the appropriate restriction enzymes, separated by agarose gel electrophoresis, and blotted onto a nylon membrane. Use of a

TABLE 4. Distribution of IS elements entrapped under different growth conditions[a]

Growth condition	No. of plasmids carrying:								Total out of 96 clones
	IS*401*	IS*402*	IS*406*	IS*407*	IS*408*	IS*Bmu1*	IS*Bmu2*	IS*Bmu3*	
Normal	0	0	0	1	2	0	18	0	21
Oxidative	0	0	0	1	4	0	21	0	26
High temperature	2	1	6	1	4	0	15	2	31
Starvation	0	0	0	0	1	1	17	0	19

[a] IS*401* and IS*407* belong to the IS*3* family, IS*402* and IS*Bmu2* belong to the IS*5* family, IS*406* belongs to the IS*256* family, and IS*408,* IS*Bmu1,* and IS*Bmu3* belong to the IS*21* family.

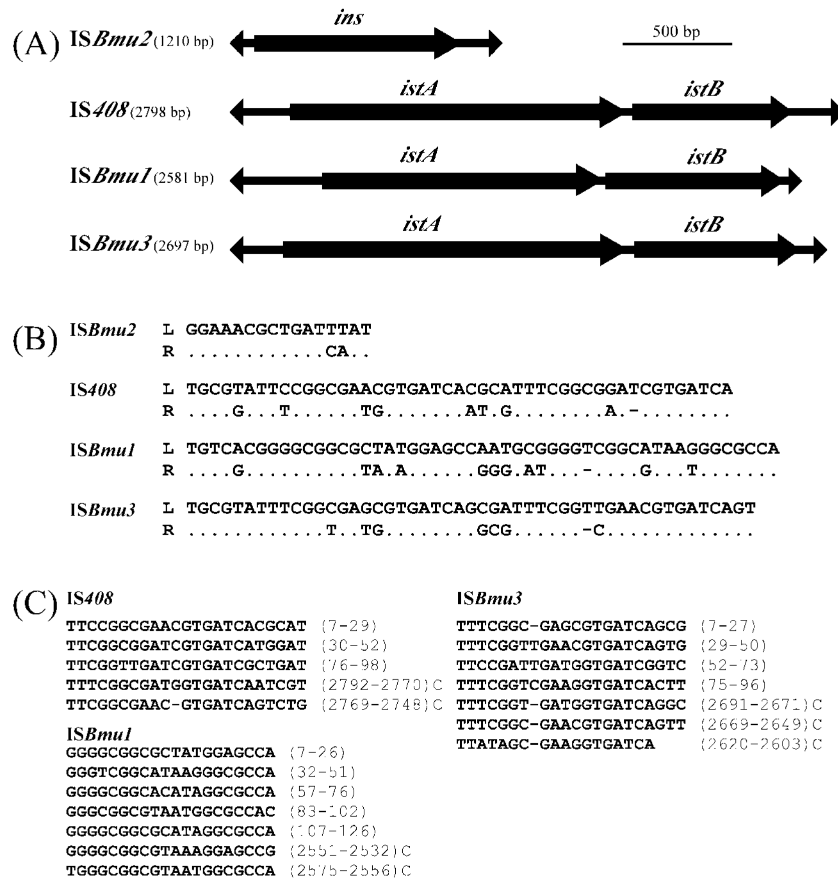

FIG. 2. IS elements identified in this study. (A) Structures of IS*408*, IS*Bmu1*, IS*Bmu2*, and IS*Bmu3*. (B) Nucleotide sequences of the terminal IRs. The left (L) and right (R) ends of each IS element are defined as those located upstream and downstream, respectively, of the *ins* or *istAB* genes. The same nucleotides are represented by dots. Note that some gaps (indicated by hyphens) were introduced for maximum matching. (C) The alignment of multiple terminal repeats with their coordinates in the IS*21* family. Hyphens indicate gaps. C, complementary strand.

portion of each IS element as a probe revealed that the ATCC 17616 genome contained three, four, nine, and two copies of IS*408*, IS*Bmu1*, IS*Bmu2*, and IS*Bmu3*, respectively (data not shown).

Effect of growth conditions on IS transposition frequency. In this study, the global IS transposition frequency was calculated using the following formula: (number of sucrose- and TET-resistant clones per milliliter of culture × ratio of IS-mediated-sucrose-resistant clones among 32 sucrose-resistant clones)/ number of TET-resistant cells per milliliter of culture. The IS transposition frequency under the high-temperature condition (1.6×10^{-5}/viable cell) was more than sevenfold higher than that observed under the three other growth conditions. Three additional experiments were further carried out to determine the global IS transposition frequencies under the high-temperature and normal growth conditions, and the transposition under the former condition was 16-fold higher than that in the latter condition (data not shown). As is shown in Table 4, the transposition of four IS elements, IS*401*, IS*402*, IS*406*, and IS*Bmu3*, was observed only under the high-temperature condition, showing their preferential transposition under high-temperature conditions. This result again emphasized the induction of IS transposition at the high-temperature condition.

DISCUSSION

The experimental scheme developed in this study allowed us to do the following things: (i) screen a large number of sucrose-resistant clones without their cultivation in liquid medium and (ii) identify and classify the previously characterized IS elements by colony PCR with mixed primer pairs. Employment of this scheme led to the successful entrapment of the five previously reported IS elements as well as three novel IS elements. Our scheme was also effective for quantitatively analyzing the transposition of these eight IS elements. The scheme employed in this study will be useful for the quantitative analysis of IS transposition in other bacterial strains.

With respect to the genomes of strains belonging to the Bcc, the complete sequence of a *Burkholderia cenocepacia* strain (J2315) and the draft sequences of two *B. cepacia* strains (R1808 and R18194) are available in public databases. We surveyed the distribution of the eight ATCC 17616-derived IS elements in the three strains. IS*401*, IS*406*, IS*408*, and their highly homologous sequences were not found among these three strains. IS*402*, IS*407*, and their isoforms are abundant in J2315 and R1808, and the latter strain additionally carries the isoforms of IS*Bmu2* and IS*Bmu3*. In contrast to R1808,

R18194 did not carry any of the eight IS elements or their isoforms, suggesting that R18194 is a unique strain from a standpoint of the distribution of IS elements. Various isoforms of IS*407* were also distributed in pathogenic *Burkholderia* species, such as *Burkholderia mallei* and *Burkholderia pseudomallei*, and in other various environmental *Burkholderia* strains (25), thus indicating that IS*407* or its highly homologous element must have been residing in a common ancestral strain prior to diversification to establish the Bcc.

Extensive studies of prokaryotic mobile DNA elements revealed that their transposition activities were modulated by several growth conditions and that two alternative, and in some cases mutually related, mechanisms govern transposition activity (27). According to one of these mechanisms, the growth conditions control the expression of transposition-related genes and/or the activities of the gene products. In the other type of mechanism, the growth conditions regulate the activities of various fundamental host factors that subsequently modulate transposition activity. The following candidate host factors have thus far been suggested: (i) the so-called DNA chaperones IHF, HU, H-NS, and FIS, which are important for the maintenance of proper DNA and genome architectures; (ii) cold- and heat shock protein chaperone systems; and (iii) recombination systems, including the SOS system. However, it has been reported that the effects of both the growth conditions and the host factors were in general specific for each mobile element (23). Taking these effects into consideration, we investigated in this study the transposition of the *B. multivorans* ATCC 17616 IS elements under normal conditions as well as under the following three conditions. The first of these three conditions was starvation, since this strain was isolated from a soil environment that was poor in nutrients (33). The two other conditions were high-temperature and oxidative stress conditions. The main reason why we employed the two latter conditions was that the Bcc strains, including *B. multivorans*, are often associated with animals. Upon infection of animals, Bcc cells are likely to be subjected to attack by the host defense system, which generates high temperatures as well as superoxide, a typical reactive oxygen species that disrupts the bacterial DNA molecules and therefore induces the host-cell SOS system (7). Among the four growth conditions examined in this study, only the high-temperature condition led to more than a sevenfold increase in the transposition activity of all of the IS elements except for IS*Bmu1*, and transposition of IS*401*, IS*402*, IS*406*, and IS*Bmu3* was detected only under this condition (Table 4). The higher transposition activities of these IS elements at 42°C in the ATCC 17616 cells contrasted with the decreased transposition activities of various mobile DNA elements (e.g., Tn*3*, IS*1*, IS*30*, and IS*911*) in *E. coli* cells at this temperature (27). The temperature sensitivities of transposition in *E. coli* have been considered to be the intrinsic properties of the transposases (12). The observation of a simultaneous increase in ATCC 17616 cells of the transposition activities of the seven IS elements belonging to four different IS families (Table 4) suggested that some common host factors affect transposition at high temperatures. Some unknown factors that inhibit transposition might be depleted at higher temperatures. Alternatively, other unknown factors might contribute to changing the donor and/or target DNA architecture into a form that enables the IS transposition machinery to work more effectively. Whatever the mechanism might be, the observed increase in IS transposition frequency indicated that the IS elements examined here play an important role in cells exposed to high temperatures, thus generating genetic diversity in a population of *B. multivorans* ATCC 17616; therefore, these IS elements also appear to be important for adaptation. To the best of our knowledge, this is the first report demonstrating an elevation in the transposition activity of several IS elements at a high temperature.

ACKNOWLEDGMENTS

This work was supported by the Research for the Future Programs of the Japan Society for the Promotion of Science and by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

The complete and preliminary sequence data for various *Burkholderia* genomes were obtained from the DOE Joint Genome Institute at http://www.jgi.doe.gov/JGI_microbial/html/ and from the Sanger Institute at ftp://ftp.sanger.ac.uk/pub/pathogens/.

REFERENCES

1. **Barsomian, G., and T. G. Lessie.** 1986. Replicon fusions promoted by insertion sequences on *Pseudomonas cepacia* plasmid pTGL6. Mol. Gen. Genet. **204:**273–280.
2. **Berger, B., and D. Haas.** 2001. Transposase and cointegrase: specialized transposition proteins of the bacterial insertion sequence IS*21* and related elements. Cell. Mol. Life Sci. **58:**403–419.
3. **Burkholder, W.** 1950. Sour skin, a bacterial rot of onion bulbs. Phytopathology **40:**115–118.
4. **Cheng, H. P., and T. G. Lessie.** 1994. Multiple replicons constituting the genome of *Pseudomonas cepacia* 17616. J. Bacteriol. **176:**4034–4042.
5. **Coenye, T., P. Vandamme, J. R. Govan, and J. J. LiPuma.** 2001. Taxonomy and identification of the *Burkholderia cepacia* complex. J. Clin. Microbiol. **39:**3427–3436.
6. **Cole, S. T., R. Brosch, J. Parkhill, T. Garnier, C. Churcher, D. Harris, S. V. Gordon, K. Eiglmeier, S. Gas, C. E. Barry III, F. Tekaia, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, B. G. Barrell, et al.** 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature **393:**537–544.
7. **Farr, S. B., and T. Kogoma.** 1991. Oxidative stress responses in *Escherichia coli* and *Salmonella typhimurium.* Microbiol. Rev. **55:**561–585.
8. **Ferrante, A. A., and T. G. Lessie.** 1991. Nucleotide sequence of IS*402* from *Pseudomonas cepacia.* Gene **102:**143–144.
9. **Figurski, D. H., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. Proc. Natl. Acad. Sci. USA **76:**1648–1652.
10. **Gaffney, T. D., and T. G. Lessie.** 1987. Insertion-sequence-dependent rearrangements of *Pseudomonas cepacia* plasmid pTGL1. J. Bacteriol. **169:**224–230.
11. **Gay, P., D. Le Coq, M. Steinmetz, T. Berkelman, and C. I. Kado.** 1985. Positive selection procedure for entrapment of insertion sequence elements in gram-negative bacteria. J. Bacteriol. **164:**918–921.
12. **Haren, L., M. Betermier, P. Polard, and M. Chandler.** 1997. IS*911*-mediated intramolecular transposition is naturally temperature sensitive. Mol. Microbiol. **25:**531–540.
13. **Hasebe, A., and S. Iida.** 2000. The novel insertion sequences IS*1417*, IS*1418*, and IS*1419* from *Burkholderia glumae* and their strain distribution. Plasmid **44:**44–53.
14. **Hasebe, A., S. Tsushima, and S. Iida.** 1998. Isolation and characterization of IS*1416* from *Pseudomonas glumae*, a new member of the IS*3* family. Plasmid **39:**196–204.
15. **Heeb, S., Y. Itoh, T. Nishijyo, U. Schnider, C. Keel, J. Wade, U. Walsh, F. O'Gara, and D. Haas.** 2000. Small, stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. Mol. Plant-Microbe Interact. **13:**232–237.
16. **Hübner, A., and W. Hendrickson.** 1997. A fusion promoter created by a new insertion sequence, IS*1490*, activates transcription of 2,4,5-trichlorophenoxyacetic acid catabolic genes in *Burkholderia cepacia* AC1100. J. Bacteriol. **179:**2717–2723.
17. **Isles, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming, and H. Levison.** 1984. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. J. Pediatr. **104:**206–210.
18. **Jäger, W., A. Schäfer, J. Kalinowski, and A. Pühler.** 1995. Isolation of insertion elements from gram-positive *Brevibacterium*, *Corynebacterium* and *Rhodococcus* strains using the *Bacillus subtilis sacB* gene as a positive selection marker. FEMS Microbiol. Lett. **126:**1–6.

19. **Komatsu, H., Y. Imura, A. Ohori, Y. Nagata, and M. Tsuda.** 2003. Distribution and organization of auxotrophic genes on the multichromosomal genome of *Burkholderia multivorans* ATCC 17616. J. Bacteriol. **185:**3333–3343.
20. **Lessie, T. G., and T. D. Gaffney.** 1986. Catabolic potential of Pseudomonas cepacia, vol. 10. AcademicPress, Inc., Orlando, Fla.
21. **Lessie, T. G., W. Hendrickson, B. D. Manning, and R. Devereux.** 1996. Genomic complexity and plasticity of *Burkholderia cepacia.* FEMS Microbiol. Lett. **144:**117–128.
22. **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.
23. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Miche, L., D. Faure, M. Blot, E. Cabanne-Giuli, and J. Balandreau.** 2001. Detection and activity of insertion sequences in environmental strains of *Burkholderia.* Environ. Microbiol. **3:**766–773.
26. **Mira, A., L. Klasson, and S. G. Andersson.** 2002. Microbial genome evolution: sources of variability. Curr. Opin. Microbiol. **5:**506–512.
27. **Nagy, Z., and M. Chandler.** 2004. Regulation of transposition in bacteria. Res. Microbiol. **155:**387–398.
28. **Parke, J. L., and D. Gurian-Sherman.** 2001. Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. Annu. Rev. Phytopathol. **39:**225–258.
29. **Riehle, M. M., A. F. Bennett, and A. D. Long.** 2001. Genetic architecture of thermal adaptation in *Escherichia coli.* Proc. Natl. Acad. Sci. USA **98:**525–530.
30. **Schneider, D., D. Faure, M. Noirclerc-Savoye, A. C. Barriere, E. Coursange, and M. Blot.** 2000. A broad-host-range plasmid for isolating mobile genetic elements in gram-negative bacteria. Plasmid **44:**201–207.
31. **Schweizer, H. P.** 1992. Allelic exchange in *Pseudomonas aeruginosa* using novel ColE1-type vectors and a family of cassettes containing a portable *oriT* and the counter-selectable *Bacillus subtilis sacB* marker. Mol. Microbiol. **6:**1195–1204.
32. **Scordilis, G. E., H. Ree, and T. G. Lessie.** 1987. Identification of transposable elements which activate gene expression in *Pseudomonas cepacia.* J. Bacteriol. **169:**8–13.
33. **Stanier, R. Y., N. J. Palleroni, and M. Doudoroff.** 1966. The aerobic *Pseudomonas*: a taxonomic study. J. Gen. Microbiol. **43:**159–271.
34. **Vandamme, P., B. Holmes, M. Vancanneyt, T. Coenye, B. Hoste, R. Coopman, H. Revets, S. Lauwers, M. Gillis, K. Kersters, and J. R. Govan.** 1997. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int. J. Syst. Bacteriol. **47:**1188–1200.
35. **Wood, M. S., A. Byrne, and T. G. Lessie.** 1991. IS*406* and IS*407*, two gene-activating insertion sequences for *Pseudomonas cepacia.* Gene **105:**101–105.
36. **Wood, M. S., C. Lory, and T. G. Lessie.** 1990. Activation of the *lac* genes of Tn*951* by insertion sequences from *Pseudomonas cepacia.* J. Bacteriol. **172:**1719–1724.