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Cloning and Sequencing of IS*1086*, an *Alcaligenes eutrophus* Insertion Element Related to IS*30* and IS*4351*

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A new insertion sequence (IS), designated IS*1086*, was isolated from *Alcaligenes eutrophus* CH34 by being trapped in plasmid pJV240, which contains the *Bacillus subtilis sacB* and *sacR* genes. The 1,106-bp IS*1086* element contains partially matched (22 of 28 bp) terminal-inverted repeats and a long open reading frame. Hybridization data suggest the presence of one copy of IS*1086* in the strain CH34 heavy-metal resistance plasmid pMOL28 and at least two copies in its chromosome. Analysis of the IS*1086* nucleotide sequence revealed striking homology with two other IS elements, IS*30* and IS*4351*, suggesting that they are three close members in a family of phylogenetically related insertion sequences. One open reading frame of the *Spiroplasma citri* phage SpV1-R8A2 B was also found to be related to this IS family but to a lesser extent. Comparison of the G+C contents of IS*30* and IS*1086* revealed that they conform to their respective hosts (46 versus 50% for IS*30* and *Escherichia coli* and 64.5% for IS*1086* and *A. eutrophus*). The pressure on the AT/GC ratio led to a very different codon usage in these two closely related IS elements. Results suggesting that IS*1086* transposition might be activated by some forms of stress are discussed.

Insertion sequences (IS) are a class of procaryotic transposable elements (class I) capable of integrating into numerous sites within genomes via a transposition pathway totally independent of homologous recombination. IS are defined genetic entities (usually 0.75 to 1.6 kb). They contain only the genetic determinants for their transposition, which can proceed through either a conservative or a replicative mode or both (for reviews, see references 4, 9, 12, 15, and 17). IS elements are responsible for the occurrence of a number of genetic rearrangements, including insertions and deletions which can result in gene activation or inactivation. We have attempted to identify such IS elements in *Alcaligenes eutrophus* CH34. This strain was isolated near a metallurgical plant from sediments highly contaminated with heavy metals (19). It carries two large plasmids, pMOL28 and pMOL30, which harbor genes encoding multiple resistance to heavy metals. pMOL28 (163 kb) encodes resistance to nickel, cobalt, chrome, mercury, and thallium, while pMOL30 (238 kb) specifies resistance to cadmium, zinc, cobalt, copper, lead, mercury, and thallium (20).

Another remarkable feature of *A. eutrophus* CH34, whose optimal growth temperature is around 30°C, is the high degree of mortality occurring when it is grown at 37°C and the very high proportion of mutants detected among the survivors (21, 28). Cultures grown at 30°C and plated at 37°C give rise to survivors at a frequency of 10^{-5} to 10^{-4} compared with the viable count at 30°C, and 5 to 80% of these survivors exhibit recognizable mutations such as auxotrophy for lysine (Lys^-) or threonine (Thr^-), inability to grow autotrophically (Aut^-), inability to use nitrate (Nit^-), inability to use NH_4^+, and inability to use tyrosine as a carbon source accompanied by overexcretion of a dark-red pigment (Tyu^-) (21). This phenomenon was named "thermospontagenesis" (21). Substantial rearrangements in the pMOL28 and pMOL30 plasmids in some of the mutants recovered after thermospontagenesis were also observed. One such rearranged plasmid is pMOL50 (210 kb), a pMOL28 derivative (163 kb) which kept all the metal resistance markers of its parent (21, 28). This appearance of mutations at a high frequency led us to investigate the movement of mobile elements during the exposure at 37°C. The suicide plasmid pJV240 was introduced in *A. eutrophus* AE104, a plasmid-free derivative of strain CH34. pJV240 is an IncQ broad-host-range plasmid bearing the *sacB-sacR* region of the *Bacillus subtilis* chromosome (10, 34). The *sacB* gene encodes levansucrase, which is toxic when synthesized in gram-negative bacteria propagated on media supplemented with sucrose (11). Cultures of AE104(pJV240) grown at 30°C were plated at 30 and 37°C on plates with or without sucrose, in the hope of trapping mobile sequences in the *sacB-sacR* region of the plasmid.

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. Bacterial strains and plasmids are listed in Table 1.

Luria broth (LB) (30) and Luria agar (LA; LB supplemented with 1.5% agar) were used as liquid and solid media to grow *Escherichia coli* and *A. eutrophus* strains. Kanamycin was used at a concentration of 1,000 μg/ml.

Restriction enzymes and T4 ligase were purchased from Bethesda Research Laboratories, Inc., Gaithersburg, Md. Nitrocellulose membranes (GeneScreen; NEN, Boston, Mass.) were used in Southern blotting experiments (29).

Construction of pJV240. The pLS306 plasmid contains the

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TABLE 1. Bacterial strains and plasmids used

Strain	Relevant plasmid	Relevant plasmid markers	Reference
A. eutrophus			
CH34	pMOL28	Nic$^+$ Cob$^+$ Chr$^+$	20
	pMOL30	Cad$^+$ Zin$^+$ Cob$^+$	
AE104			
AE245	pMOL50	Nic$^+$ Cob$^+$ Chr$^+$	28
E. coli			
DH5αF′	F′	φ80d*lacZ*ΔM15	13
CM656	pJV240	Ap Km Sac$^+$ IncQ	34
CM404	pRK2013	Km Tra$^+$	8
CM444	R388	Su Tp Tra$^+$ IncW	7
HB101			3

B. subtilis sacB and *sacR* genes on a *Bam*HI-*Pst*I fragment (10) which was inserted into the pKT240 broad-host-range plasmid vector (1) to generate pJV240 (34).

Triparental mating. Plate matings were carried out by incubating three strains (partners) on an LB plate of rich medium with appropriate controls. One partner contains the plasmid (Tra$^-$ Mob$^+$) to be mobilized in the final recipient (second partner). The third partner contains the mobilizing plasmid.

Two types of triparental matings were used. (i) The mobilizing plasmid pRK2013 is used to mobilize pJV240 in *A. eutrophus*. The counterselection of *E. coli* strains is made through the carbon source (use of azelaic acid, which is a good substrate for *A. eutrophus*). (ii) Plasmid R388 is used to mobilize the Suc^r derivatives of pJV240 in HB101 (see below). Counterselection occurred by selection at 42°C with appropriate antibiotics.

Introduction of pJV240 into AE104 and isolation of sucrose-resistant derivatives. pJV240 was introduced from *E. coli* CM656 into AE104, a plasmid-free derivative of CH34, by a triparental mating. The third partner, *E. coli* CM404, carries pRK2013, which can mobilize pJV240 but cannot be maintained in nonenteric bacteria (8). Transconjugants were selected for their kanamycin-resistant phenotype. Suc^r mutants were selected by plating cultures grown at 30°C on LA plates supplemented with kanamycin with or without 5% sucrose. The plates were incubated for 3 days at either 30 or 37°C. The plasmids from the Suc^r mutants found at both temperatures were transferred in *E. coli* HB101 by triparental matings in which mobilization was mediated by the IncW plasmid R388 carried by strain CM444.

Plasmid DNA was extracted by standard procedures (29) for *E. coli* and by the method of Prakash et al. (24) for *A. eutrophus*. Plasmid DNA was analyzed with restriction enzymes according to the supplier's instructions.

DNA sequencing and sequence analysis. The *Bam*HI-*Eco*RI fragment from one derivative of pJV240 containing the insertion was subcloned into M13mp18 and M13mp19. Part of the sequence was determined by using the M13 universal primers. Oligonucleotides synthesized by Cyclone DNA synthesizer (New Brunswick Scientific, New Brunswick, N.J.) were used as primers to complete DNA sequencing. The nucleotide sequence of the fragment was determined by the dideoxy-chain termination method (30) with both strands of DNA by using 1 to 2 μg of single-stranded DNA as a template, [^{35}S]dATP (NEN), and the Sequenase version 2.0 DNA sequencing kit (U.S. Biochemicals, Cleveland, Ohio), as recommended by the manufacturer.

Sequence comparison was carried out by using the FASTA, LFASTA, and TFASTA programs (23). Data bank searches included GenBank, the EMBL nucleotide sequence data base (release 27), and the Swissprot Protein Sequence Database (release 18).

Sequence analysis was performed by using the PC/GENE program package (IntelliGenetics Inc., Mountain View, Calif.).

Analysis of the genomic distribution of IS*1086*. Total DNA was extracted from CH34 and digested with *Bam*HI, *Pst*I, *Xho*I, *Eco*RI, and *Hin*dIII. Southern blotting was performed after the genomic DNA had been subjected to electrophoresis in a 0.8% agarose gel.

An *Nco*I-*Xma*III IS*1086* fragment was labeled with [^{32}P]ATP (NEN) and used as a hybridization probe.

Nucleotide sequence accession number. The EMBL Data Library submission number for IS*1086* is X58441.

RESULTS

Isolation of sucrose-resistant insertion mutants. A culture of strain AE104(pJV240) grown at 30°C was plated on medium with and without sucrose and incubated at 30 or 37°C in order to determine the frequency of sucrose-resistant mutants obtained with or without direct selection on sucrose after incubation at these two temperatures.

When AE104(pJV240) was plated on LB medium without sucrose and incubated at 30°C, no sucrose-resistant mutant could be found among 1,000 colonies that were transferred from the original plates without sucrose onto plates containing 5% sucrose. Plating on medium containing 5% sucrose and incubation at 30°C resulted in a frequency of 2×10^{-6} CFU compared with plating on medium without sucrose (10^4 survivors per ml for 5×10^9 cells per ml plated).

When AE104(pJV240) was plated on medium without sucrose and incubated at 37°C, a strong decrease in the number of colonies due to thermospontagenesis was observed, resulting in a frequency of survivors of 5×10^{-5}.

Finally, when AE104(pJV240) was grown at 30°C and plated at 37°C on medium with sucrose, the frequency of colonies obtained under these conditions was expected to equal the product of the frequency of survivors at 37°C and the frequency of sucrose-resistant mutants obtained at 30°C and hence to be around 10^{-10}. Surprisingly, the number of survivors found on medium with sucrose after incubation at 37°C was only 3 to 100 times lower than the number of survivors obtained on medium without sucrose at the same temperature (frequency, 2×10^{-5} to 5×10^{-7}). This result was confirmed by the following observation: among the survivors (frequency of 5×10^{-5}), on medium without sucrose incubated at 37°C, 14.5% had become sucrose resistant, as determined by testing the colonies on medium with sucrose. This suggests that a very efficient mutation mechanism is active during thermospontagenesis.

To examine which types of mutations were responsible for sucrose resistance at 30 and 37°C, plasmids from 30 independent sucrose-resistant mutants obtained at each temperature were transferred to *E. coli* HB101 by triparental matings. The transconjugants recovered were purified and examined for their plasmid content. At 37°C, 12 of 30 mutated pJV240 plasmids had increased in length by approximately 1.1 kb. At 30°C, 12 of 30 mutated pJV240 plasmids also had a similar increase of 1.1 kb. This indicated that an enlargement of pJV240 occurs with equal frequencies at both temperatures. Restriction analysis and hybridization studies showed that although the insertion had occurred at different

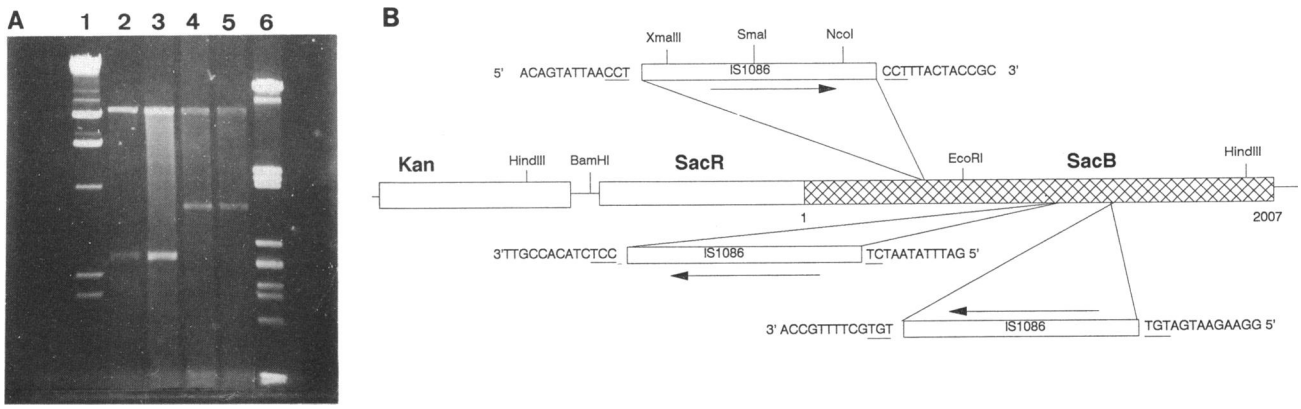

FIG. 1. (A) *Bam*HI-*Pst*I double-digestion restriction patterns for pJV240 (lane 2) and plasmids of three Suc^r mutants (lanes 3 to 5). Two of them (lanes 4 and 5) display a heavier restriction fragment. Lambda phage DNA digested with *Hin*dIII (lane 1) and with *Pst*I (lane 6) was used as molecular weight markers. (B) Restriction map of the *sacB-sacR* region and IS*1086*, with localization of various IS*1086* insertions and their flanking sequences. Arrows indicate different orientations of insertions on two strands. Target sequences are underlined. Two independently isolated IS*1086* elements were mapped in the same site (at nucleotide 504 in the *sacB* gene).

positions in the *sacRB* operon, it was the same DNA insertion in all cases.

Figure 1A shows the restriction patterns of three mutated (at 37°C) pJV240 plasmids digested by *Bam*HI and *Pst*I. The sizes of the fragments showed that two of these mutated plasmids had acquired an insertion of approximately 1.1 kb.

Digestion with various enzymes of the heavier *Bam*HI-*Pst*I *sacB* fragment and comparison with the corresponding fragment of the parental pJV240 led to the restriction map for the insertion shown in Fig. 1B. The presence of these restriction sites was confirmed for other insertions which are shown in Fig. 1B.

Complete nucleotide sequence of IS*1086*. The IS which maps near the junction between *sacB* and *sacR* (Fig. 1B) was entirely sequenced as described in Materials and Methods. The complete insertion is 1,106 bp long. It possesses all the characteristics expected of an IS: 3 bp (CCT) of the target sequence have been duplicated and flank the insert, which is bordered by a 28-bp inverted repeat with 6 bases mismatched (see Fig. 2 and 4). Hereafter we shall refer to it as IS*1086*. The left and right IS-*sacB* junctions were sequenced in three other plasmids.

Among these four independently isolated insertions obtained at 37°C, two mapped at exactly the same target site and generated the same CCT duplication (3 bp). For a third insertion, the target site was different and a TGT triplet of the target had been duplicated. The fourth insertion was flanked by CT on one side and CCT on the other.

The fourth insertion occurred before a CCT, generating an incomplete (from CCT to CT) or an incorrect (from CC to CT) duplication of 2 bp. Thus, IS*1086* may have a preferred target and usually duplicates 3 bp.

Open reading frames (ORFs) of IS*1086*. The IS*1086* coding capacity was analyzed by the method of Kolaskar and Reddy (18). The results are shown in Table 2.

On one strand, two distinct ATG codons could be considered as translational start signals corresponding to a 339-amino-acid polypeptide and a 325-amino-acid polypeptide (Fig. 2). Two putative ribosome binding sites are also present just upstream of two ATGs, as indicated in Fig. 2. Further experiments are obviously required to identify the actual initiation codon. It is plausible, however, that a single ORF could span up to 92% of the entire nucleotide sequence of IS*1086*. Because of the presence of translational consensus signals and a striking homology with two other IS (see below), the putative ORF of IS*1086* is likely to be functional. Three types of promoters have been reported for *A. eutrophus* (16, 31, 33). No corresponding consensus preceding the two potential initiation codons just described could be detected.

On the opposite strand, no transcriptional or translational initiation signal could be found upstream of a potential ORF starting at position 704 and consisting of 109 amino acids (Table 2).

The DNA sequence was also screened for palindromic sequences, and only one was found between nucleotides 131 and 156 (Fig. 2).

Homology with other IS. To determine whether the IS*1086* sequence is related to other known DNA sequences, particularly to those of IS elements, data banks were screened. Two sequences displaying significant levels of homology with IS*1086* were found. Both are IS elements: IS*30* isolated from *E. coli* (5, 6) and IS*4351* isolated from *Bacteroides fragilis* (25). Figure 3 shows an alignment of the three sequences. The best alignment is observed in the C-terminal quarter of the three putative ORFs.

A search of the EMBL nucleotide sequence data base (release 27) by using the TFASTA program revealed in addition limited but significant similarity between the putative IS*1086* transposase and one of the proteins potentially encoded by the *Spiroplasma citri* phage SpV1-R8A2 B (ORF3) (26). The phage genome consists of a circular single-stranded DNA of 8,273 bases. ORF3 of the phage also shows homology with IS*30* and IS*4351* putative transposases. Table 3 compares the percent identity and percent similarity between each pair of these four elements.

TABLE 2. Coding capacity of IS*1086*

IS*1086* strand	Initiation codon position	Termination codon position	No. of amino acids
1	56	1072	339
	98	1072	325
2	704	1030	109

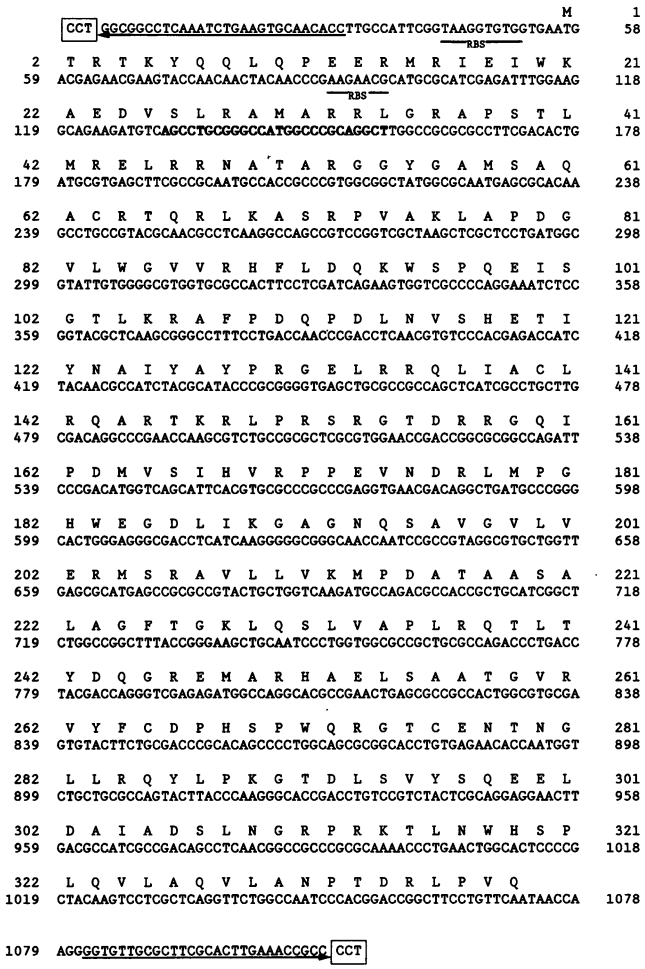

FIG. 2. IS*1086* nucleotide sequence. Target repeats are boxed. Imperfect inverted repeats are underlined with an arrow. Two putative translation initiation codon ATGs are located at bp 56 and 98. Two potential ribosome binding sites (RBS) are shown. The palindromic sequence is indicated in boldface.

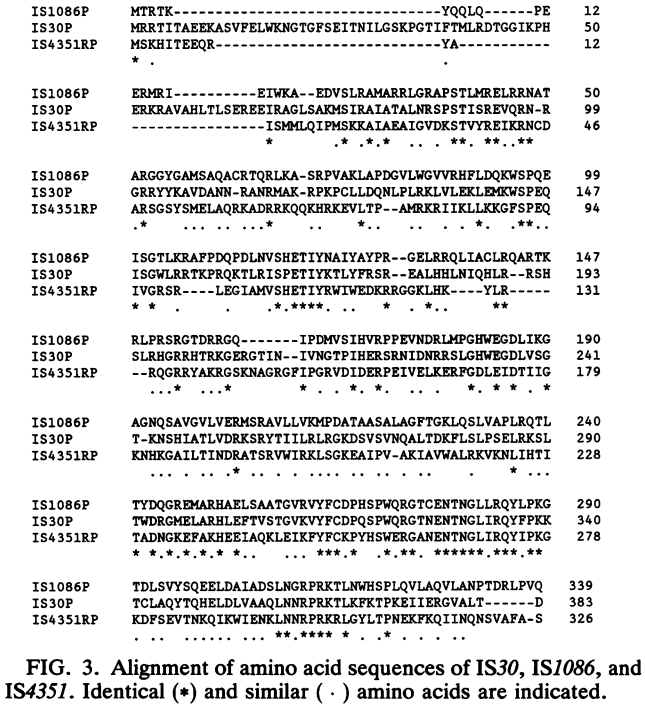

FIG. 3. Alignment of amino acid sequences of IS*30*, IS*1086*, and IS*4351*. Identical (*) and similar (·) amino acids are indicated.

Homology within inverted repeats. The degree of homology between the inverted repeat of IS*1086* and the equivalent sequences of IS*30* and IS*4351* is shown in Fig. 4. These three elements share two conserved blocks of 4 and 5 nucleotides (. . . TCAA . . . . . GCAAC). In the case of the inverted repeat of SpV1-R8A2 B, the left and the right repeats are adjacent on the circular genome and are separated by 3 bp around nucleotide 4737. Contrary to the ORF region whose sequence homology with SpV1-R8A2 B is on the borderline of what is considered significant, the inverted repeat of IS*1086* shares a very striking identity (76%) with that of SpV1-R8A2 B (Fig. 4). The block GCAAC is common to both repeats in the three IS elements and in the phage.

Comparison of the G+C contents of IS*1086*, IS*4351*, and IS*30*. Because the genomes of *A. eutrophus*, *E. coli*, and *B. fragilis* differ in G+C content, it was of interest to determine whether the three related IS had G+C contents which correspond to those of their host organisms. The results are shown in Table 4: the G+C contents of the three IS closely match those of their hosts. As also emphasized in Table 4, the bias toward G/C or A/T (22) resides essentially at silent sites (i.e., sites accepting synonymous substitutions in codons).

Genomic distribution of IS*1086*. After Southern blotting of CH34 genomic DNA digested with several restriction enzymes, the IS*1086* probe (see Materials and Methods) was found to hybridize with two *Eco*RI, two *Hin*dIII, two *Xho*I, and two *Pst*I fragments in addition to a fragment from pMOL28 (see below). As no site for these endonucleases is present in IS*1086*, at least two copies of the IS should exist in the chromosome of CH34.

The two large plasmids, pMOL28 and pMOL30, from *A. eutrophus* CH34 and the rearranged plasmid found after thermospontagenesis, pMOL50 (a derivative of pMOL28) (28), were also digested with *Eco*RI, and the restriction fragments were hybridized with the same IS*1086* probe after blotting. The probe hybridized only with one pMOL28 4.8-kb *Eco*RI fragment (data not shown). This fragment did not exist in pMOL50, in which it was replaced by a 3.7-kb fragment (Fig. 5). This new fragment did not hybridize with the IS*1086* probe, although it strongly hybridized with the pMOL28 4.8-kb *Eco*RI fragment (data not shown), suggesting that IS*1086* was lost during the rearrangement which generated pMOL50. The relative position of the 4.8-kb

TABLE 3. Identical and conserved amino acids between IS*1086*, IS*30*, IS*4351*, and SpV1-R8A2 B

Sequence	% Identity or similarity with[a]:			
	IS*1086*	IS*30*	IS*4351*	SPV1-R8A2 B
IS*1086*	100	60.9	53.0	53.4
IS*30*	42.8	100	52.4	49.1
IS*4351*	31.2	32.3	100	52.2
SpV1-R8A2 B	27.2	25.6	30.2	100

[a] Percent conserved similarities between the four elements are shown above the diagonal, and percent identities are shown below.

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SpV1R8A2L   ATGCATCC TGTA A T CTGA A GT GCAAC AAATC
SpV1R8A2R   ***A**TA **** * A A*** * ** ***** *****

IS1086L      GGCGGCC TCAA A T CTGA A GT GCAAC ACC
IS1086R      ******TT **** G * GC** * *C ***** ***

IS4351L      GCTGAAT TCAA C T TGCA A AT GCAAC
IS4351R      CT***G* **** * * *AT* * ** *****

IS30L        TGTAGAT TCAA T T GGTC A AC GCAAC A
IS30R        ******* **** * C T*** * *T ***** *
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FIG. 4. Alignment of inverted repeats of three IS elements and bacteriophage SpV1-R8A2 B. Two well-conserved blocks are boxed. Matched sites between the left and right repeats are indicated (*).

*Eco*RI fragment which shelters IS*1086* in pMOL28 is not yet known.

DISCUSSION

We have identified a new mobile DNA element in *A. eutrophus* CH34. This element is expected to be an IS, for the following reasons. (i) It was inserted in the *sacB* gene after selection for Suc^r (i.e., *sacB*) mutants. (ii) It displays significant homology with two previously described IS, IS*30* from *E. coli* and IS*4351* from *B. fragilis*. (iii) Its primary structure is similar to that of an IS (9): it is 1.1 kb long, which is typical of IS; it contains imperfect inverted repeats of 28 bp; and it is flanked in most cases by a direct duplication of 3 bp of the transposition target sequence. This is different from IS*30*, which generates a 2-bp direct repeat (6). Like IS*30* and IS*4351*, IS*1086* contains a unique potential ORF of 339 codons which covers 92% of the total length of the element. The corresponding protein is likely to be the transposase.

IS*1086* was found in the mutated pJV240 plasmid of about 50% of the Suc^r mutants, whether they had been obtained at 30°C on plates with sucrose or at 37°C on plates with or without sucrose. The frequency of Suc^r mutants (i.e., the ratio of Suc^r colonies to the viable count) was higher (1,000×) at 37°C than at 30°C, whether they were recovered with or without selection on sucrose. Since we know that the Suc^r mutants do not constitute a fraction of the population which is resistant to exposure to 37°C (data not shown), these results suggest that IS*1086* transposition (as well as other mutagenic events) is activated at 37°C. It is also intriguing that the pJV240 *sac* genes which originate from *B. subtilis* have a G+C content different from that of the *A. eutrophus* host, which may be the reason for the high frequency of insertion observed in those genes.

The amino acid sequence of the large IS*1086* ORF shows

TABLE 4. G+C contents[a] at silent sites

IS	No. of nucleotides					% G or C
	A	C	G	T	Total	
IS*1086*						
Total	200	358	300	159	1,017	65
Silent sites	40	183	94	43	360	77
Is*30*						
Total	374	259	268	251	1,152	46
Silent sites	140	107	75	112	434	42
IS*4351*						
Total	350	177	250	201	178	43
Silent sites	111	76	71	87	345	43

[a] G+C contents for *A. eutrophus*, *E. coli*, and *B. fragilis* are 69.5, 50, and 43%, respectively.

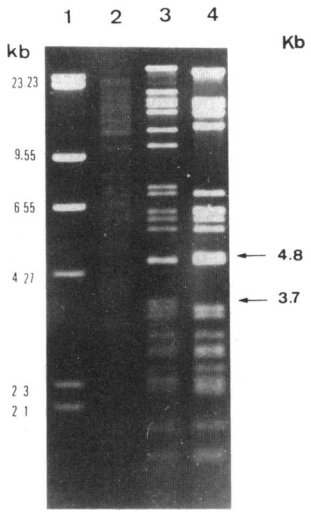

FIG. 5. Results of digestion of pMOL28 and pMOL50 (a derivative of pMOL28 from thermospontagenesis) with *Eco*RI. The 4.8-kb fragment (lane 4) which contains IS*1086* in pMOL28 is replaced by a 3.7-kb fragment in pMOL50 (lane 3). Lane 2 is a CH34 chromosomal *Eco*RI digestion.

homology with those of *E. coli* IS*30* and *B. fragilis* IS*4351*. A low degree of conservation in the amino acid sequence of the long ORF might indicate an ancient origin for the family constituted by these IS elements, as suggested by Schwartz et al. (32) for elements belonging to the IS*2*-IS*3* family. However, intra- or interspecies gene transfer events among eubacteria as well as selective pressure (e.g., on base composition and codon usage) on these elements when they migrate into a new genus may result in a rapid divergence that could be mistaken for a common ancestry. The mean G+C content of IS*30* (46%) is still a little lower than the genomic G+C content of its *E. coli* host (50%), particularly at silent codon positions, suggesting that IS*30* might be a recent immigrant in *E. coli*. Also supporting this view is the finding that AUA, AGG, AGA, and CGA codons, which are infrequent in *E. coli* genes, are more frequent in IS*30* (35).

Besides the two or three small conserved blocks existing at the beginning and in the middle of the IS*30*-, IS*1086*-, and IS*4351*-encoded proteins, the identity is most striking towards the C-terminal end. In view of the conserved blocks of bases in the inverted repeats of the three elements, this suggests that the well-conserved region carries the end-binding domains of the transposases, which is similar to the organization of transposases in the IS*3* family (32) and in IS*10* (14).

In spite of their sequence similarity, the G/C bias of these IS elements leads naturally to a different codon usage; for instance, 92% of lysine codons in IS*1086* are AAG whereas only 25% are in IS*30*.

The homology between IS*1086* and part of the *S. citri* phage Spv1-R8A2 B genome is interesting. Although the phage genome is a single-stranded circular DNA, it has homology with its host genome, and one of its ORFs (ORF4) displays a low level of homology with *Salmonella* phage P22 integrase. It was therefore suggested that the corresponding protein could catalyze integration of the Spv1-R8A2 B genome into the host chromosome (2, 26). Our results suggest that, alternatively, the protein encoded by ORF3 could act on the adjacent inverted repeat to catalyze inte-

gration of the phage genome, in which case SpV1-R8A2 B could be a new kind of mutator or transposing phage whose genome is packaged as a single strand.

Since *S. citri* is gram positive according to Woese (36), we postulate that the IS*1086* family is present in a large range of eubacterial species. Similar findings for other IS elements, for example, IS*257*, have been reported previously (27). If intra- or interspecies gene transfer is the basis of the relationship between IS*30*, IS*4351*, and IS*1086*, it would be interesting to know which mechanisms (if any) would act to adapt the invading genetic information to the host G+C content and at what pace.

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REFERENCES

1. **Bagdasarian, M. M., R. Lurz, B. Rückert, and M. Bagdasarian.** 1983. Activity of hybrid *trp-lac* (tac) promoter of *E. coli* in *P. putida*. Construction of broad-host-range, controlled expression vectors. Gene **26**:273–282.
2. **Bové, J. M., P. Carle, M. Gernier, F. Laignet, J. Renaudin, and C. Saillard.** 1989. Molecular cellular biology of *Spiroplasma*, p. 244–364. *In* R. F. Whitcomb and J. G. Tully (ed.), The mycoplasmas, vol. 5. Academic Press, New York.
3. **Boyer, H. W., and D. Rolland-Dussoix.** 1969. A complementation analysis of restriction and modification of DNA in Escherichia coli. J. Mol. Biol. **41**:59.
4. **Calos, M. P., and J. H. Miller.** 1980. Transposable elements. Cell **20**:579–595.
5. **Caspers, P., B. Dalrymple, S. Iida, and W. Arber.** 1984. IS30, a new insertion of *E. coli* K-12. Mol. Gen Genet. **196**:68–73.
6. **Dalrymple, B., P. Caspers, and W. Arber.** 1984. Nucleotide sequence of the procaryotic mobile genetic element IS30. EMBO J. **3**:2145–2149.
7. **Datta, N., and R. W. Hedges.** 1971. Trimethoprim resistance conferred by W plasmids in Enterobacteriaceae. J. Gen. Microbiol. **72**:349–356.
8. **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.
9. **Galas, D. J., and M. Chandler.** 1989. Bacterial insertion sequences, p. 109–162. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
10. **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.
11. **Gay, P., D. Le Coq, M. Steinmetz, E. Ferrari, and J. A. Hoch.** 1983. Cloning structural gene *sacB*, which codes for exoenzyme levansucrase of *Bacillus subtilis*: expression of the gene in *Escherichia coli*. J. Bacteriol. **153**:1424–1431.
12. **Grindley, N. D. F., and R. R. Reed.** 1985. Transpositional recombination in procaryotes. Annu. Rev. Biochem. **54**:863–896.
13. **Hanahan, D.** 1985. Techniques for transformation of *E. coli*, p. 109. *In* D. M. Glover (ed.), DNA cloning: a practical approach, vol. 1. IRL Press, McLean, Va.
14. **Huisman, O., P. R. Errada, L. Signon, and N. Kleckner.** 1989. Mutational analysis of IS10's outside end. EMBO J. **8**:2101–2109.
15. **Iida, S., J. Meyer, and W. Arber.** 1983. Procaryotic IS elements, p. 159–221. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press Inc., New York.
16. **Jendrossek, D., N. Kruger, and A. Steinbüchel.** 1990. Characterization of alcohol dehydrogenase genes of derepressible wild-type *Alcaligenes eutrophus* H16 and constitutive mutants. J. Bacteriol. **172**:4844–4851.
17. **Kleckner, N.** 1981. Transposable elements in procaryotes. Annu. Rev. Genet. **15**:341.
18. **Kolaskar, A. S., and B. V. B. Reddy.** 1985. A method to locate protein coding sequences in DNA of procaryotic systems. Nucleic Acids Res. **13**:185–194.
19. **Mergeay, M., C. Houba, and J. Gerits.** 1978. Extrachromosomal inheritance controlling resistance to cadmium, cobalt and zinc ions: evidence from curing a Pseudomonas. Arch. Int. Physiol. Biochim. **86**:440–441.
20. **Mergeay, M., D. Nies, H. G. Schlegel, J. Gerits, P. Charles, and F. Van Gijsegem.** 1985. *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J. Bacteriol. **162**:328–334.
21. **Mergeay, M., A. Sadouk, L. Diels, M. Faelen, J. Gerits, J. Denecke, and B. Powell.** 1987. High level spontaneous mutagenesis revealed by survival at non-optimal temperature in *Alcaligenes eutrophus* CH34. Arch. Int. Physiol. Biochim. **95**:B36.
22. **Muto, A., and S. Osawa.** 1987. The guanine and cytosine content of genomic DNA and bacterial evolution. Proc. Natl. Acad. Sci. USA **84**:166–169.
23. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85**:2444–2448.
24. **Prakash, R. K., R. A. Schilperoort, and M. P. Nuti.** 1981. Large plasmids of fast-growing rhizobia: homology studies and location of structural nitrogen fixation (*nif*) genes. J. Bacteriol. **145**:1129–1136.
25. **Rasmussen, J. L., D. A. Odelson, and F. L. Macrina.** 1987. Complete nucleotide sequence of insertion element IS*4351* from *Bacteroides fragilis*. J. Bacteriol. **169**:3573–3580.
26. **Renaudin, J., P. Aullo, J. C. Vignault, and J. M. Bové.** 1990. Complete nucleotide sequence of the genome of *Spiroplasma citri* virus SpV1-R8A2 B. Nucleic Acids Res. **18**:1293.
27. **Rouch, D. A., and R. A. Skurray.** 1989. IS257 from Staphylococcus aureus: member of an insertion sequence superfamily prevalent among Gram-positive and Gram-negative bacteria. Gene **76**:195–205.
28. **Sadouk, A., S. Taghavi, and M. Mergeay.** Unpublished data.
29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
31. **Schubert, P., N. Krüger, and A. Steinbüchel.** 1991. Molecular analysis of *Alcaligenes eutrophus* poly(3-hydroxybutyrate) biosynthetic operon: identification of the N terminus of poly(3-hydroxybutyrate) synthase and identification of the promoter. J. Bacteriol. **173**:168–175.
32. **Schwartz, E., M. Kroger, and B. Rak.** 1988. IS150: distribution, nucleotide sequence and phylogenetic relationships of a new *E. coli* insertion element. Nucleic Acids Res. **16**:6790–6802.
33. **Tran-Betcke, A., U. Warnecke, C. Böcker, C. Zaborosch, and B. Friedrich.** 1990. Cloning and nucleotide sequences of the genes for the subunits of NAD-reducing hydrogenase of *Alcaligenes eutrophus* H16. J. Bacteriol. **172**:2920–2929.
34. **Vanderleyden, J.** Personal communication.
35. **Wada, K., Y. Wada, H. Doi, F. Ishibashi, T. Gojobori, and T. Ikemura.** 1991. Codon usage tabulated from the GenBank genetic sequence data. Nucleic Acids Res. **19**(Suppl.):1981–1986.
36. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51**:221–271.